CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

203100Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

ADDENDUM TO THE OFFICE OF CLINICAL PHARMACOLOGY REVIEW

NDA: 203100	Submission Date: October 27, 2011
Brand Name	Stribild
Generic Name	Elvitegravir/Cobicistat/Emtricitabine/Tenofovir
Reviewer	Vikram Arya, Ph.D., FCP Stanley Au, Pharm.D., BCPS Leslie Chinn, Ph.D.
Pharmacometrics Reviewer	Jeffry Florian, Ph.D.
Pharmacometrics Team Leader	Yaning Wang, Ph.D.
Clinical Pharmacology Team Leader	Kellie Reynolds, Pharm.D.
OCP Division	Division of Clinical Pharmacology 4
OND Division	Division of Antiviral Products
Applicant	Gilead Sciences Inc.
Formulation; strength(s) to-be-marketed	Fixed dose combination tablet: 150/150/200/300 mg (EVG/Cobi/FTC/TDF)
Proposed Indication for the Application	Treatment of HIV-1 infection in treatment naïve adults
Review Type(s)	505 (b)(1) New Drug Application, standard review

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Subsequent to the finalization of the Clinical Pharmacology review for NDA 203100 on July 2, 2012 the following additional issues were reviewed or discussed:

A) The applicant proposed to include a statement in the elvitegravir/cobicistat /emtricitabine/tenofovir prescribing information (section 7.2 and section 12.3) stating that there is minimal metabolism of cobicistat through CYP2D6. A new in vitro report (AD-216-2106) was submitted to support this information.
B) The long term stability of the desipramine and digoxin anaytes was evaluated to support including drug-drug interaction data for desipramine and digoxin when coadminstered with cobicistat in the prescribing information.
C) The clinical pharmacology postmarketing requirements (PMRs) were finalized.
D) Several sections of the prescribing information (label) were updated.

A) New in vitro report-AD-216-2106

A new in vitro report was submitted. The in vitro report evaluated the metabolites that are formed through CYP3A4 and CYP2D6 metabolism of cobicistat.

1. Title

Metabolite Profiles of Cobicistat Generated by Human CYP2D6 and CYP3A4

2. Objectives

The objective of the study was to determine the metabolites that are formed through CYP3A4 and CYP2D6 metabolism of cobicistat.

3. Methods

Reference standards were synthesized for cobicistat and three cobicistat metabolites: GS-341842 (M25, E5), GS-9612 (GS-364751, M31, E3) and GS-9454 (GS-342006, M21, E1).

Figure 1-Chemical structures for cobicistat and cobicistat metabolites

Cobicistat
Exact Mass: 775.35

GS-341842
Exact Mass: 636.31

GS-9612
Exact Mass: 791.35

GS-9454
Exact Mass: 634.37

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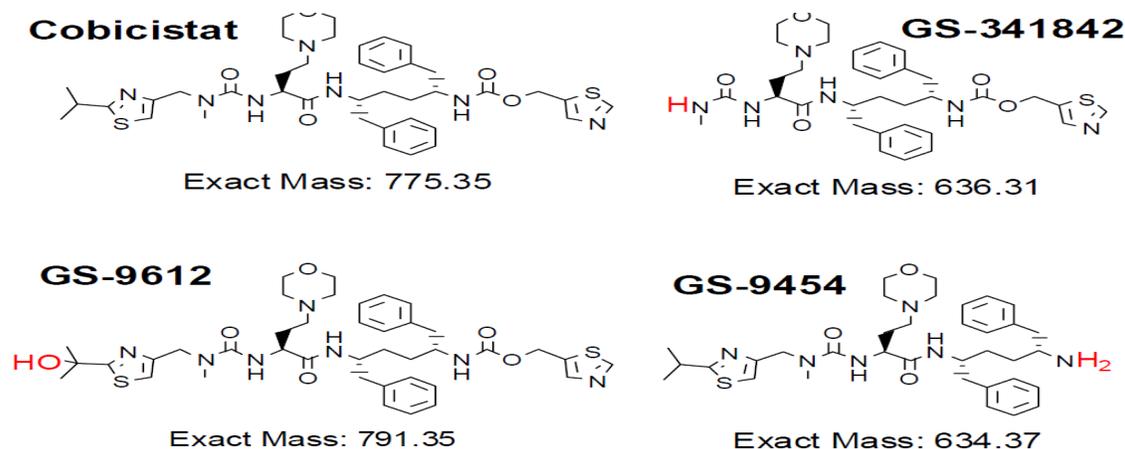
Cobicistat was incubated with human hepatic microsomes or recombinant CYP2D6 or CYP3A4 for 2 hours at 37°C. A 20 μM concentration of cobicistat was evaluated, which exceeds the C_{max} concentration for cobicistat 150 mg once daily in healthy subjects of approximately 2 μM. Samples were subsequently analyzed using an UPLC instrument.

4. Results

In both the human microsomes and recombinant CYP3A4, the most abundant metabolite that was detected was the M31 metabolite (m/z=792.3), with the M25 (m/z=637.35) and M21 (m/z=635.3) metabolites also detected. In recombinant CYP2D6, the most abundant metabolite was the M31 metabolite, and the second most abundant metabolite was an unidentified metabolite (m/z=808.3) that was not isolated from human microsomes and recombinant CYP3A4. The M21 metabolite was also detected in recombinant CYP2D6.

Chromatograms displaying the observed peaks for human hepatic microsomes, and recombinant CYP3A4 and CYP2D6 after incubating cobicistat are displayed in Figure 2.

Figure 2-Chromatograms for human microsomes, and recombinant CYP3A4 and CYP2D6 after incubating cobicistat (note: cobicistat m/z=776.3).

<u>Human microsomes</u>
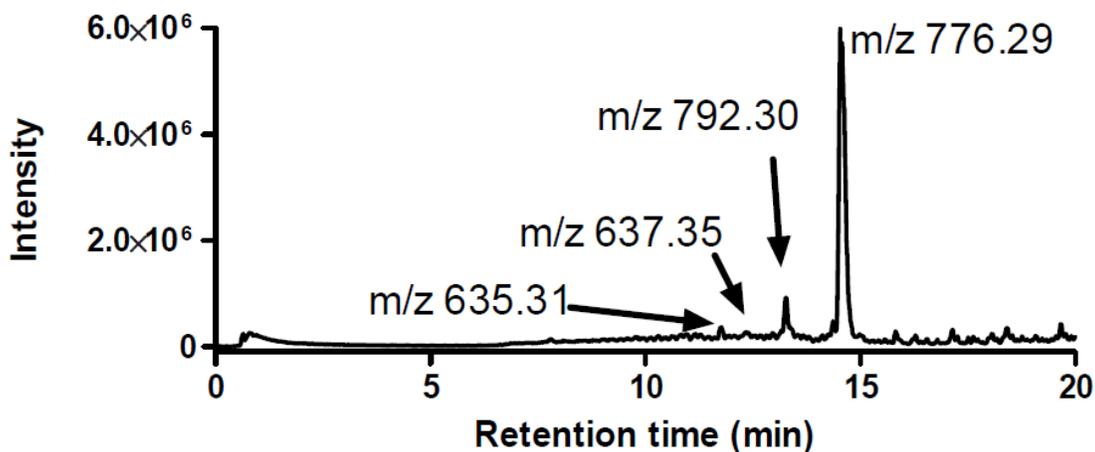
Recombinant CYP3A4
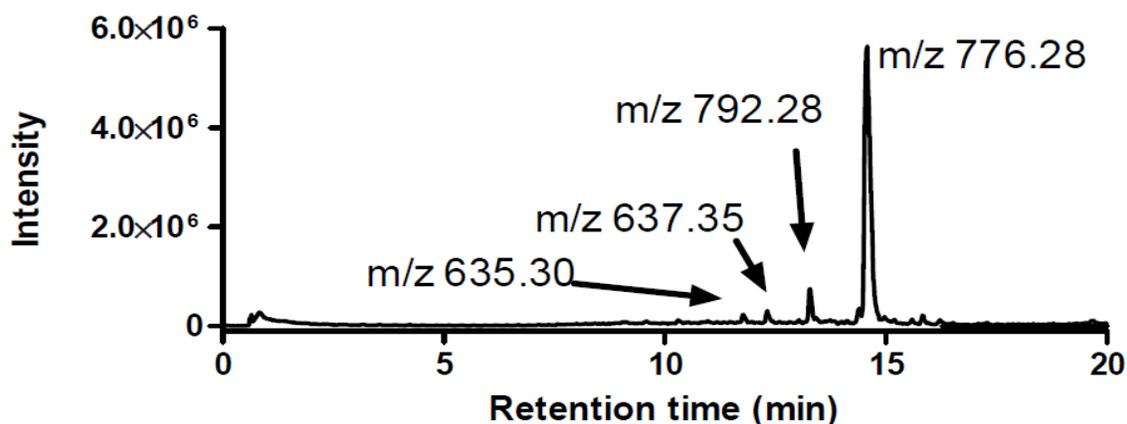
Recombinant CYP2D6
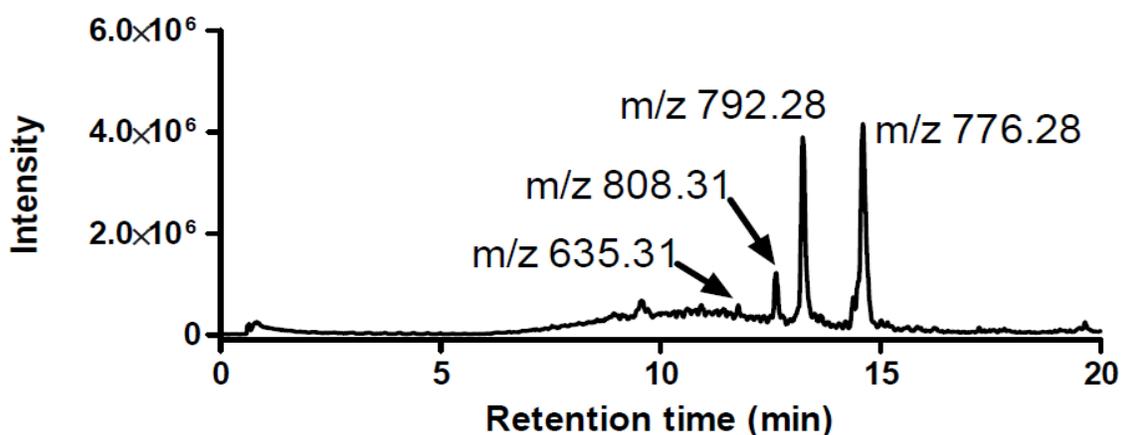
5. Conclusions

For cobicistat, the metabolism profile of recombinant CYP3A most closely matched the metabolism profile obtained from human hepatic microsomes. In the human mass balance trial (GS-US-216-111), the mean percentage of total ^{14}C radioactivity that was detected using high performance liquid chromatography for the M31 metabolite was 14% and 0.7% in feces and urine, respectively. Therefore, CYP2D6 is not anticipated to be a primary metabolism pathway for cobicistat.

B) Bioanalytical issues

The applicant was requested to provide long term analyte stability data for desipramine and digoxin to support the inclusion of drug-drug interaction data for desipramine and digoxin when coadminstered with cobicistat in the prescribing information.

For the GS-US-216-112 trial, desipramine was stored at -70ºC for approximately 27 days at the trial site and at -20ºC for approximately 36 days at the bioanalytical

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laboratory. Desipramine long term stability data at -20ºC was reviewed as part of the Clinical Pharmacology review for NDA 203100 that was finalized on July 2, 2012. For the addendum, additional long term stability data for desipramine at -70ºC was submitted by the applicant. The submitted desipramine long term sample stability data demonstrated that desipramine was stable for 91 days at -20°C and 28 days at -70ºC in K_2EDTA anticoagulated plasma.

Digoxin was stored at -70ºC at the trial site and at the bioanalytical laboratory for the GS-US-216-112 trial. The total duration of digoxin long term stability data at -70ºC that is needed for the GS-US-216-112 trial is approximately 55 days. The applicant submitted acceptable long term stability data for digoxin at -70ºC for 75 days in lithium heparin plasma. However, the digoxin samples for the GS-US-216-112 trial were drawn in tubes using K_2EDTA as the anticoagulant. To determine the potential impact of the differences in the anticoagulant used, two issues were considered:

1) For the incurred sample reanalysis, out of 97 samples that were reanalyzed, one sample did not meet the acceptance criteria. The digoxin bioanalytical report for the GS-US-216-112 trial states that the total number of samples was 920. Based on the results the incurred sample reanalysis, no issues were identified.

2) The digoxin Cmax and $AUC_{(0-inf)}$ pharmacokinetic parameters for a single 0.5 mg dose (when administered by itself) that were reported for the GS-US-216-112 trial are similar to the pharmacokinetic parameters that were reported from a published trial (see Table 1 below).

Table 1-Comparison of geometric mean digoxin C_{max} and $AUC_{(0-inf)}$ values for a single 0.5 mg dose

	GS-US-216-112	Schmitt, et, al[1]
C_{max} (ng/mL)	1.7	1.87
$AUC_{(0-inf)}$ [ng*hr/mL]	30.9	27.0

[1] Schmitt C, et al. Effect of saquinavir/ritonavir on P-glycoprotein activity in healthy volunteers using digoxin as a probe. Int J Clin Pharmacol Ther. 2010 Mar; 48(3):192-9.

Overall, while the impact of the differences in anticoagulants on digoxin long term stability has not been directly evaluated, no issues were identified that are anticipated to impact the reported digoxin plasma concentration data.

C) Clinical Pharmacology related Post Marketing Requirements (PMRs)

The PMRs for Stribild were under discussion at the time of finalizing the clinical pharmacology review on July 2, 2012. Following discussions within the review team and with the applicant, the following clinical pharmacology related PMRs were agreed upon:

- Conduct a pharmacokinetic (PK) sub-trial of the renal safety trial in women to evaluate the potential for drug-drug interaction between Stribild and commonly used oral contraceptives. Intensive pharmacokinetic data on each oral contraceptive, when given alone and when co-administered with Stribild, should be collected in an adequate number of subjects.

  Rationale:

  Oral contraceptives are a convenient option for contraception and widely used in the HIV infected population. The results from a drug-drug interaction trial of STRIBILD and Ortho Tricyclen Lo showed a significant increase in the exposure of the progestational component. Due to the safety concerns associated with increase in exposure of the progestational component, drug-drug interaction information of STRIBILD with other commonly used oral contraceptives will provide information on the safe and effective use of "Stribild-Oral Contraceptive" combination in HIV infected patients.

- Conduct an *in vivo* drug-drug interaction trial of Stribild and telaprevir.

  Rationale:

  Telaprevir is a substrate of CYP3A and p-gp; therefore, the systemic concentration of telaprevir is expected to increase when telaprevir is co-administered with STRIBILD. In addition, telaprevir is an inhibitor of CYP3A therefore; systemic concentrations of elvitegravir and cobicistat are expected to increase when STRIBILD is co-administered with telaprevir.

  The results from an *in vivo* drug-drug interaction trial between STRIBILD and telaprevir will provide quantitative drug-drug interaction information for the safe and effective use of the combination in patients who are co-infected with HIV and Hepatitis C (HCV).

- Conduct an *in vivo* drug-drug interaction trial of Stribild and buprenorphine/naloxone.

  Rationale:

  Buprenorphine/naloxone is used to treat opioid dependence (by alleviating the withdrawal symptoms) in HIV-1 infected patients. Buprenorphine is a substrate of CYP3A4 and STRIBILD is a CYP3A4 inhibitor; therefore, there is potential for increase in the systemic concentration of buprenorphine when buprenorphine/naloxone is administered with STRIBILD.

  The results from an *in vivo* drug-drug interaction trial of STRIBILD and buprenorphine/naloxone will provide quantitative drug-drug interaction information for the safe and effective use of the combination in HIV-1 infected patients.

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- Conduct an in vivo drug-drug interaction trial of Stribild and Methadone.

  Rationale:

  Methadone is used to treat opioid dependence (by alleviating the withdrawal symptoms) in HIV-1 infected patients. Methadone is a substrate of CYP3A4 and CYP2D6 and STRIBILD is an inhibitor of CYP3A4 and weak inhibitor of CYP2D6; therefore, there is potential for increase in the systemic concentration of methadone when methadone is administered with STRIBILD.

  The results from an *in vivo* drug-drug interaction trial of STRIBILD and methadone will provide quantitative drug-drug interaction information for the safe and effective use of the combination in HIV-1 infected patients.

D) Updates to the Prescribing Information

Major clinical pharmacology related updates to the prescribing information after finalizing the clinical pharmacology review include:

1) Changes to table 5 in Section 7.4 (Established and Other Potentially Significant Interactions)

Section (s) Impacted	Information in Final Clinical Pharmacology Review (Finalized 7/2/2012)	Information in Final Prescribing Information	Comments
Section 7.4: Anticoagulants: Warfarin	Effect on concentration column indicates (b) (4)	Effect on concentration column indicates "Effect on warfarin unknown"	Applicant's proposed language "effect on warfarin unknown" is based on the inhibition effect of Stribild on CYP3A enzymes and induction effect of Stribild on CYP2C9 enzymes.
Section 7.1 (Potential for Stribild to Affect Other Drugs)	No information regarding potential for EVG to induce CYP2C9 enzymes	(b) (4)	The results of study JTK303-AD-023 showed that EVG has the potential to induce CYP2C9 enzyme.
Section 7.4: Antifungals	No clinical recommendation regarding voriconazole	An assessment of benefit/risk ratio is recommended to justify use of voriconazole with Stribild.	Clinical recommendation regarding voriconazole was under discussion at the time of finalizing the clinical pharmacology

			review.
Section 7.4: HMG-CoA reductase inhibitors Atorvastatin [(b) (4)]	The "effect on concentration" column indicates [(b) (4)]	The "effect on concentration" column indicates "↑ atorvastatin"	Based on consultation with the Acting Clinical Pharmacology Team Leader for Metabolic and Endocrinology Team, [(b) (4)] was excluded from the clinical recommendation table. The decision to exclude [(b) (4)] was based on the fact [(b) (4)] [(b) (4)]
Section 7.4: Hormonal Contraceptives	Clinical Recommendation indicates: [(b) (4)]	Clinical Recommendation indicates: The effects of increases in the concentration of the progestational component norgestimate are not fully known and can include increased risk of insulin resistance, dyslipidemia, acne, and venous thrombosis. The potential risks and benefits associated with coadministration of norgestimate/ethinyl estradiol with STRIBILD should be considered, particularly in women who have risk factors for these events. Coadministration of STRIBILD with other hormonal contraceptives	Based on discussions internally and with the applicant, it was determined that the recommendation should be revised to focus more on the adverse events associated with increase in exposure of the norgestimate component. The statement [(b) (4)] was deleted [(b) (4)]

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		(eg, contraceptive patch, contraceptive vaginal ring, or injectable contraceptives) or oral contraceptives containing progestogens other than norgestimate has not been studied; therefore, alternative (non hormonal) methods of contraception can be considered.	(b) (4)

2) Changes to section 12.3

- In the Absorption and Bioavailability sub-section:
    i. A table was included to show the population pharmacokinetic parameters of elvitegravir, cobicistat, emtricitabine, and tenofovir following oral administration of Stribild in HIV infected patients.
    ii. ⬛⬛⬛ (b) (4) was replaced with "Stribild should be taken with food".

- In the Metabolism sub-section, ⬛⬛⬛ (b) (4) ⬛⬛⬛ was replaced with "Cobicistat is metabolized by CYP3A and to a minor extent by CYP2D6 enzymes and does not undergo glucuronidation".
- Tables 7 and 8 were updated to show the mean ratio of the pharmacokinetic parameters and the 90 % CI.
- Table 8 was updated to include the pharmacokinetic parameters of desipramine and digoxin based on the results of trial GS-US-216-0112.

Additional minor changes were made to the Highlights section, other sections of the Prescribing Information, and the Patient Information Section to provide consistent information throughout the Prescribing Information of Stribild.

Reference ID: 3177545

OFFICE OF CLINICAL PHARMACOLOGY (OCP) REVIEW

NDA: 203-100	Submission Date: October 27, 2011
Brand Name	STRIBILD
Generic Name	Elvitegravir/Cobicistat/Emtricitabine/Tenofovir
Reviewers	Vikram Arya, Ph.D., FCP Stanley Au, Pharm.D., BCPS Leslie Chinn, Ph.D.
Pharmacometrics Reviewer	Jeffry Florian, Ph.D.
Pharmacometrics Team Leader	Yaning Wang, Ph.D.
Clinical Pharmacology Team Leader	Kellie Reynolds, Pharm.D.
OCP Division	Division of Clinical Pharmacology 4
OND Division	Division of Antiviral Products (DAVP)
Applicant	Gilead Sciences Inc.
Formulation; strength(s) to-be-marketed	Tablets. 150/150/200/300 mg (EVG/Cobi/FTC/TDF)
Proposed Indication	Treatment of HIV-1 infection in treatment naïve adults
Review Type	505 (b)(1) New Drug Application, Standard Review

1 Executive Summary

Elvitegravir/Cobicistat/Emtricitabine/Tenofovir (EVG/Cobi/FTC/TDF) is a new, 4-component fixed dose combination (FDC) tablet. EVG is a new chemical entity that belongs to the class of HIV-1 integrase strand-transfer inhibitors. Cobi, a new chemical entity, is a structural analog of ritonavir and an inhibitor of CYP3A enzymes. Cobi enhances (or boosts) the systemic exposures of EVG. Emtricitabine (FTC) and Tenofovir (TDF) are nucleoside- and nucleotide reverse transcriptase inhibitors (NRTIs and N$_{(t)}$RTIs), respectively, and are approved either individually (Emtriva® and Viread®, respectively), as a combination (Truvada®; TVD), or as part of a combination (Atripla®; and Complera®),

The **proposed dosing regimen** of the FDC tablet is 150/150/200/300 mg (EVG/Cobi/FTC/TDF) once daily. The doses of emtricitabine (200 mg) and tenofovir (300 mg) in the FDC tablet are identical to the doses of emtricitabine and tenofovir in approved products.

The **proposed indication** for the FDC tablet is the treatment of HIV-1 infection in adults age 18 and older who are antiretroviral (ARV) treatment naïve and have no known substitutions associated with resistance to the individual components. **FDC tablet is intended for use as a complete regimen in treatment naïve HIV-1 infected patients and will not be combined with other antiretroviral drugs.**

The safety and efficacy data for FDC were collected in two pivotal phase III trials (GS-US-236-0102 and GS-US-236-0103) and one phase II trial (GS-US-236-0104). All the aforementioned trials were conducted in treatment naïve, HIV-1 infected patients using the proposed commercial FDC tablets.

The applicant provided the results from clinical pharmacology trials to support the prescribing information for FDC. The clinical pharmacology trials were conducted using EVG (co-administered with low dose ritonavir; 12 *in vivo* trials), Cobi (7 *in vivo* trials), EVG and cobi given together (6 *in vivo* trials), and FDC (7 *in vivo* trials). In addition, the applicant provided the results of 43 *in vitro* studies to evaluate the absorption and disposition properties and the effect of enzymes and transporters on EVG (18 studies) and Cobi (25 studies).

1.1   Recommendation

The Office of Clinical Pharmacology (OCP) has reviewed the information in this NDA and the information provided supports the approval of the application.

1.2   Postmarketing Commitments or Requirements

The post marketing commitments were under discussion at the time of completion of this review. As part of post marketing commitments, the applicant

may be asked to conduct drug-drug interaction trials of STRIBILD with telaprevir ^{(b) (4)}

1.3 Summary of Important Clinical Pharmacology and Biopharmaceutics Findings

Exposure-response analyses focused on the two new molecular entities, EVG (efficacy and safety) and Cobi (safety). Exposure-response (efficacy) analyses were not performed for Cobi because the primary role of Cobi is to increase the systemic exposure of EVG and Cobi does not have antiviral activity. Exposure-response safety analyses for EVG and Cobi focused on adverse events of interest from the Phase III trials. Additional exposure-response safety analyses for Cobi evaluated changes in renal function due to Cobi exposure.

In trial GS-US-236-0102, 87.6% of EVG/Cobi/FTC/TDF subjects had virologic success compared to 84.1% of subjects in the Atripla group. In trial GS-US-236-0103, 89.5 % of EVG/Cobi/FTC/TDF subjects had virologic success compared to 86.8 % of subjects in the Atazanavir/ritonavir (+Truvada) group. Additional details regarding trial design and summary of major findings are provided in section 2.2.

1.3.1 Dose Selection and Exposure-response (efficacy) analyses

EVG:

The Phase III EVG dose (150 mg) was selected based on the results of the Phase 2 trial GS-US-183-0105 that demonstrated greater decreases in viral load at week 24 with 125/100 mg EVG boosted with ritonavir (EVG/r) compared to 20/100 or 50/100 mg. A final dose of EVG/r 150/100 mg was selected based on the results of a relative bioavailability trial (GS-US-183-0140) that demonstrated similarity in EVG systemic exposures between 125 mg EVG using the Phase 2 formulation (+100 mg ritonavir) and 150 mg EVG using a new formulation (+100 mg ritonavir). The EVG/Cobi/FTC/TDF tablet provides similar EVG exposure compared to 150 mg EVG + 100 mg ritonavir.

EVG exposure-response analysis for efficacy in treatment naïve HIV-1 infected patients was based on intensive and sparse pharmacokinetic data available from 373 subjects who received FDC in GS-US-236-0102 and GS-US-236-0103. A relatively flat exposure-response relationship was identified across the EVG exposures (EVG AUC (min-max) was 4358-69754 ng·hr/mL; C_{tau} (min-max) was 58-2341 ng/mL).

Cobi:

The dose of Cobi (150 mg) was selected based on the similarity of EVG C_{tau} and AUC after administration of EVG (150 mg) + Cobi (150 mg) and EVG (150 mg) + ritonavir (100 mg) in trial GS-US-236-0101.

Exposure-response (efficacy) analyses were not performed for Cobi because the primary role of Cobi is to increase the systemic exposure of EVG and Cobi does not have antiviral activity.

1.3.2   **Exposure-safety analyses**

The exposure-safety analyses for EVG evaluated whether there was a potential relationship between predicted EVG AUC_{tau} and C_{tau} and the most common adverse events observed during the Phase III trials (e.g., headaches, diarrhea, and nausea). For all EVG analyses, no relationship was observed between predicted AUC_{tau} or C_{tau} and the adverse event of interest.

Exposure-safety analyses for Cobi assessed both associations with common adverse events observed during Phase III trials (e.g., headaches, diarrhea, and nausea) and changes in calculated creatinine clearance. Cobi exposure-response analysis was based on intensive PK sampling in 61 subjects. Similar Cobi exposures (AUC_{tau} and C_{tau}) were observed between subjects with and without the key adverse events of interest.

An exposure-response analysis on changes in renal function versus Cobi AUC_{tau} was also conducted as elevations in serum creatinine and subsequently, reduced estimated glomerular filtration rate (eGFR), was observed in the FDC treatment arms. No relationship between Cobi AUC_{tau} and change in eGFR was observed based on the available Cobi pharmacokinetic data.

The applicant performed an iohexol study that evaluated actual glomerular filtration rate (aGFR). Results indicate no statistically significant changes ($p < 0.05$) in GFR from baseline were observed on days 7 and 14 for placebo, cobicistat, or ritonavir. In addition, the reviewers performed an analysis evaluating eGFR from the Phase III trials.   In patients with decreased baseline renal function, smaller maximal decrease in eGFR was observed.   This observation of smaller maximal decreases in eGFR in subjects with lower baseline eGFR provides additional information that decreases in eGFR observed during Phase III and attributed to Cobi may be attributed to inhibition of tubular secretion of creatinine as hypothesized by the applicant.

Finally, an imbalance in renal adverse events was observed between the FDC treatment arms and active comparator arms from Phase III (9 subjects on a FDC regimen, 2 subjects on Atripla, and 1 subject on atazanavir/ritonavir). Of these subjects, 6 discontinued FDC therapy due to renal adverse events.   No exposure-response relationship regarding renal adverse events between EVG exposures could be identified from the available data. In addition, exposure-response relationships for cobicistat, emtricitabine and tenofovir could not be evaluated due to the limited number of subjects with cobicistat, tenofovir or emtricitabine pharmacokinetic data (n=61).

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1.3.3　　　**Absorption**

- EVG and Cobi are highly permeable compounds.
- EVG and Cobi are P-glycoprotein substrates.
- The results of the drug-drug interaction trials of EVG and Cobi, administered together with drugs that alter the intra gastric pH (famotidine and omeprazole; trials GS-US-216-0120 and GS-US-216-0122) or EVG, co-administered with low dose ritonavir (GS-US-183-0119) suggest that there is no significant effect of changes in pH on the absorption of EVG and Cobi.
- The results of trial GS-US-183-0119 suggest that STRIBILD and antacid administration should be separated by two hours. The interaction observed between STRIBILD and antacid is due to complexation with divalent cations (and not due to alterations in pH). .
- FDC tablets should be taken with food. The results of a food effect trial (GS-US-236-0105) conducted using FDC tablets showed that the mean AUC_{inf} of EVG increased by 34 % and 87 % under light meal and high fat meal conditions respectively, as compared to administration of FDC tablets under fasting conditions. Food altered cobi exposures by < 20 %.

1.3.4　　　**Distribution**

EVG:

The % unbound of EVG, as determined by equilibrium dialysis, is ~ 0.7 %. The blood-to-plasma ratio of ^{14}C EVG radioactivity is ~0.73 indicating that EVG was predominantly distributed to plasma relative to the cellular components of the blood.

Cobi:

At 1 μM, the % unbound of Cobi in human plasma is ~ 6.3 %. The blood-to-plasma ratio of total ^{14}C Cobi radioactivity was less than or equal to 0.75 indicating that Cobi is excluded from the cellular components of the blood.

FTC and TDF:

The fraction unbound of FTC and TDF in human plasma is > 95 % and > 99 %, respectively.

1.3.5　　　**Metabolism**

EVG:

The majority of EVG metabolism in human liver microsomes is mediated by CYP3A4, although metabolism *via* CYP3A5 and CYP1A1 was also detected.

Cobi:

The *in vitro* metabolism of cobi was evaluated in human hepatocytes and hepatic microsomal fractions. The results showed that CYP3A4 and CYP2D6 are involved in the metabolism of Cobi.

FTC and TDF are not significantly metabolized.

1.3.6 Excretion

EVG:

The major route of elimination of EVG and its metabolites is hepatobiliary excretion. The results of the mass balance trial conducted using a single dose of 50 mg [^{14}C] EVG (co-administered with 100 mg ritonavir) showed that the majority of the radioactivity was excreted in feces (94.8 %: 30.8 % EVG; 33.8 % GS-9202 [metabolite of EVG produced by CYP3A4 mediated metabolism of EVG] and low levels of other metabolites), while urinary recovery was low (6.7%: only metabolites were detected in the urine; no unchanged EVG was detected in the urine).

COBI:

The major route of elimination of Cobi and its metabolites is hepatobiliary excretion. The results of the mass balance trial showed that following single dose administration of [^{14}C] Cobi, the majority (86.2% of the administered dose; 27 % of the administered dose recovered as Cobi followed by known oxidative metabolites E3 [14 %] and E1 [5.5 %]) of the radioactivity was excreted in the feces, while urinary recovery was 8.2% (6.3 % as unchanged Cobi; metabolites were present in low amounts).

FTC and TDF:

FTC and TDF are primarily eliminated unchanged by a combination of glomerular filtration and active tubular secretion.

1.3.7 Intrinsic factors

Renal Impairment:

FDC tablets can be given to subjects with eGFR ≥ 70 mL/min without any dose adjustments. eGFR ≥ 70 mL/min was one of the inclusion criteria in pivotal Phase III trials to avoid early discontinuations due to the expected inhibitory effect of Cobi on creatinine secretion. Because the dosing frequency of tenofovir needs to be adjusted when given to patients with eGFR ≤ 50 mL/min, FDC will be discontinued in patients with eGFR ≤ 50 mL/min. For patients with eGFR

between 50 and 70 mL/min, the pharmacokinetics of the individual components of the FDC is not expected to be different from patients with eGFR ≥ 70 mL/min. However, due to additional considerations related to the safety profile of FDC in patients with eGFR 50-70 mL/min, FDC should not be initiated in patients with eGFR 50-70 mL/min.

Hepatic Impairment:

FDC tablet can be given to subjects with either mild- or moderate hepatic impairment without any dose adjustments. The pharmacokinetics of FDC tablet in subjects with severe hepatic impairment has not been evaluated; therefore, FDC tablets are not recommended for use in patients with severe hepatic impairment.

Other Intrinsic Factors:

Gender, race, and age were not found to influence either exposure or exposure-response relationship for elvitegravir. No dose adjustment is recommended based on body weight, body surface area, or body mass index. No exploration of exposures for cobicistat was performed with respect to covariates due to the limited cobicistat pharmacokinetic data available.

1.3.8   **Extrinsic factors**

Drug-Drug Interactions:

Overall Considerations:

- Clinical recommendations regarding administration of FDC with other antiretroviral drugs are not applicable because FDC will not be co-administered with other antiretroviral drugs.
- The results obtained from drug-drug interaction trials using EVG and Cobi given together are applicable to the FDC tablet because the results of trial GS-US-236-0110 showed that the mean systemic exposures of EVG and Cobi after administration of the FDC tablet were similar to the mean systemic exposures of EVG and Cobi given together. Tenofovir and emtricitabine do not contribute to drug-drug interactions.
- The applicant did not conduct drug-drug interaction trials of STRIBILD with   (b) (4)   telaprevir; DDI trials with methadone and buprenorphine/naloxone are currently ongoing.

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Table 1 shows the drugs that will be contraindicated with [STRIBILD].

Table 1: Drugs Contraindicated with [STRIBILD]

Drug Class	Drugs within class that are contraindicated with STRIBILD	Clinical Comment
Alpha 1-Adrenoreceptor Antagonist:	Alfuzosin	Potential for increased alfuzosin concentrations, which can result in hypotension.
Antimycobacterial	Rifampin	Rifampin is a potent inducer of CYP450 metabolism. STRIBILD should not be used in combination with rifampin, as this may cause significant decrease in the plasma concentration of elvitegravir and cobicistat. This may result in loss of therapeutic effect to STRIBILD.
Ergot Derivatives	Dihydroergotamine Ergotamine Methylergonovine	Potential for serious and/or life-threatening events such as acute ergot toxicity characterized by peripheral vasospasm and ischemia of the extremities and other tissues.
GI Motility Agent	Cisapride	Potential for serious and/or life-threatening events such as cardiac arrhythmias
Herbal Products	St. John's wort (Hypericum perforatum)	Patients taking STRIBILD should not use products containing St. John's wort because co-administration may result in reduced plasma concentrations of elvitegravir and cobicistat. This may result in loss of therapeutic effect and development of resistance.
HMG CoA Reductase Inhibitors:	Lovastatin Simvastatin	Potential for serious reactions such as myopathy including rhabdomyolysis.
Neuroleptic	Pimozide	Potential for serious and/or life-threatening events such as cardiac arrhythmias
Phosphodiesterase-5 (PDE5) Inhibitor	Sildenafil when dosed as REVATIO® for the treatment of pulmonary arterial hypertension	A safe and effective dose in combination with STRIBILD has not been established for sildenafil (REVATIO®) when used for the treatment of pulmonary hypertension. There is increased potential for sildenafil-associated adverse events (which include visual disturbances, hypotension, priapism, and syncope).
Sedative/hypnotics:	Triazolam Orally administered midazolam	Triazolam and orally administered midazolam are extensively metabolized by CYP3A4. Co-administration of triazolam or orally administered midazolam with STRIBILD may cause large increases in the concentration of these benzodiazepines. The potential exists for serious and/or life threatening events such as prolonged or increased sedation or respiratory depression.

Table 2 shows established and other potentially significant drug interactions based on which alteration in dose or regimen may be recommended. The "*" proceeding the name of the drug indicates that a drug-drug interaction trial was conducted.

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Table 2: Established and other potentially significant drug interactions based on which alteration in dose or regimen may be recommended (* indicates that a drug-drug interaction trial was conducted)

Concomitant Drug Class: Drug Name	Effect on Concentration[b]	Clinical Comment
Acid Reducing Agents: Antacids* (for example aluminium and magnesium hydroxide)	↓ elvitegravir	Elvitegravir plasma concentrations are lower when STRIBILD is ⟨(b) (4)⟩ with antacids. It is recommended to separate STRIBILD and antacid administration by at least 2 hours. Note: No dose adjustment is needed when STRIBILD is combined with either H$_2$ receptor antagonists or proton pump inhibitors.
Antiarrhythmics: e.g: amiodarone bepridil disopyramide flecainide systemic lidocaine mexiletine propafenone quinidine digoxin*	↑ antiarrhythmics ↑ digoxin	Concentrations of these antiarrhythmic drugs may be increased when coadministered with STRIBILD. Caution is warranted and therapeutic concentration monitoring, if available, is recommended for antiarrhythmics when co-administered with STRIBILD. ⟨(b) (4)⟩
Antibacterials: clarithromycin telithromycin	↑ clarithromycin ↑ telithromycin ↑ cobicistat	Concentrations of clarithromycin and/or cobicistat may be altered when clarithromycin is coadministered with STRIBILD. Patients with CLcr greater than or equal to 60 mL/min: No dose adjustment of clarithromycin is required Patients with CLcr between 50 mL/min and 60 mL/min The dose of clarithromycin should be reduced by 50%. Concentrations of telithromycin and/or cobicistat may be increased when telithromycin is coadministered with STRIBILD.
Anticoagulants: warfarin	⟨(b) (4)⟩	Concentrations of warfarin may be ⟨(b) (4)⟩ upon coadministration with STRIBILD. It is recommended that the international normalized ratio (INR) be monitored upon coadministration with STRIBILD.
Anticonvulsants: e.g.: carbamazepine	↑ carbamazepine	Coadministration ⟨(b) (4)⟩ with STRIBILD

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oxcarbazepine phenobarbital phenytoin	↓ elvitegravir ↓ cobicistat	may significantly decrease cobicistat and elvitegravir plasma concentrations, which may result in loss of therapeutic effect and development of resistance. Alternative anticonvulsants should be considered.
clonazepam ethosuximide	↑ clonazepam ↑ ethosuximide	Concentrations of clonazepam and ethosuximide may be increased when administered with STRIBILD. Clinical monitoring is recommended upon co-administration with STRIBILD.
Antidepressants: Selective Serotonin Reuptake Inhibitors (SSRIs) e.g. Paroxetine

Tricyclic Antidepressants (TCAs)

e.g.

amitryptyline desipramine imipramine nortryptaline

buproprione trazodone | ↑ SSRIs ↑ TCAs ↑ trazodone | Concentrations of these antidepressant agents may be increased when coadministered with STRIBILD. Careful dose titration of the antidepressant and monitoring for antidepressant response is recommended, |
| Antifungals: itraconazole ketoconazole* voriconazole | ↑ elvitegravir ↑ cobicistat ↑ itraconazole ↑ ketoconazole ↑voriconazole | Concentrations of ketoconazole, itraconazole and voriconazole may increase upon coadministration with STRIBILD. When administering with STRIBILD, the maximum daily dose of ketoconazole or itraconazole should not exceed 200 mg per day. |
| Anti-gout: colchicine | ↑ colchicine | STRIBILD should not be coadministered with colchicine to patients with renal or hepatic impairment.

Treatment of gout-flares – coadministration of colchicine in patients receiving STRIBILD:

0.6 mg (1 tablet) x 1 dose, followed by 0.3 mg (half tablet) 1 hour later. Treatment course to be repeated no earlier than 3 days.

Prophylaxis of gout-flares – coadministration of colchicine in patients receiving STRIBILD:

If the original regimen was 0.6 mg twice a day, the regimen should be adjusted to 0.3 mg once a day. If the original regimen was 0.6 mg once a day, the regimen should be adjusted to 0.3 mg once every other day. |

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		Treatment of familial Mediterranean fever – coadministration of colchicine in patients receiving STRIBILD: Maximum daily dose of 0.6 mg (may be given as 0.3 mg twice a day).
Antimycobacterial: rifabutin* rifapentine	↓ elvitegravir ↓ cobicistat	Coadministration of rifabutin and rifapentine with STRIBILD may significantly decrease elvitegravir and cobicistat plasma concentrations, which may result in loss of therapeutic effect and development of resistance. Coadministration of STRIBILD with rifabutin or rifapentine is not recommended.
Beta-Blockers: e.g. metoprolol timolol	↑ beta-blockers	Concentrations of beta-blockers may be increased when coadministered with STRIBILD. Clinical monitoring is recommended and a dose decrease of the beta blocker may be necessary when these agents are coadministered with STRIBILD.
Calcium Channel Blockers: e.g. amlodipine diltiazem felodipine nicardipine nifedipine verapamil	↑ calcium channel blockers	Concentrations of calcium channel blockers may be increased when coadministered with STRIBILD. Caution is warranted and clinical monitoring is recommended upon coadministration with STRIBILD.
Corticosteroid: Systemic: Dexamethasone	↓ elvitegravir ↓ cobicistat	Systemic dexamethasone, a CYP3A inducer, may significantly decrease elvitegravir and cobicistat plasma concentrations, which may result in loss of therapeutic effect and development of resistance.
Corticosteroid: Inhaled/Nasal: Fluticasone	↑ fluticasone	Concomitant use of inhaled fluticasone and STRIBILD may increase plasma concentrations of fluticasone, resulting in reduced serum cortisol concentrations. Alternative corticosteroids should be considered, particularly for long term use.
Endothelin Receptor Antagonists: bosentan	↑ bosentan	Coadministration of bosentan in patients on STRIBILD: In patients who have been receiving STRIBILD for at least 10 days, start bosentan at 62.5 mg once daily or every other day based upon individual tolerability. Coadministration of STRIBILD in patients on bosentan: Discontinue use of bosentan at least 36 hours prior to initiation of STRIBILD. After at least 10 days following the initiation of STRIBILD, resume bosentan at 62.5 mg once daily or every other day based upon individual tolerability.
HMG CoA Reductase Inhibitors: Atorvastatin (b) (4)	↑ HMG-CoA reductase inhibitors	Initiate with the lowest starting dose of (b) (4) and titrate carefully while monitoring for safety.

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(b) (4)		
Hormonal Contraceptives: norgestimate/ethinyl estradiol	↑ norgestimate ↓ ethinyl estradiol	(b) (4)
Immuno-suppressants: e.g. cyclosporine (b) (4) sirolimus tacrolimus	↑ immunosuppressants	Concentrations of immunosuppressive agents may be increased when coadministered with STRIBILD. Therapeutic monitoring of the immunosuppressive agents is recommended upon coadministration with STRIBILD.
Inhaled Beta Agonist: salmeterol	↑ salmeterol	Coadministration of salmeterol and STRIBILD is not recommended. Coadministration of salmeterol with STRIBILD may result in increased risk of cardiovascular adverse events associated with salmeterol, including QT prolongation, palpitations, and sinus tachycardia.
Neuroleptics: e.g. perphenazine risperidone thioridazine	↑ neuroleptics	A decrease in dose of the neuroleptic may be needed when coadministered with STRIBILD.
Phosphodiesterase-5 (PDE5) Inhibitors: sildenafil tadalafil vardenafil	↑ PDE5 inhibitors	Co-administration with STRIBILD may result in an increase in PDE-5 inhibitor associated adverse events, including hypotension, syncope, visual disturbances, and priapism. Use of PDE-5 inhibitors for pulmonary arterial hypertension (PAH): Use of sildenafil is contraindicated when used for the treatment of pulmonary arterial hypertension (PAH). The following dose adjustments are recommended for the use of tadalafil with STRIBILD: Co-administration of tadalafil in patients on STRIBILD:

		In patients receiving STRIBILD for at least 1 week, start tadalafil at 20 mg once daily. Increase tadalafil dose to 40 mg once daily based upon individual tolerability.
		Co-administration of STRIBILD in patients on tadalafil:
		Avoid use of tadalafil during the initiation of STRIBILD. Stop tadalafil at least 24 hours prior to starting STRIBILD. After at least one week following initiation of STRIBILD, resume tadalafil at 20 mg once daily. Increase tadalafil dose to 40 mg once daily based upon individual tolerability.
		Use of PDE-5 inhibitors for erectile dysfunction:
		Sildenafil at a single dose not exceeding 25 mg in 48 hours,
		Vardenafil at a single dose not exceeding 2.5 mg in 72 hours, or
		Tadalafil at a single dose not exceeding 10 mg in 72 hours can be used with increased monitoring for PDE-5 inhibitor associated with adverse events.
Sedative/hypnotics: Benzodiazepines: e.g. Parenterally administered midazolam clorazepate diazepam estazolam flurazepam buspirone zolpidem	↑ sedatives/hypnotics	Concomitant use of parenteral midazolam with STRIBILD may increase plasma concentrations of midazolam. Co-administration should be done in a setting that ensures close clinical monitoring and appropriate medical management in case of respiratory depression and/or prolonged sedation. Dosage reduction for midazolam should be considered, especially if more than a single dose of midazolam is administered. Co-administration of oral midazolam with STRIBILD is CONTRAINDICATED. With other sedative/hypnotics, dose reduction may be necessary and (b) (4) monitoring is recommended.

a.   This table is not all inclusive.
b.   ↑ = increase, ↓ = decrease
*: Indicates that a drug-drug interaction trial was conducted.

- ### GS-US-216-0106 (DDI Trial of FDC with Oral Contraceptives):

  The applicant conducted a drug-drug interaction trial to assess the effect of FDC on the pharmacokinetics of individual components (norgestimate [NGM] and ethinyl estradiol [EE]) of a representative hormonal contraceptive medication, Ortho Tri-Cyclen® Lo. The results of the trial showed that:

  - The mean C_{max}, C_{tau}, and AUC_{tau} of NGMN (norgestromin; the primary and pharmacologically active metabolite of norgestimate, also known

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as 17-desacetyl norgestimate) increased by 108 %, 167 %, and 126 % respectively, after co-administration of NGM/EE with EVG/Cobi/FTC/TDF as compared with when NGM/EE was administered alone.

- The mean C_{tau}, and AUC_{tau} of EE decreased by 43% and 25 % after co-administration of NGM/EE with EVG/Cobi/FTC/TDF as compared with when NGM/EE was administered alone. There was no significant change in the C_{max} of EE.

The clinical significance of the increased NGMN exposures and the benefit-vs-risk assessment of using FDC with oral contraceptives in general was discussed with the Division of Reproductive and Urology Products (DRUP). The response to the formal consult request and follow up discussions indicate that there is a potential risk of rare thromboembolic events which may occur due to higher NGMN exposures; however, the rarity of these thromboembolic events makes it challenging to quantify the risk. On the other hand, Ortho Tricyclen® Lo is a widely used oral contraceptive and is a convenient option for contraception. Further, the results of the trial indicate that the efficacy of Ortho Tricyclen Lo is not expected to decrease when given with FDC because the progestin component is considered more important (than the estrogen component) for contraceptive efficacy. Based on the aforementioned considerations, it was determined that a) describing the PK changes of NGMN and EE in the package insert, b) indicating that DDIs between FDC and oral contraceptives containing other progestins has not been conducted and cannot be predicted based on the available data, c) not recommending an increase in the dose of EE to 35 μg [(b) (4)] and d) suggesting healthcare professionals may consult with experts in the area of pregnancy and women's health concerns before co-administration of FDC and Ortho Tricyclen Lo will convey sufficient benefit-to-risk information regarding use of FDC and Ortho Tri Cyclen® Lo.

Extrapolation of Drug-Drug Interactions:

Clinical recommendations pertaining to concomitant use of FDC and several non-antiretroviral drugs that were not studied are based on Cobi's inhibition of CYP3A.

Overall, the clinical recommendations will be similar to the clinical recommendations when the non-antiretroviral drugs are combined with other potent CYP3A inhibitors such as ritonavir.

- Drugs that are contraindicated with ritonavir and boosted protease inhibitors (PIs) because of CYP3A inhibition will be contraindicated with FDC.
- *Drugs for which dose adjustment is recommended when combined with strong CYP3A inhibitors:* The clinical recommendation will be similar to the clinical recommendations when the drugs are co-administered with other

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strong CYP3A inhibitors such as ritonavir.

- *Drugs that are CYP3A substrates and for which plasma concentration is monitored in a clinical setting:* The clinical recommendation will be to exercise caution and monitor the concentrations of the concomitantly administered drug (similar to the recommendation with other strong CYP3A inhibitors such as ritonavir).
- *Drugs that are CYP3A inducers and may significantly decrease the concentration of EVG and Cobi:* The clinical comment will recommend against co-administration.

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2 Question based review (QBR)

2.1 General Attributes of the Drug

2.1.1 What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product as they relate to clinical pharmacology review?

Elvitegravir/Cobicistat/Emtricitabine/Tenovofir (EVG/Cobi/FTC/TDF) is an immediate release tablet containing elvitegravir 150 mg, cobicistat 150 mg, emtricitabine 200 mg and tenofovir disoproxil fumarate 300 mg (equivalent to 245 mg of tenofovir disoproxil). The tablets are green, capsule shaped, film coated, and debossed with GSI on one side and "1" on the other side.

ELVITEGRAVIR	COBICISTAT
Emperical Formula: $C_{23}H_{23}ClFNO_5$	Emperical Formula: $C_{40}H_{53}N_7O_5S_2$
Formula Weight: 447.9	Formula Weight: 776
EMTRICITABINE	TENOFOVIR DISOPROXIL FUMARATE
Emperical Formula: $C_8H_{10}FN_3O_3S$	Emperical Formula: $C_{23}H_{24}N_5O_{14}P$
Formula Weight: 247.24	Formula Weight: 635.52
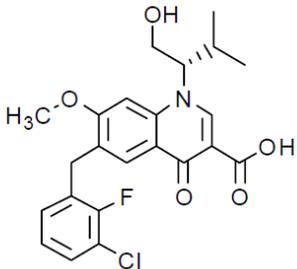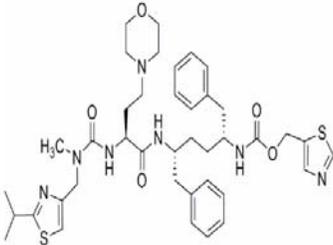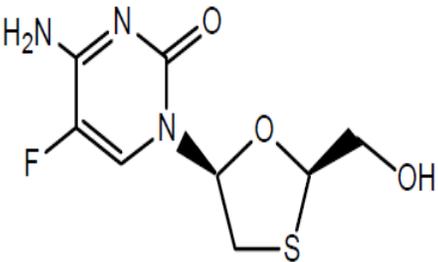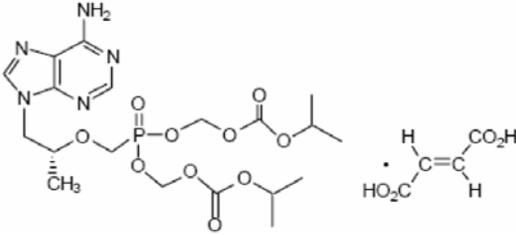
EVG/Cobi/FTC/TDF is a bilayer tablet with EVG and Cobi in one layer (referred to as the EVG/Cobi layer) and the other layer containing FTC and TDF (referred to as the FTC/TDF layer). Table 3 shows the qualitative and quantitative composition of EVG/Cobi/FTC/TDF tablets.

Table 3: Qualitative and quantitative composition of EVG/COBI/FTC/TDF tablets

Components	% w/w	Unit Formula (mg/unit)	Quality Standard	Function
Tablet Core				
Elvitegravir	(b) (4)	150.0[a]	In-House	Active
Cobicistat on Silicon Dioxide		(b) (4)	In-House	Active
Emtricitabine		200.0[b]	In-House	Active
Tenofovir Disoproxil Fumarate		300.0[b,d]	In-House	Active
Hydroxypropyl Cellulose		(b) (4)	NF, Ph. Eur.	(b) (4)
Sodium Lauryl Sulfate			NF, Ph. Eur., JP	
Silicon Dioxide			In-House or NF, Ph. Eur.,	
Lactose Monohydrate			NF, Ph. Eur., JP	
Microcrystalline Cellulose			NF, Ph. Eur., JP	
Croscarmellose Sodium			NF, Ph. Eur., JP	
Magnesium Stearate			NF, Ph. Eur., JP	
	(b) (4)		USP, Ph. Eur.	
Film Coat				
	(b) (4)		In-House	
			USP, Ph. Eur.	(b) (4)

2.1.2 What is the proposed mechanism of action and therapeutic indication(s)?

EVG is a new chemical entity that belongs to the class of HIV-1 integrase strand-transfer inhibitors (INSTIs) that prevents integration of HIV-1 genetic material into

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the host-cell genome.

Cobi, a new chemical entity, is a mechanism based inhibitor of cytochrome P450 3A (CYP3A) enzymes and enhances (or "boosts") the systemic exposure of CYP3A substrates such as EVG.

FTC and TDF are approved nucleoside or nucleotide reverse transcriptase inhibitors. Both drugs work by inhibiting reverse transcriptase, a key enzyme involved in viral replication.

The proposed indication for the FDC tablet is the treatment of HIV-1 infection in adults ages 18 and older who are antiretroviral (ARV) treatment naïve or have no known substitutions associated with resistance to the individual components.

2.1.3 What are the proposed dosage(s) and route(s) of administration?

The proposed dose of EVG/Cobi/FTC/TDF in HIV-1 infected patients with CrCl ≥ 70 mL/min is 150/150/200/300 mg once daily administered orally with food.

2.2 General Clinical Pharmacology

2.2.1 What are the design features of the clinical studies used to support dosing or claims?

The safety and efficacy data of the fixed dose combination (FDC) tablet were collected in two pivotal phase III trials (GS-US-236-0102 and GS-US-236-0103) and one phase II trial (GS-US-236-0104). All the aforementioned trials were conducted in treatment naïve, HIV-1 infected patients using the FDC tablets.

- **GS-US-236-0102** is a randomized active control trial to assess the safety and efficacy of FDC versus Atripla (Emtricitabine/Tenofovir/Efavirenz; ATR) in HIV-1 infected, treatment naïve, adult patients. Patients were randomized in a 1:1 ratio to either treatment group 1 (FDC once daily + placebo for ATR once daily) or treatment group 2 (ATR + placebo for FDC once daily). The primary endpoint was HIV-1 RNA < 50 copies/mL at week 48. The secondary endpoint was to evaluate the efficacy, safety, and tolerability of the 2 treatment regimens through 96 weeks of treatment.

  Table 4 shows the virologic outcome at week 48, (HIV-1 RNA cutoff at 50 copies/mL, snapshot analysis, ITT analysis set)

Table 4: Virologic outcome at week 48, (HIV-1 RNA cutoff at 50 copies/mL, snapshot analysis, ITT analysis set)

HIV-1 RNA Category	QUAD (N=348)	ATR (N=352)	QUAD vs. ATR p-value[a]	Difference in Percentages (95.2% CI)[b, c]
Virologic Success at Week 48				
HIV-1 RNA < 50 copies/mL	305 (87.6%)	296 (84.1%)	0.17	3.6% (-1.6% to 8.8%)
Virologic Failure at Week 48	25 (7.2%)	25 (7.1%)		
HIV-1 RNA >= 50 copies/mL	13 (3.7%)	11 (3.1%)		
Discontinued Study Drug Due to Lack of Efficacy	4 (1.1%)	2 (0.6%)		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA >= 50 copies/mL[d]	8 (2.3%)	12 (3.4%)		
No Virologic Data in Week 48 Window[e]	18 (5.2%)	31 (8.8%)		
Discontinued Study Drug Due to AE/Death	10 (2.9%)	19 (5.4%)		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA < 50 copies/mL[d]	8 (2.3%)	11 (3.1%)		
Missing Data During Window but on Study Drug	0	1 (0.3%)		

a   P-value for the superiority test comparing the percentages of virologic success was from the CMH test stratified by baseline HIV-1 RNA stratum.

b   Difference in percentages of virologic success and its 95.2% CI were calculated based on baseline HIV-1 RNA stratum-adjusted MH proportion.

c   At each of the 2 IDMC meetings, an analysis of efficacy was performed at the alpha level of 0.001; therefore, for the primary endpoint analysis, a 95.2% CI (corresponding to an alpha level of 0.048) was constructed to preserve the overall alpha level of 0.05. As such, the primary analysis CI is described as a 95% CI.

d   Discontinuation due to other reasons includes subjects who discontinued study drug due to investigator's discretion, withdrew consent, lost to follow-up, subject noncompliance, protocol violation, and pregnancy.

e   Week 48 window is between Day 309 and 378 (inclusive).

- **GS-US-236-0103** is an ongoing active control trial to assess the safety and efficacy of the FDC versus ritonavir boosted Atazanavir (ATV) + Truvada (TVD) in HIV-1 infected, treatment naïve, adult patients. 715 HIV-1 infected patients were randomized in a 1:1 ratio to either treatment group 1 (FDC once daily + placebo for RTV, ATV and Truvada) or group 2 (ATV 300 mg, RTV 100 mg, and Truvada once daily + placebo for FDC once daily). The primary endpoint was HIV-1 RNA < 50 copies/mL at week 48. The secondary endpoint was to evaluate the efficacy, safety, and tolerability of the 2 treatment regimens through 96 weeks of treatment. The double blind phase is ongoing; however the 48-week primary endpoint has been analyzed and interim 48-week data was provided in the application.

Table 5 shows the virologic outcome at week 48, (HIV-1 RNA cutoff at 50

copies/mL, snapshot analysis, ITT analysis set).

Table 5: Virologic outcome at week 48, (HIV-1 RNA cutoff at 50 copies/mL, snapshot analysis, ITT analysis set)

HIV-1 RNA Category	QUAD (N=353)	ATV/r+TVD (N=355)	QUAD vs. ATV/r+TVD p-value[a]	Difference in Percentages (95.2% CI)[b,c]
Virologic Success at Week 48				
HIV-1 RNA < 50 copies/mL	316 (89.5%)	308 (86.8%)	0.22	3.0% (-1.9% to 7.8%)
Virologic Failure at Week 48	19 (5.4%)	19 (5.4%)		
HIV-1 RNA >= 50 copies/mL	7 (2.0%)	8 (2.3%)		
Discontinued Study Drug Due to Lack of Efficacy	4 (1.1%)	0		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA >= 50 copies/mL[d]	8 (2.3%)	11 (3.1%)		
No Virologic Data in Week 48 Window[e]	18 (5.1%)	28 (7.9%)		
Discontinued Study Drug Due to AE/Death	11 (3.1%)	18 (5.1%)		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA < 50 copies/mL[d]	7 (2.0%)	9 (2.5%)		
Missing Data During Window but on Study Drug	0	1 (0.3%)		

a   P-value for the superiority test comparing the percentages of virologic success was from the CMH test stratified by baseline HIV-1 RNA stratum.

b   Difference in percentages of virologic success and its 95.2% CI were calculated based on baseline HIV-1 RNA stratum-adjusted MH proportion.

c   At each of the 2 IDMC meetings, an alpha penalty of 0.001 was applied; therefore, for the primary endpoint analysis, a 95.2% CI (corresponding to an alpha level of 0.048) was constructed to preserve the overall alpha level of 0.05. As such, the primary analysis CI is described as a 95% CI.

d   Discontinuation due to other reasons includes subjects who discontinued study drug due to investigator's discretion, withdrew consent, lost to follow-up, subject noncompliance, protocol violation, and pregnancy.

e   Week 48 window is between Day 309 and 378 (inclusive).

- **GS-US-236-0104** is an ongoing Phase 2, active control study evaluating the safety and efficacy of the FDC versus Atripla (Emtricitabine/Tenofovir/Efavirenz; ATR) in HIV-1 infected, treatment naïve, adult patients. Patients were randomized in a 2:1 ratio to either FDC + placebo for ATR or ATR +placebo for FDC, respectively. The primary efficacy endpoint was HIV-1 RNA < 50 copies/mL at week 24. The study is ongoing in an open label extension phase; data through week 96 was provided by the applicant .

Table 6 shows the percentage of subjects with plasma HIV-1 RNA < 50 copies/mL at week 24 (ITT analysis set).

Table 6: Percentage of subjects with plasma HIV-1 RNA < 50 copies/mL at week 24 (ITT analysis set)

Week 24[a, b]	QUAD n/N (%)	ATR n/N (%)	QUAD vs ATR[c] p-value	QUAD vs ATR[c] Difference in Percentages (95% CI)
Missing = Failure[d]				
< 50 copies/mL	43/48 (89.6%)	20/23 (87.0%)	0.72	2.8% (−14.5% to 20.1%)
95% CI	77.3% to 96.5%	66.4% to 97.2%	—	—
Missing/ART Switch = Failure[e]				
< 50 copies/mL	43/48 (89.6%)	19/23 (82.6%)	0.39	7.2% (−11.7% to 26.0%)
95% CI	77.3% to 96.5%	61.2% to 95.0%	—	—
Missing = Excluded[f]				
< 50 copies/mL	43/45 (95.6%)	20/21 (95.2%)	0.90	0.7% (−13.4% to 14.8%)
95% CI	84.9% to 99.5%	76.2% to 99.9%	—	—

a   HIV-1 RNA results were from HIV Cobas Amplicor PCR version 1.5 assay only.
b   The 95% CI for percentage estimate of HIV-1 RNA < 50 copies/mL for each treatment was obtained using Exact method.
c   P-values were from the Cochran-Mantel-Haenszel (CMH) tests stratified by baseline HIV-1 RNA category (<= 100,000 or > 100,000 copies/mL). Difference in percentages of success and its 95% CI were calculated based on baseline HIV-1 RNA stratum-adjusted Mantel-Haenszel proportion.
d   For missing = failure: Denominator for percentage was the number of subjects in the ITT analysis set. P-value and percentage difference (95% CI) were based on a binary response: success (HIV-1 RNA < 50 copies/mL) and failure (HIV-1 RNA >= 50 copies/mL or missing).
e   For missing/ART switch: Denominator for percentage was the number of subjects in the ITT analysis set. P-value and percentage difference (95% CI) were based on a binary response: success (HIV-1 RNA<50) and failure (HIV-1 RNA>=50, missing, ART switch). Subjects who discontinued study drug and had no follow-up information on new ART were treated as having an ART switch. The commercial ATR switch for subjects who were randomized to and treated in ATR group was not considered an ART switch.
f   For missing = excluded: Denominator for percentage was the number of subjects in the ITT analysis set with nonmissing HIV-1 RNA value at each visit. P-value, percentage difference, and its 95% CI were based on a binary response: success (HIV-1 RNA < 50 copies/mL) and failure (HIV-1 RNA >= 50 copies/mL).

2.2.2   What is the basis for selecting the response endpoints (i.e., clinical or surrogate endpoints) or biomarkers (collectively called pharmacodynamics [PD]) and how are they measured in clinical pharmacology and clinical studies?

The primary efficacy endpoint was plasma HIV-1 RNA viral load < 50 copies/mL at week 48 (in trials GS-US-236-0102 and GS-US-236-0103) and week 24 (in trial GS-US-236-0104). The use of HIV-1 viral load has been validated as a surrogate marker to demonstrate the efficacy of antiretroviral drugs developed for the treatment of HIV-1 infection.

2.2.3   Are the active moieties in the plasma (or other biological fluid)

appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

The relevant analytes were measured in plasma using validated LC/MS/MS analytical methods. EVG and Cobi were measured in all trials; measurement of other analytes (FTC, TFV, and other non-antiretroviral drugs) was dependent on the objectives of the trial.

2.2.4 Exposure-response

2.2.4.1 What are the characteristics of the exposure-response relationships (dose response, concentration-response) for efficacy?

Exposure-response efficacy analyses focused on EVG because the primary role of Cobi is to increase the systemic exposure of EVG and Cobi is not expected to have antiviral activity.

Based on the results of the Phase III trials using FDC tablet, a flat exposure-response relationship was observed between elvitegravir C_{0h} and AUC versus the primary study endpoint of percentage of patients achieving virologic success (plasma viral load <50 HIV-1 RNA copies/mL) at Week 48 (Figure 1). This assessment was based on data available from 373 patients administered FDC in Phase III trials (GS-US-236-0102; GS-US-236-0103). To identify if there was any loss in efficacy at lower exposures, the exposure response relationship was further explored by separating the groups into deciles (i.e. ten groups of equivalent size). The observed percentage of patients achieving virologic success was flat across all deciles. The median C_{tau} in the lowest decile was 156 ng/mL with a response of 87%. Virologic success in deciles with higher C_{tau} values (234–916 ng/mL) ranged between 84–97%. A similar trend was observed for $AUC_{tau.}$ Finally, no significant covariates such as age, gender, race, or baseline viral load were identified as predictive of virologic success. In patients with available PK data, a trend of lower virologic response was observed in patients with different baseline viral load (patients <100,000 copies/mL: 92% (n=214); patients 100,000-500,000 copies/mL: 87% (n=135); patients >500,000 copies/mL: 79% (n=24)). The virologic response rates between patients with viral load <100,000 copies/mL and 100,000-500,000 copies/mL was similar.

Figure 1: Percentage of Patients Achieving Virologic Success (<50 Copies/mL) Versus Elvitegravir C0h (left) and AUCτ (right) from GS-US-236-0102 and GS-US-236-0103
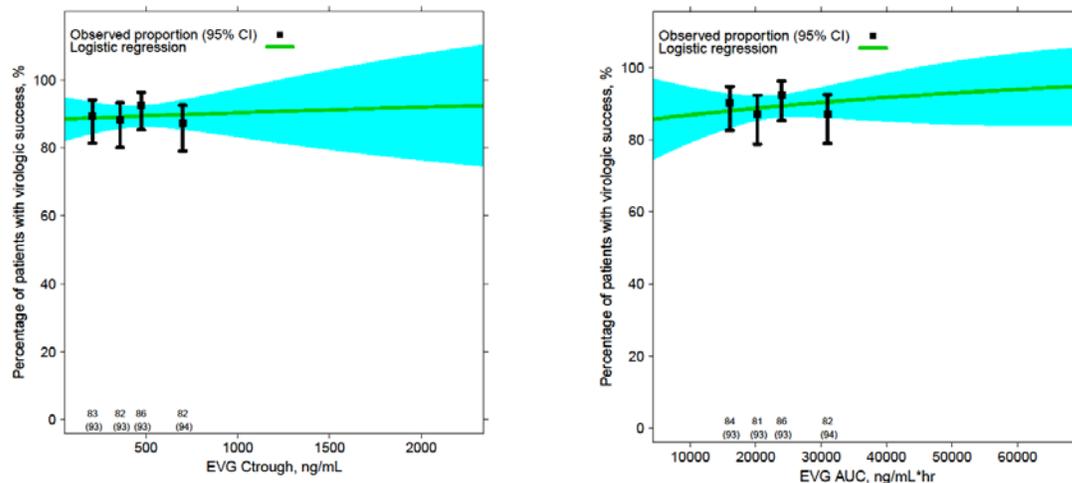
Exposure-response efficacy relationships for other components of the FDC regimen could not be evaluated due to the limited number of subjects with TDF or FTC pharmacokinetic data available (n=61).

2.2.4.2 What are the characteristics of the exposure-response relationships (dose response, concentration-response) for safety?

An exposure-response relationship could not be established for most common adverse events observed during the Phase III studies for EVG (e.g., headaches, diarrhea, and nausea). Logistic regression models were evaluated for EVG C_{0h} and AUC and no significant relationships identified.

It was observed that administration of the FDC tablet led to an increase in serum creatinine and a subsequent decrease in eGFR. This change in serum creatinine was attributed to cobicistat based on observations from preclinical and Phase I/II studies. The sponsor hypothesized that this increase in serum creatinine was due to cobicistat inhibition of tubular secretion of creatinine and performed dedicated sub-studies with cystatin-C and iohexol to confirm this observation. In addition to this analysis, the review team explored relationships between baseline eGFR and maximum on treatment decrease in eGFR. It was observed that cobicistat's effects on eGFR are dependent on baseline status, with smaller changes in patients with initial lower baseline eGFR. These data support the applicant's assertion that cobicistat inhibits tubular secretion of creatinine.

Finally, an imbalance in renal adverse events was observed between the FDC treatment arms and active comparator arms from Phase III (9 subjects on a FDC regimen, 2 subjects on Atripla, and 1 subject on atazanavir/ritonavir). Of these

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subjects, 6 discontinued FDC therapy due to renal adverse events. No exposure-response relationship between renal adverse events and elvitegravir exposures could be identified from the available data. Exposure-response relationships for other components of the FDC regimen could not be evaluated due to the limited (n=61) pharmacokinetic data available for cobicistat, TDF, or FTC.

2.2.4.3 Does this drug prolong the QT or QTc interval?

Neither elvitegravir nor cobicistat were found to prolong the QT interval based on separate thorough QT (TQT) studies.

EVG:

In the TQT study, two doses of EVG/rtv were used: 125/100 mg and 250/100 mg. The highest upper bounds of the two-sided 90% CI for the time-matched, baseline-adjusted mean difference of QTcF between the two treatment groups of elvitegravir/ritonavir and placebo in the TQT study were 7.3 msec and 8.1 msec, less than 10 msec, the level suggested as the threshold for regulatory concern in ICH E14. The largest mean differences were less than 5 msec for each elvitegravir/ritonavir treatment group vs placebo.

Exposure-response analysis does not reveal a significant relationship between QT interval and plasma elvitegravir concentrations.

Cobi:

No significant QTc prolongation effect of cobicistat (250 mg and 400 mg) was detected in the TQT study. The highest upper bounds of the 2-sided 90% CI for the mean difference of cobicistat (250 mg and 400 mg) and placebo were below 10 ms, the threshold for regulatory concern as described in ICH E14 guidelines. The largest lower bound of the two-sided 90% CI for the $\Delta\Delta$QTcI for moxifloxacin was greater than 5 ms, and the moxifloxacin profile over time is adequately demonstrated, indicating that assay sensitivity was established.

The single supratherapeutic dose (400 mg) produces mean C_{max} values of 2.7-fold higher than the mean C_{max} for the therapeutic dose at steady state (150 mg). These concentrations are likely to be above those for the predicted worst case scenario (double dosing or drug interaction). At these concentrations there would be no prolongations of the QT-interval.

2.2.4.4   Is the dose and dosing regimen selected by the applicant consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

The dose and dosing regimen selected by the applicant are consistent with the known dose-concentration-response relationship. In HIV-1 infected patients from the Phase III studies receiving the FDC tablet, a flat exposure-response relationship was identified for treatment-naïve subjects regardless of baseline viral load, race, or gender for elvitegravir AUC and C_{tau} ranging from 4358–69754 ng·hr/mL and 58–2341 ng/mL, respectively. This result follows from the Phase II dose-finding study (GS-US-183-0105) that evaluated the safety, efficacy and pharmacokinetics of ritonavir boosted elvitegravir vs a comparator ritonavir boosted protease inhibitor (CPI/r) in combination with background therapy. Three doses of EVG (+rtv) were used: 20/100 mg, 50/100 mg and 125/100 mg.

Table 7 shows the average change from baseline in HIV-1 RNA after the various treatments at week 24.

Table 7: Average change from baseline in HIV-1 RNA after the various treatments at week 24

$DAVG_{24}$ (log$_{10}$ copies/mL)	CPI/r (N = 63)	EVG/r 50/100 mg (N = 71)	EVG/r 125/100 mg (N = 73)
Mean ± SD	−1.19 ± 1.17	−1.44 ± 1.07	−1.66 ± 1.20
Median	−1.00	−1.34	−1.57
P-value vs CPI/r [a]	—	0.29	0.021
Difference (95% CI) [b]	—	−0.19 (−0.54 to 0.17)	−0.42 (−0.77 to −0.06)

The lowest dose of ritonavir-boosted elvitegravir (20/100 mg) resulted in high rates of virologic rebound (mean change from baseline in HIV-1 RNA was -1.54 log$_{10}$ copies/mL and -1.07 log$_{10}$ copies/mL at Weeks 2 and 8, respectively). At Week 16, 60 of the 71 subjects randomized to 20/100 mg group switched to open-label elvitegravir/ritonavir 125/100 mg (34 of whom also added a protease inhibitor); the remaining 11 subjects discontinued the study.

The results of trial GS-US-183-0105 identified elvitegravir/ritonavir 125/100 q.d. as the appropriate dose for Phase III evaluation (difference in EVG dose results from a change in formulation between Phase II and Phase III). Similarly, of the most common adverse events identified during the Phase III trials, no exposure-response relationship was identified for either elvitegravir or cobicistat exposures.

Based on GS-US-236-0101 a cobicistat dose of 150 mg is necessary to achieve boosted elvitegravir C_{tau} similar to that attained from elvitegravir boosted with

ritonavir 100 mg. In addition, the elvitegravir exposure when boosted with 150 mg cobicistat is similar after initial and repeated dosing. As such, the cobicistat dose is appropriate for rapidly achieving therapeutic elvitegravir exposures.

The tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) doses within the FDC pill are the approved adult dose (TDF 300 mg; FTC 200 mg) for each of these nucleoside or nucleotide reverse transcriptase inhibitors.

2.2.5   What are the PK characteristics of the drug and its major metabolite?

The discussion of the pharmacokinetic parameters of EVG and Cobi is based on the information collected after administration of FDC tablets. For information on the pharmacokinetic parameters of EVG (+rtv) and Cobi when given alone, please refer to the individual study reviews of EVG (+rtv) and Cobi.

2.2.5.1   What are the single dose and multiple dose PK parameters?

The single dose pharmacokinetics of the individual components of the FDC tablet was evaluated in trial GS-US-236-0105 (food effect trial). The multiple dose pharmacokinetics of the individual components of the FDC tablet was evaluated in trial GS-US-236-0110 (relative bioavailability trial; one of the formulations evaluated was the Phase III [and commercial] formulation).

In trial GS-US-236-0105, the FDC tablet was administered as a single dose under fasting conditions, with a light meal (373 kcal, 20 % fat), and with a high fat meal (800 kcal, 50 % fat). In trial GS-US-236-0110, two different formulations of the FDC were administered with food (approximately 400 kcal and 13 gms fat). For comparison of single- and multiple dose pharmacokinetic parameters of the individual components of the FDC tablet, the single dose data is taken from the group which received the FDC tablet with a light meal in trial GS-US-236-0105 and the multiple dose data is based on formulation 2 (Phase III and commercial formulation) from trial GS-US-236-0110 where the subjects received the FDC formulation with food (fat content similar to the fat content of a light meal).

EVG:

Table 8 shows the comparison of the mean pharmacokinetic parameters of EVG on day 1 (from trial GS-US-236-0105) and day 10 (from trial GS-US-236-0110) after administration of the FDC Phase III formulation.

Table 8: Comparison of the mean pharmacokinetic parameters of EVG on day 1 (from trial GS-US-236-0105) and day 10 (from trial GS-US-236-0110) after administration of the FDC Phase III formulation

EVG PK Parameter	Day 1[a] (N = 24)	Day 10 (N = 36)
AUC_{inf} or AUC_{tau} (ng·h/mL)	21,100 (28)	22,500 (27)
C_{max} (ng/mL)	1760 (32)	1920 (24)
C_{24} (ng/mL)	355 (54)	508 (41)
T_{max} (h)	4.5 (4.0, 5.0)	4.5 (4.0, 4.5)
$T_{1/2}$ (h)	5.3 (4.7, 6.7)	13 (8.6, 14)

Data are mean (%CV) or median (Q1, Q3) and are shown to 3 significant digits (2 for time parameters).

a   Data are from the treatment with a light meal.

Cobi:

Table 9 shows the comparison of the mean pharmacokinetic parameters of Cobi on day 1 (from trial GS-US-236-0105) and day 10 (from trial GS-US-236-0110) after administration of the FDC Phase III formulation

Table 9: Comparison of the mean pharmacokinetic parameters of Cobi on day 1 (from trial GS-US-236-0105) and day 10 (from trial GS-US-236-0110) after administration of the FDC Phase III formulation

Cobi PK Parameter	Day 1[a]	Day 10[b]
AUC_{inf} or AUC_{tau} (ng*hr/mL)	8012	11288
C_{max} (ng/mL)	1243	1528
C_{24} (ng/mL)	16.4	45.5
T_{max} (h)	4.0	4
$T_{1/2}$ (h)	3.04	3.54

Data are mean (% CV); a: N = 24; b: N =36

Although the mean systemic exposure of Cobi after multiple dose was higher as compared to the mean systemic exposure of Cobi after single dose, no additional increase in CYP3A inhibition was observed, as shown by similarity in EVG exposures between day 1 and day 10. In addition, the results from trial GS-US-216-0101 suggest that despite the non-linear increase in COBI exposure (when COBI was administered alone), minimal differences were observed in the CYP3A4 inhibitory effect of COBI across the dose range evaluated (50-200 mg).

FTC:

A comparison of the single- and multiple dose pharmacokinetic parameters of FTC suggests that the mean C_{max} and AUC of FTC after single dose administration of the FDC tablet (1811 ng/mL and 10,738 ng*hr/mL, respectively) were similar to the mean C_{max} and AUC of FTC after multiple dose administration of the FDC tablet (1932 ng/mL and 12,491 ng*hr/mL, respectively)

TDF:

A comparison of the single- and multiple dose pharmacokinetic parameters of TDF suggests that the mean C_{max} and AUC of TDF after single dose administration of the FDC tablet were 386 ng/mL and 3139 ng*hr/mL, respectively. The mean C_{max} and AUC of TDF after multiple dose administration of the FDC tablet were 485.6 ng/mL and 4066.2 ng*hr/mL, respectively. A comparison of the single- and multiple dose pharmacokinetic parameters of tenofovir suggest that the mean C_{max} and AUC after multiple dose administration were 26 % and 30 % higher, respectively, as compared with single dose administration.

The mean systemic exposures of tenofovir after administration of the FDC tablet were ~26 % higher than the mean systemic exposures of tenofovir when tenofovir is given alone. Of note, the increase in tenofovir exposures when given as FDC (as compared with when tenofovir is given alone) is similar to the increase in tenofovir exposures when tenofovir is co-administered with boosted PIs such as atazanavir/ritonavir (~37 % higher tenofovir exposures) and DRV/rtv (~22 % higher tenofovir exposures) as compared with when tenofovir is given alone. The increase in the mean exposures of tenofovir when given as FDC is hypothesized to be related to the inhibitory effect of Cobi on Pgp-mediated transport of TDF in the gut.

2.2.5.2   How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?

EVG:

Table 10 shows the comparison of the mean pharmacokinetic parameters of EVG after once daily administration of the FDC in HIV infected patients and healthy subjects (data taken from trial GS-US-236-0110; formulation 2).

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Table 10: Comparison of the mean pharmacokinetic parameters of EVG after once daily administration of the FDC in HIV infected patients and healthy subjects (data taken from trial GS-US-236-0110; formulation 2)

EVG	AUC_{tau} (ng•h/mL)	C_{max} (ng/mL)	C_{tau} (ng/mL)
Population PK[a] (N = 419)	23,000 (33)	1730 (23)	451 (58)
Healthy Subjects (N = 36)	22,500 (27)	1920 (24)	508 (41)

Data are mean (%CV) and are shown to 3 significant digits.

a   All the QUAD STR-treated subjects in GS-US-236-0103 and GS-US-236-0104 and all the QUAD STR-treated subjects who participated in the PK substudy in GS-US-236-0102 were included.

There were no significant differences in the mean pharmacokinetic parameters of EVG between healthy subjects and HIV-1 infected patients.

Cobi:

Table 11 shows the comparison of the mean pharmacokinetic parameters of Cobi after once daily administration of the FDC in HIV infected patients and healthy subjects (data taken from trial GS-US-236-0110; formulation 2).

Table 11: Comparison of the mean pharmacokinetic parameters of Cobi after once daily administration of the FDC in HIV infected patients and healthy subjects (data taken from trial GS-US-236-0110; formulation 2)

COBI	AUC_{tau} (ng•h/mL)	C_{max} (ng/mL)	C_{tau} (ng/mL)
HIV-1 Infected Subjects (N = 62)[a]	8300 (46)[b,c]	1140 (36)[d]	49.1 (260)[b,e,f]
Healthy Subjects (N = 36)	11,300 (29)	1530 (26)	45.5 (88)

Data are mean (%CV) and are shown to 3 significant digits.

a   All the QUAD STR-treated subjects who participated in the PK substudy in GS-US-236-0102, GS-US-236-0103, or GS-US-236-0104 were included.

b AUC_{tau} and C_{tau} were not calculated for Subject 2191-7159 for any analyte because concentration values were missing for some postdose time points.

c   n = 61

d   n = 62

e   n = 53

f   Missing C_{tau} (except for Subject 2191-7159) was due to values below the limit of quantitation and was excluded from this analysis.

The mean pharmacokinetic parameters of Cobi were lower in HIV-1 infected patients as compared to healthy volunteers; however, these differences in the

mean pharmacokinetic parameters of Cobi did not lead to differences in CYP inhibition by Cobi (as shown by similarity in the mean pharmacokinetic parameters of EVG between healthy volunteers and HIV-1 infected patients).

FTC:

After administration of the FDC tablet, the steady state mean pharmacokinetic parameters of FTC were similar in healthy subjects and HIV-1 infected patients.

TDF:

After administration of the FDC tablet, the steady state mean pharmacokinetic parameters of tenofovir were similar in healthy subjects and HIV-1 infected patients.

2.2.5.3   What are the characteristics of drug absorption?

EVG:

EVG is a high permeability compound.

The *in vitro* bidirectional permeability of [^{14}C] EVG was studied using monolayers of LLC-PK1 cells transfected with an expression vector for human MDR1 or with the empty control expression vector. [^{14}C] Mannitol (low permeability control) and [^{14}C] digoxin were assessed in parallel. Table 12 shows the transport of EVG and control compounds across monolayers of LLC-PK1 cells.

Table 12: Transport of EVG and control compounds across monolayers of LLC-PK1 cells transfected with control vector or expression vector for human MDR1

| Compound | Time (h) | Cleared Volume (µL/mg Cellular Protein) | | | | | |
| | | Control Cells | | | MDR1-Expressing Cells | | |
		A-B	B-A	Ratio	A-B	B-A	Ratio
EVG	1	179.2 ± 16.7	207.8 ± 11.2	1.2	64.1 ± 17.3	961.9 ± 75.8	15.0
	2	412.7 ± 20.0	512.8 ± 34.4	1.2	139.4 ± 25.2	1891.6 ± 126.1	13.6
	4	606.4 ± 18.7	912.6 ± 62.9	1.5	212.1 ± 34.7	3085.4 ± 65.7	14.5
Digoxin	1	21.6 ± 1.1	44.6 ± 10.3	2.1	25.3 ± 9.0	245.8 ± 27.0	9.7
	2	48.6 ± 9.5	96.7 ± 16.1	2	54.9 ± 5.1	498.1 ± 69.8	9.1
	4	103.0 ± 20.1	229.8 ± 27.4	2.2	97.4 ± 12.3	1000.2 ± 125.8	10.3
Mannitol	1	24.1 ± 12.8	16.2 ± 1.2	0.7	53.9 ± 36.4	42.9 ± 18.2	0.8
	2	36.6 ± 10.4	35.7 ± 4.0	1	97.0 ± 63.6	83.6 ± 27.4	0.9
	4	89.9 ± 14.4	73.5 ± 6.3	0.8	176.9 ± 100.2	131.7 ± 33.8	0.7

A-B = apical to basal; B-A = basal to apical; EVG = elvitegravir; MDR1 = P-glycoprotein

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The permeability of EVG in the control cells was independent of the direction (B-A/A-B ratio < 2) and was 6-12 fold higher than the permeability of mannitol, suggesting that EVG is a high permeability compound.

Please refer to section 2.4.2.4 for information about the involvement of P-gp in EVG permeability.

Cobi:

Cobi is a high permeability compound.

Table 13 shows the bidrectional permeability of Cobi through the Caco-2 cells monolayers.

Table 13: Bidrectional permeability of Cobi through the Caco-2 cells monolayers

Direction	Target Conc. (µM)	Initial Conc. (µM)	Recovery (%)	P_{app} (10^{-6} cm/s)	Efflux Ratio
Cell-Free		1.2	ND	9.45	
Forward	1	1.4	73.8	7.61	1.1
Reverse		1.3	55.0	8.51	

COBI = cobicistat; P_{app} = apparent permeability; ND = not determined due to missing donor well concentration at 120 minutes

At 1 µM, the forward permeability of Cobi was 7.61 X 10^{-6} cm/s.

2.2.5.4   What are the characteristics of drug distribution?

EVG:

The protein binding of EVG was independent of the concentration (range evaluated was 0.1-10 µg/mL) in plasma from rats, dogs, monkeys and humans. The free fraction (%) in human plasma, or in a physiological concentration of human serum albumin (HSA) averaged 0.7 %, indicating that EVG is primarily bound to albumin in human plasma.

The results of the mass balance trial showed that the blood-to-plasma ratio of ^{14}C EVG radioactivity was ~0.73 and time independent, indicating the EVG was predominantly distributed to plasma relative to the cellular components of the blood.

Cobi:

At 1 µM Cobi in human plasma, the free fraction (%) was 6.3 %. The free fraction (%) of Cobi was similar across the concentration range (1-30 µM)

evaluated.

The results of the mass balance trial showed that the blood-to-plasma ratio of total ^{14}C Cobi radioactivity was time independent and less than or equal to 0.75 indicating that Cobi is excluded from the cellular components of the blood.

FTC and TDF:

Per the approved package insert, the fraction unbound FTC and TDF was > 95 % and > 90 %, respectively, in human plasma.

2.2.5.5   Does the mass balance trial suggest renal or hepatic as the major route of elimination?

The results of the mass balance trials conducted with EVG [GS-US-183-0126] and Cobi [GS-US-216-0111] are summarized below:

EVG:

The major route of elimination of EVG and its metabolites is hepatobiliary excretion. The results of the mass balance trial conducted using a single dose of 50 mg [^{14}C] EVG (co-administered with low dose ritonavir) showed that the majority of the radioactivity was excreted in feces (94.8 %), while urinary recovery was low (6.7%; no unchanged EVG in the urine). Although radioactivity was recovered up to 312 hours in feces and up to 168 hours in urine after dosing, the majority of the fecal recovery (87.5 %) and urinary recovery (5.6 %) occurred within 120 hours and 24 hours of dosing, respectively.

Cobi:

The major route of elimination of Cobi and its metabolites is hepatobiliary excretion. The results of the mass balance trial showed that following single dose administration of [^{14}C] Cobi, the majority of the radioactivity was excreted in the feces (86.2% of the administered dose), while urinary recovery was low (8.2%). Fecal and urine collection was performed up to 504 hours.

FTC and TDF:

FTC and TDF are primarily eliminated unchanged by a combination of glomerular filtration and active tubular secretion.

2.2.5.6   What are the characteristics of drug metabolism?

EVG:

The *in vitro* metabolism was evaluated in human hepatic microsomal fractions

and the oxidation and glucuronidation was evaluated using recombinant enzymes and microsomal fractions with enzyme selective inhibitors. The *in vivo* metabolism of [^{14}C] EVG was evaluated based on plasma, urine, and feces sampling in a mass balance trial.

The results of the *in vitro* assessment indicate that CYP mediated metabolism and glucuronidation play a major role in the biotransformation of EVG. The results of the mass balance trial showed that the predominantly circulating species in plasma was EVG (~94 % of the radioactivity); the remaining radioactivity was comprised of metabolites formed from CYP3A mediated hydroxylation (GS-9202; M1) and glucuronidation (GS-9200; M4) pathways. The radioactivity in the feces (pooled samples) was accounted for mainly by EVG and GS-9202 (30.8 % and 33.9 %, respectively), and radioactivity in the urine was present as the glucuronide of EVG.

Cobi:

The *in vitro* metabolism was evaluated in human hepatocytes and hepatic microsomal fractions. The *in vivo* metabolism of ^{14}C Cobi was evaluated based on plasma, urine, and feces sampling in a mass balance trial.

The results of the *in vitro* metabolism studies showed that CYP3A4 and CYP2D6 were involved in the metabolism of Cobi.

The results of the mass balance trial showed that following administration of ^{14}C Cobi, the predominantly circulating species in plasma was Cobi (~98.6 % of the circulating radioactivity). The majority of radioactivity in the feces comprised of Cobi; M21 and M31 metabolites were also detected in the feces. In the urine, the majority of radioactivity was recovered as Cobi; low levels of metabolites M21 and M31 were also detected.

FTC:

Following administration of radiolabelled FTC, approximately 86 % of FTC is recovered in the urine and 13 % is recovered as active metabolites. The metabolites of emtricitabine include 3'-sulfoxide diaseteromers and their glucuronic acid conjugate. Emtricitabine is eliminated by a combination of glomerular filtration and active tubular secretion.

TDF:

Approximately 70-80 % of the intravenous dose of tenofovir is recovered as unchanged drug in the urine. Tenofovir is eliminated by a combination of glomerular filtration and active tubular secretion.

2.2.5.7 What are the characteristics of drug excretion?

See section 2.2.5.5

2.2.5.8 Based on the PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

EVG:

The applicant did not evaluate the degree of linearity or non-linearity of EVG, when EVG was co-administered with Cobi (either as EVG + Cobi or the FDC tablet). The applicant evaluated the systemic exposures of various doses of EVG, when EVG was co-administered with 100 mg ritonavir.

Table 14 shows the mean systemic exposures of EVG after administration of various doses of EVG and 100 mg ritonavir.

Table 14: Mean systemic exposures of EVG after administration of various doses of EVG and 100 mg ritonavir

EVG Plasma PK Parameter [a]	EVG/r 20/100 mg (N = 11)	EVG/r 50/100 mg (N = 12)	EVG/r 125/100 mg (N = 12)
C_{max} (ng/mL) Mean (%CV)	265.79 (72.77)	753.71 (30.10)	1442.20 (33.97)
AUC_{tau} (ng•h/mL) Mean (%CV)	3029.25 (84.58)	8701.86 (40.84)	16,789.54 (33.06)
C_{tau} (ng/mL) Mean (%CV)	67.28 (176.31)	211.03 (77.51)	262.99 (52.13)

The mean systemic exposures of EVG were more than dose proportional between 25 (+100 mg RTV) mg and 50 (+100 mg RTV) mg EVG but less than dose proportional between 50 (+100 mg RTV) mg and 125 (+100 mg RTV) mg EVG.

Cobi:

Table 15 shows the mean pharmacokinetic parameters after multiple dose (once daily) administration of Cobi.

Table 15: Mean pharmacokinetic parameters after multiple dose administration of Cobi

GS-9350 Plasma PK Parameters	GS-9350 50 mg (N = 12)	GS-9350 100 mg (N = 11)	GS-9350 200 mg (N = 12)
C_{max} (ng/mL) Mean (%CV)	170.0 (70.1)	563.3 (30.7)	1854.8 (28.0)
AUC_{tau} (ng·h/mL) Mean (%CV)	827.0 (81.6)	3435.8 (34.3)	16108.3 (34.3)
C_{tau} (ng/mL) Mean (%CV)	0.4 (346.4)	4.9 (87.0)	126.6 (74.9)
T_{max} (h) Median (Q1, Q3)	4.50 (3.50, 4.50)	4.50 (4.50, 4.53)	4.50 (4.50, 4.50)
$T_{\frac{1}{2}}$ (h) Median (Q1, Q3)	2.19 (1.34, 2.48)	3.12 (2.55, 3.36)	5.20 (4.12, 6.10)
T_{last} (h) Median (Q1, Q3)	12.00 (8.03, 16.00)	24.00 (16.00, 24.00)	24.00 (24.00, 24.00)
CL_{ss}/F (mL/h) Mean (%CV)	154288.3 (106.9)	33190.3 (43.6)	13952.5 (38.4)

Within the Cobi dose range evaluated (50-200 mg), there was a greater than dose proportional increase in the mean systemic exposure of Cobi with increasing dose.

2.2.5.9   How do the PK parameters change with time following multiple dosing?

Please refer to the response to question 2.2.5.1.

2.2.5.10   What is the inter- and intra-subject variability of PK parameters in volunteers and patients, and what are the major causes of variability?
EVG:

Overall, in both healthy and HIV-1 infect subjects, low to moderate inter-individual variability was observed for elvitegravir when boosted with cobicistat or ritonavir. Based on the population pharmacokinetic analysis, the inter-individual variability for the apparent oral clearance (CL/F) and apparent volume of distribution (V/F) was 32% and 49%.

In healthy subjects, based on pharmacokinetic data from GS-US-236-0101 for 150 mg elvitegravir boosted with either 100 or 150 mg cobicistat, the mean inter-individual variability (% coefficient of variation) for AUC_{tau}, C_{max} , and C_{tau} were 25-30%, 26-28%, and 53-60%, respectively. This variability in AUC_{tau}, C_{max}, and C_{tau} is consistent with the variability of 100 mg elvitegravir boosted with 100 mg ritonavir (23%, 32%, and 41%, respectively).

The data were insufficient to inform intra-subject PK variability for EVG. Major causes of PK variability for EVG included body surface area and Cobi AUC_{tau}. The effect of BSA on EVG exposure corresponded to -15% to +18% changes in apparent clearance over the range of body surface areas included in the Phase III trials. These changes in apparent clearance were not anticipated to be clinically significant, and no dose adjustments for EVG based on body surface area are recommended. When PK data from EVG coadministered with 100 and 150 mg Cobi were included in the population PK model, a significant relationship between EVG exposure and Cobi AUC_{tau} was identified. However, when considering only 150 mg Cobi, an effect of Cobi AUC on EVG exposure could not be identified, which supports the use of 150 mg Cobi for boosting EVG exposure.

Cobi:

In healthy subjects, based on pharmacokinetic data from GS-US-236-0101 for 100 and 150 mg cobicistat when administered as part of a FDC, the mean inter-individual variability (% coefficient of variation) for AUC_{tau}, C_{max} , and C_{tau} were 32-35%, 28-30%, and 107-124%, respectively.

The data was insufficient to inform intra-subject PK variability and major causes of PK variability for Cobi.

TDF:

In healthy subjects, based on pharmacokinetic data from GS-US-236-0101 for 300 mg TDF when administered as part of a FDC with EVG, Cobi, and FTC, the mean inter-individual variability (% coefficient of variation) for AUC_{tau}, C_{max}, and C_{tau} were 20%, 29%, and 26%, respectively. These mean inter-individual variability values are similar to that observed for 300 mg TDF administered with 200 mg FTC as Truvada (AUC_{tau}, C_{max}, and C_{tau} were 23%, 25%, and 25%, respectively).

The data was insufficient to inform intra-subject PK variability and major causes of PK variability for TDF.

FTC:

In healthy subjects, based on pharmacokinetic data from GS-US-236-0101 for 200 mg FTC when administered as part of a FDC with EVG, Cobi, and TDF, the mean inter-individual variability (% coefficient of variation) for AUC_{tau}, C_{max}, and C_{tau} were 19%, 23%, and 27%, respectively. These mean inter-individual variability values are similar to that observed for 200 mg FTC administered with 300 mg TDF as Truvada (AUC_{tau}, C_{max}, and C_{tau} were 22%, 26%, and 26%, respectively).

The data was insufficient to determine intra-subject PK variability and major

causes of PK variability for FTC.

2.3 Intrinsic Factors

2.3.1   What intrinsic factors (age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, & organ dysfunction) influence exposure &/or response and what is the impact of any differences in exposure on the PDs? What dosage regimen adjustments, if any, are recommended for each of these subgroups?

Gender, race, and age were not found to influence either the exposure or exposure-response relationship for elvitegravir (administered as FDC tablet). Body weight, body surface area, and body mass index were identified as statistically significant covariates during model development. However, it was primarily the lowest quartile of these groups where higher elvitegravir exposures were observed. Given that there were no identified exposure-response efficacy or safety relationships and as elvitegravir is administered as part of a fixed dose combination, no dose adjustments are recommended based on body weight, body surface area, or body mass index. No exploration of exposures for cobicistat were performed with respect to covariates due to the limited number of patients with cobicistat pharmacokinetic data available.

2.3.2   Based upon what is known about exposure-response relationships and their variability and the groups studied, healthy volunteers vs. patients vs. specific populations (examples shown below), what dosage regimen adjustments, if any, are recommended for each of these groups? If dosage regimen adjustments are not based upon exposure-response relationships, describe the alternative basis for the recommendation.

2.3.2.1   Elderly

The Phase II and III trials included 24 subjects > 55 years of age, but only 3 subjects ≥ 65 years of age (i.e. 65, 66, and 72 years of age). While population pharmacokinetic analysis did not indicate an age effect for elvitegravir pharmacokinetics, the number of patients ≥ 65 years of age was not sufficient to determine whether they would respond differently from younger subjects.

2.3.2.2   Pediatrics

The safety and effectiveness of FDC in HIV-1 pediatric subjects has not been established.

2.3.2.3   Gender

Gender did not have an effect on elvitegravir exposures based on the available

pharmacokinetic data from males (n = 384) and females (n = 35) from Phase II and III trials in HIV-1 infected patients. No dose adjustments are recommended based on gender.

2.3.2.4 Race

The percentage of subjects from the Phase II and III study populations that were white, black, or Asian were 70%, 22%, and 4%, respectively, with the remainder undocumented or unknown. Based on a population pharmacokinetic analysis of these HIV-1 infected subjects, race did not have an effect on elvitegravir exposures. No dose adjustments are recommended based on race.

2.3.2.5 Renal impairment

The effect of renal impairment on the pharmacokinetics of Cobi boosted EVG was evaluated in trial GS-US-216-0124. Table 16 shows the statistical comparison of the mean pharmacokinetic parameters of EVG in subjects with severe renal impairment (eGFR< 30 mL/min) and subjects with normal renal function (eGFR ≥ 90 mL/min).

Table 16: Statistical comparison of the mean pharmacokinetic parameters of EVG in subjects with severe renal impairment and subjects with normal renal function

EVG PK Parameter	Geometric Least-Squares Mean		Geometric Least-Squares Means Ratio (%) (90% CI)
	Test Severe Renal Impairment $eGFR_{CG} < 30$ mL/min (N = 12)	Reference Normal Renal Function $eGFR_{CG} \geq 90$ mL/min (N = 11)	
AUC_{tau} (ng•h/mL)	25316.69	33530.63	75.50 (62.82, 90.75)
C_{max} (ng/mL)	2154.03	3200.46	67.30 (54.78, 82.68)
C_{tau} (ng/mL)	491.26	711.29	69.07 (51.82, 92.06)

Table 17 shows the statistical comparison of the mean pharmacokinetic parameters of Cobi in subjects with severe renal impairment and subjects with normal renal function.

Table 17: Statistical comparison of the mean pharmacokinetic parameters of Cobi in subjects with severe renal impairment and subjects with normal renal function

| COBI PK Parameter | Geometric Least-Squares Mean | | Geometric Least-Squares Means Ratio (%) (90% CI) |
	Test Severe Renal Impairment eGFR$_{CG}$ < 30 mL/min (N = 12)	Reference Normal Renal Function eGFR$_{CG}$ ≥ 90 mL/min (N = 11)	
AUC$_{tau}$ (ng·h/mL)	17313.3	13797.50	125.48 (98.57, 159.73)
C$_{max}$ (ng/mL)	2041.71	1667.84	122.42 (99.82, 150.13)
C$_{tau}$ (ng/mL)	89.02	78.88	112.85 (56.75, 224.40)

The results of this trial showed that the pharmacokinetics of EVG and Cobi was not significantly altered in subjects with severe renal impairment (eGFR < 30 mL/min) as compared with subjects with normal renal function (eGFR ≥ 90 mL/min). Considering that elimination of EVG and Cobi through the renal route is minimal (as shown in the mass balance trials of EVG and Cobi) and the FDC tablet will only be used in subjects with CrCL ≥ 70 mL/min (eGFR ≥ 70 mL/min was one of the inclusion criteria in pivotal Phase III trials to prevent early discontinuations due to the expected inhibitory effect of Cobi on creatinine secretion), renal impairment is not expected to significantly alter the pharmacokinetics of either EVG and Cobi.

The applicant also evaluated the free fraction (%) of EVG and Cobi in trial GS-US-216-0124. The free fraction (%) of EVG in normal matched control subjects and subjects with severe renal impairment (eGFR < 30 mL/min) was 1.16 and 1.42, respectively. The free fraction (%) of Cobi in normal matched control subjects and subjects with severe renal impairment (eGFR < 30 mL/min) was 2.47 and 2.49, respectively.

The dosing recommendations based on the renal impairment trial (GS-US-216-0124) conducted with EVG and cobi given together can be extended to the FDC tablet (EVG/Cobi/Emtricitabine/Tenofovir) based on the following considerations:

- Similarity in exposures of EVG and Cobi when given either as EVG/Cobi or the FDC tablet.
- No significant differences in systemic exposures (and hence no dose adjustment) of either emtricitabine or tenofovir between subjects with normal renal function (CrCL≥ 90 mL/min) and subjects with CrCl between 70-90 mL/min

FDC tablets can be given to subjects with eGFR ≥ 70 mL/min without any dose adjustments. eGFR ≥ 70 mL/min was one of the inclusion criteria in pivotal Phase III trials to avoid early discontinuations due to the expected inhibitory

effect of Cobi on creatinine secretion. Because the dosing frequency of tenofovir needs to be adjusted when given to patients with eGFR ≤ 50 mL/min, FDC will be discontinued in patients with eGFR ≤ 50 mL/min. For patients with eGFR between 50 and 70 mL/min, the pharmacokinetics of the individual components of the FDC is not expected to be different from patients with eGFR ≥ 70 mL/min. However, due to additional considerations related to the safety profile of FDC in patients with eGFR 50-70 mL/min, FDC should not be initiated in patients with eGFR 50-70 mL/min.

2.3.2.6   Hepatic impairment

The effect of hepatic impairment on the pharmacokinetics of Cobi boosted EVG was evaluated in trial GS-US-183-0133.

Table 18 shows the statistical comparison of the mean pharmacokinetic parameters of EVG in subjects with moderate hepatic impairment and subjects with normal hepatic function.

Table 18: Statistical comparison of the mean pharmacokinetic parameters of EVG in subjects with moderate hepatic impairment and subjects with normal hepatic function

PK Parameter	GLSMs		GLSM Ratio (%) (90% CI)
	Reference Treatment: Normal Matched Control Group (N=10)	Test Treatment: Moderate Hepatic Impairment Group (N=10)	
AUC_{tau} (ng•h/mL)	20537.29	27722.39	134.99 (103.09, 176.75)
C_{tau} (ng/mL)	335.30	602.29	179.63 (111.03, 290.60)
C_{max} (ng/mL)	1880.67	2657.00	141.28 (108.80, 183.45)

GLSMs were obtained using a mixed-effects model. The model included treatment, sequence, and period as fixed effects, and subject-within-sequence as a random effect.

Table 19 shows the statistical comparison of the mean pharmacokinetic parameters of Cobi in subjects with moderate hepatic impairment and subjects with normal hepatic function.

Table 19: Statistical comparison of the mean pharmacokinetic parameters of Cobi in subjects with moderate hepatic impairment and subjects with normal hepatic function

PK Parameter	GLSMs		GLSM Ratio (%) (90% CI)
	Reference Treatment: Normal Matched Control Group (N=10)	Test Treatment: Moderate Hepatic Impairment Group (N=10)	
AUC_{tau} (ng•h/mL)	9358.61	9334.67	99.74 (76.01, 130.89)
C_{tau} (ng/mL)	33.30	69.17	207.70 (117.13, 368.31)
C_{max} (ng/mL)	1250.46	1076.63	86.10 (65.35, 113.43)

GLSMs were obtained using a mixed-effects model. The model included treatment, sequence, and period as fixed effects, and subject-within-sequence as a random effect.

The mean C_{max} and AUC of EVG increased by 41 % and 35 %, respectively in subjects with moderate hepatic impairment as compared with subjects with normal hepatic function. The range of systemic exposures of EVG observed in subjects with moderate hepatic impairment is within the range of EVG exposures observed in Phase 3 trials. EVG exposures in Phase 3 trials have not been associated with any major adverse events.

The protein binding of EVG and Cobi was also determined in trial GS-US-183-0133. The free fraction (%) of EVG in normal matched control subjects and subjects with moderate hepatic impairment was 1.15 and 1.22, respectively. The free fraction (%) of Cobi in normal matched control subjects and subjects with moderate hepatic impairment was 2.71 and 3.23, respectively.

The dosing recommendations based on the hepatic impairment trial conducted with EVG and Cobi given together can be extended to the FDC tablet (EVG/Cobi/Emtricitabine/Tenofovir) based on the following considerations:

- Similarity in exposures of EVG and Cobi when given either as EVG/Cobi or the FDC tablet.
- Emtricitabine and Tenofovir are primarily renally excreted, hence, hepatic impairment is not expected to significantly alter either emtricitabine or tenofovir concentrations.

Recommendation: FDC tablet can be given to subjects with either mild- or moderate hepatic impairment without any dose adjustments. The pharmacokinetics of FDC tablet in subjects with severe hepatic impairment has not been evaluated, therefore, FDC tablets are not recommended for use in patients with severe hepatic impairment.

2.3.2.7   What pregnancy and lactation use information is there in the
          application?

No information regarding the use of the FDC tablet in pregnant or lactating
women was included in the NDA submission.

2.4   Extrinsic Factors

2.4.1   What extrinsic factors influence dose-exposure and/or response, and
           what is the impact of any differences in exposure on response?

The applicant evaluated the effect of two extrinsic factors on the
pharmacokinetics of the individual components of the FDC tablet: effect of food
(evaluated in trial GS-US-236-0105 and described in response to question 2.5.3)
and the drug-drug interactions (described in section 2.4.2).

2.4.2   Drug-drug interactions

2.4.2.1   Is there an in vitro basis to suspect in vivo drug-drug interactions?

Yes, there is in vitro basis to suspect in vivo drug-drug interactions. CYP3A
mediated metabolism plays a major role in the biotransformation of EVG. *In vitro*
studies showed that Cobi is metabolized by CYP3A4 and CYP2D6. Further,
Cobi is an inhibitor of CYP3A and potentially CYP2D6 enzymes and inhibits P-
glycoprotein (p-gp), BCRP, OATP1B1, and OATP1B3 transporters.

See sections 2.4.2.2 and 2.4.2.3 for more information.

2.4.2.2   Is the drug a substrate of CYP enzymes? Is metabolism influenced by
           genetics?

EVG:

In vitro metabolism study results showed that CYP3A4 was responsible for the
majority of EVG metabolism in human liver microsomes, although metabolism *via*
CYP3A5 and CYP1A1 was also detected. In liver microsomes from all species
(rats, dogs, monkeys, and/or humans), the primary oxidative metabolite was M1,
a chlorofluorophenyl hydroxide of EVG, and the primary glucuronide conjugate
was M4, an acyl-glucuronide conjugate of EVG. In the presence of the CYP3A4
inhibitor ketoconazole, metabolism of EVG in human liver microsomes was
markedly reduced (69.8 and 97.5% inhibition by 0.2 and 2 µM ketoconazole,
respectively), but the CYP2C9 inhibitor sulfaphenazole and the CYP2D6 inhibitor
quinidine did not substantially influence EVG metabolism. The results of in vitro
studies that showed CYP3A was responsible for the majority of EVG metabolism
were confirmed in human trials; results of trial GS-US-183-0102 showed that the
geometric least squares mean of EVG AUC$_{tau}$ after administration of multiple

dose EVG and a single dose of ritonavir (a potent CYP3A4 inhibitor) was 8.58-fold higher than after administration of multiple dose EVG without RTV.

Cobi:

The *in vitro* metabolism of cobi was evaluated in human hepatocytes and hepatic microsomal fractions (AD-216-2024 and AD-216-2025). Both human hepatocytes and hepatic microsomal fractions generated oxidative metabolites. In study AD-216-2025, cobi was incubated with five different cDNA expressing human cytochrome P450 enzymes: CYP1A2, CYP2C9, 2C19, CYP2D6, and CYP3A4. The results showed that CYP3A4 and CYP2D6 were involved in the metabolism of cobi.

FTC and TDF:

FTC and TDF are not significantly metabolized; both drugs are primarily excreted unchanged as a combination of glomerular filtration and active tubular secretion.

2.4.2.3   Is the drug an inhibitor and/or an inducer of CYP enzymes?

EVG:

The potential for EVG to inhibit major human drug metabolizing CYP450-enzymes was evaluated using pooled human hepatic microsomal fractions and enzyme specific substrates (JTK303-AD-027). The concentration of EVG evaluated ranged from 0-30 µg/mL

Table 20 shows the effect of EVG on the activities of major human hepatic microsomal CYP450 enzymes.

Table 20: Effect of EVG on the activities of major human hepatic microsomal CYP450 enzymes

Enzyme	Activity	EVG	Control Inhibitor[a]
		Calculated IC$_{50}$ (µg/mL)	Activity remaining (%)
CYP1A2	Ethoxyresorufin O-deethylase	> 30	5.6%
CYP2A6	Coumarin 7-hydroxylase	> 30	< 10.2%
CYP2C9	Tolbutamide 4-hydroxylase	> 30	42.5%
CYP2C19	(S) Mephenytoin 4'-hydroxylase	> 30	29.6%
CYP2D6	Bufuralol 1'-hydroxylase	> 30	< 17.5%
CYP2E1	Chlorzoxazone 6-hydroxylase	> 30	48.3%
CYP3A	Midazolam 1'-hydroxylase	> 30	< 9.6%
	Testosterone 6β-hydroxylase	28.32	5.6%

a   Control Inhibitors: CYP1A2, α Naphthoflavone (1 µM); CYP2A6, Methoxsalen (5 µM); CYP2C9, Sulfaphenazole
    (3 µM); CYP2C19, Tranylcypromine (20 µM); CYP2D6, Quinidine (4 µM); CYP2E1 Diethyldithiocarbamate
    (100 µM); CYP3A, Ketoconazole (1 µM).

The results showed that the IC_{50} of EVG was > 30 µg/mL for all the enzymes except CYP3A4 where an IC_{50} of 28.32 µg/mL was noted. As the total C_{max} of EVG is significantly lower (~2000 ng/mL) after administration of the FDC tablet formulation, EVG by itself is not expected to alter the concentration of co-administered drugs that are substrates of CYP3A4.

The induction potential of EVG was evaluated in primary cultures of human hepatocytes (JTK303-AD-023). Table 21 shows the effect of EVG and positive control inducers on enzyme activities of primary cultures of fresh human hepatocytes.

Table 21: Effect of EVG and positive control inducers on enzyme activities of primary cultures of fresh human hepatocytes

Test Article	Donor Lot	Fold Increase Compared to Vehicle Control			
		CYP1A2	CYP2C9	CYP2C19	CYP3A
EVG 0.1 µg/mL	66	1.18	0.96	ND	1.74
	68	1.08	0.92	ND	1.48
EVG 1 µg/mL	66	1.24	1.19	ND	6.41
	68	1.58	1.64	ND	6.32
EVG 10 µg/mL	66	0.63	1.49	ND	19.1
	68	1.16	2.72	ND	10.6
Positive Control[a]	66	30.6	3.14	ND	34.1
	68	48.4	4.29	NC	25.7

EVG = elvitegravir; NC: Cannot be calculated due to lack of activity in vehicle control; ND: Activity not detectable

Fractional increase = (Fold Increase of test compound – 1) / (Fold Increase of positive control – 1) x 100%

a   Positive controls: 20 µM β-naphthoflavone (CYP1A2), 20 µM rifampicin (CYP2C9, CYP2C19), 10 µM rifampicin (CYP3A)

The results suggest that at the highest concentration evaluated (10 µg/mL), EVG is a weak inducer of CYP3A4, however, when EVG is co-administered with cobi (CYP3A4), the weak inductive effect of EVG will most likely be overcome by the inhibitory effect of cobi.

Cobi

The potential for cobi to inhibit various CYP3A enzymes was evaluated *in vitro* in human hepatic microsomal fractions (AD-216-2028). Table 22 shows the effect of cobi and ritonavir on various CYP3A enzyme activities.

Table 22: Effect of cobi and ritonavir on various CYP3A enzyme activities

Activity	Calculated IC$_{50}$ (µM)	
	COBI	RTV
Midazolam 1'-hydroxylase	0.15	0.11
Testosterone 6β-hydroxylase	0.15	0.12
Terfenadine *t*-butyl-hydroxylase	0.29	0.28
Elvitegravir hydroxylase (to metabolite M1)	0.03	0.03
Atazanavir oxidation	0.04	0.04
Telaprevir oxidation	0.03	0.02

COBI = cobicistat; RTV = ritonavir

Based on *in vitro* evaluation, cobi and ritonavir showed similar IC$_{50}$ values in human liver microsomes. The results of clinical trial GS-US-216-0101 confirmed the CYP3A inhibition properties of cobi; the apparent clearance (CL/F) of midazolam (CYP substrate) was decreased by ~93 % and ~95 % in the presence of 100 mg and 200 mg cobi, respectively.

Other CYP enzymes:

The specificity of cobi towards other CYP enzymes was evaluated using pooled human hepatic microsomal fractions (AD-216-2029 and AD-216-2070). Table 23 shows the IC$_{50}$ values for inhibition of major human CYP450 enzymes by cobi, ritonavir and other positive controls.

Table 23: IC$_{50}$ values for inhibition of major human CYP450 enzymes by cobi, ritonavir and other positive controls

Enzyme	Activity	Calculated IC$_{50}$ (µM)		
		Control Inhibitor[a]	COBI	RTV
CYP1A2	Ethoxyresorufin O-deethylase	0.03	> 25	> 25
CYP2B6	Bupropion 4-hydroxylase	2.8	2.8	2.9
CYP2C8	Paclitaxel 6α-hydroxylase	0.06	30.1	5.5
CYP2C9	Tolbutamide hydroxylase	1.6	> 25	3.9
CYP2C19	(S) Mephenytoin 4'-hydroxylase	10.8	> 25	> 25
CYP2D6	Dextromethorphan O-demethylase	0.04	9.2	3.4
CYP3A	Midazolam 1'-hydroxylase	0.07	0.15	0.10
	Testosterone 6β-hydroxylase	0.09	0.15	0.11

COBI = cobicistat; CYP = cytochrome P450 enzyme; RTV = ritonavir

a   Control Inhibitors: CYP1A2, α-Napthoflavone (0–100 µM); CYP2B6, Triethylenethiophosphoramide (0–30 µM); CYP2C8 Montelukast (0–30 µM); CYP2C9, Sulfaphenazole (0–10 µM); CYP2C19, Tranylcypromine (0–100 µM); CYP2D6, Quinidine (0–10 µM); CYP3A, Ketoconazole (0–10 µM).

At concentrations up to 25 µM, neither cobi nor ritonavir inhibited CYP1A2 and CYP2C19. Cobi did not inhibit CYP2C9 (in contrast to ritonavir), however, the

inhibitory potency of cobi and ritonavir was similar for CYP2B6 (although DDI trial conducted with efavirenz [a sensitive CYP2B6 substrate] showed that Cobi is not expected to affect CYP2B6 in vivo). Cobi was less potent than ritonavir in terms of CYP2C8 inhibition. Cobi is not expected to inhibit CYP2C8 *in vivo.* Based on the C_{max} of Cobi (~2 µM), Cobi is anticipated to inhibit CYP3A4 and potentially CYP2D6.

The results of the DDI trial conducted using desimipramine as a probe substrate for CYP2D6 (GS-US-216-0112) confirmed the results of the *in vitro* findings that showed that cobi is a weak inhibitor of CYP2D6.

UGT enzymes:

The potential for cobi to inhibit the activity of UGT1A1 was evaluated (AD-216-2075). Table 24 shows the IC_{50} values of the human hepatic microsomal UGT1A1 activity for cobi, ritonavir, and atazanavir.

Table 24: IC_{50} values of the human hepatic microsomal UGT1A1 activity For cobi, ritonavir, and atazanavir

Enzyme	Activity	Calculated IC₅₀ (µM)		
		ATV	COBI	RTV
UGT1A1	β-Estradiol-3-glucuronidation	0.83	16.3	4.73

ATV = atazanavir; COBI = cobicistat; RTV = ritonavir; UGT = uridine diphosphate glucuronosyl transferase

Cobi was shown to be a less potent inhibitor of UGT1A1 as compared to the positive controls used in the study (~20-fold less potent than ATV and ~6-fold less potent than RTV).

CYP Induction:

The potential for cobi, ritonavir, and other positive control compounds to induce human drug metabolizing enzymes through activation of human hydrocarbon receptor (AhR) and human pregnane X receptor (PXR) was evaluated (AD-216-2027). Collectively, the results suggest that cobi has a low potential to activate AhR and PXR. At the mean C_{max} of cobi expected after steady state administration of the FDC tablet to HIV infected patients (total C_{max} 1140 ng/mL; ~2 µM), cobi is expected to have a weak induction effect on the CYP3A4 mRNA expression and no effect on the expression of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, UGT1A1 and MDR1. Further, any CYP3A4 induction is likely to be overcome by CYP3A inhibition by cobi as shown by the results of the cocktail study and by the increase in the systemic exposure of midazolam (CYP3A substrate) when co-administered with Cobi.

Table 25 shows the activation of the human hydrocarbon receptor (AhR) by cobi, ritonavir, and other positive control compounds.

Table 25: Activation of human hydrocarbon receptor (AhR) by cobi, ritonavir, and other positive control compounds

Concentration	Fold Induction over 0.1% DMSO Control[a]			
	Test Compounds		Positive controls	
	COBI	RTV	β-Naphthoflavone	Omeprazole
0.1 μM	—	—	2.17	—
1 μM	1.12	0.80	5.91	—
3 μM	1.28	0.69	—	—
5 μM	—	—	17.72	—
10 μM	1.60	0.80	27.31	—
25 μM	—	—	—	8.16
50 μM	—	—	—	13.46
100 μM	—	—	—	27.34
200 μM	—	—	—	67.33

COBI = cobicistat; RTV = ritonavir

a   Fold activation of human CYP1A2 promoter after 24 hours incubation at the indicated extracellular concentration

Table 26 shows the activation of the human pregnance X receptor (PXR) by cobi, ritonavir, and other positive control compounds.

Table 26: Activation of human pregnane X receptor (PXR) by cobi, ritonavir, and other positive control compounds

Concentration	Fold Induction Over 0.1% DMSO Control[a]				
	COBI	RTV	Rifampin	Mifepristone	Androstanol
0.3 μM	—	—	3.15	—	—
1 μM	1.57	3.64	6.09	—	—
3 μM	1.61	7.62	9.90	—	—
10 μM	2.24	10.14	14.30	8.58	3.38

COBI = cobicistat; PXR = pregnane X receptor; RTV = ritonavir

a   Fold activation of CYP3A4 promoter after 24 hours incubation at the indicated extracellular concentration.

The potential for cobi to induce drug metabolizing enzymes was also evaluated using primary cultures of human hepatocytes (AD-216-2071). The hepatocytes were obtained from three separate donors and four different concentrations of cobi (1, 3, 10 and 30 μM) were used.

Table 27 shows the evaluation of cobicistat's induction effect using changes in the mRNA levels (fold induction)

Table 27: Evaluation of cobicistat's induction effect using changes in the mRNA levels (fold induction)

Treatment	CYP1A2 Hu790	CYP1A2 Hu793	CYP1A2 Hu8053	CYP2B6 Hu790	CYP2B6 Hu793	CYP2B6 Hu8053	CYP3A4 Hu790	CYP3A4 Hu793	CYP3A4 Hu8053	UGT1A1 Hu790	UGT1A1 Hu793	UGT1A1 Hu8053	MDR1 Hu790	MDR1 Hu793	MDR1 Hu8053
3-MC (2 µM)	400	406	565	3.10	0.825	1.07	0.270	0.528	0.227	12.0	9.63	3.94	0.693	0.573	0.687
Phenobarbital (1000 µM)	1.55	2.46	1.78	65.3	46.4	12.1	45.2	31.9	12.2	10.8	14.5	4.78	1.96	2.30	2.00
Rifampicin (10 µM)	1.26	1.15	1.85	21.7	19.6	9.64	73.5	39.7	24.6	8.85	11.6	4.53	2.07	2.31	1.94
GS-9350 (1 µM)	1.30	1.28	0.864	1.55	0.763	1.69	6.08	6.58	3.26	1.22	1.85	1.25	0.933	0.538	0.875
GS-9350 (3 µM)	1.58	0.522	1.33	1.54	1.06	2.46	13.8	10.8	8.48	1.22	2.16	1.58	0.923	0.724	1.06
GS-9350 (10 µM)	3.10	1.27	4.60	1.32	1.01	1.99	17.6	11.8	8.32	0.980	2.12	1.61	1.14	0.914	1.01
GS-9350 (30 µM)	6.62	13.3	10.4	0.377	0.501	0.293	2.91	8.36	1.85	0.603	1.70	0.890	1.28	1.11	1.45

FTC and TDF:

The potential for CYP mediated drug-drug interactions with FTC and TDF and other drugs is considered to be low.

2.4.2.4 Is the drug a substrate and/or an inhibitor of P-glycoprotein transport processes?

EVG:

EVG is a substrate of P-glycoprotein transport process.

LLC-PK1 cells, transfected with an expression vector for human MDR1, were used to determine if EVG is a P-gp substrate (JTK303-AD-026). Table 28 shows the transport of EVG and control compounds across monolayers of LLC-PK1 cells transfected with control vector or expression vector of human MDR1.

Table 28: Transport of EVG and control compounds across monolayers of LLC-PK1 cells transfected with control vector or expression vector of human MDR1

Compound	Time (h)	Control Cells A-B	Control Cells B-A	Control Cells Ratio	MDR1-Expressing Cells A-B	MDR1-Expressing Cells B-A	MDR1-Expressing Cells Ratio
EVG	1	179.2 ± 16.7	207.8 ± 11.2	1.2	64.1 ± 17.3	961.9 ± 75.8	15.0
EVG	2	412.7 ± 20.0	512.8 ± 34.4	1.2	139.4 ± 25.2	1891.6 ± 126.1	13.6
EVG	4	606.4 ± 18.7	912.6 ± 62.9	1.5	212.1 ± 34.7	3085.4 ± 65.7	14.5
Digoxin	1	21.6 ± 1.1	44.6 ± 10.3	2.1	25.3 ± 9.0	245.8 ± 27.0	9.7
Digoxin	2	48.6 ± 9.5	96.7 ± 16.1	2	54.9 ± 5.1	498.1 ± 69.8	9.1
Digoxin	4	103.0 ± 20.1	229.8 ± 27.4	2.2	97.4 ± 12.3	1000.2 ± 125.8	10.3
Mannitol	1	24.1 ± 12.8	16.2 ± 1.2	0.7	53.9 ± 36.4	42.9 ± 18.2	0.8
Mannitol	2	36.6 ± 10.4	35.7 ± 4.0	1	97.0 ± 63.6	83.6 ± 27.4	0.9
Mannitol	4	89.9 ± 14.4	73.5 ± 6.3	0.8	176.9 ± 100.2	131.7 ± 33.8	0.7

A-B = apical to basal; B-A = basal to apical; EVG = elvitegravir; MDR1 = P-glycoprotein

The efflux ratio of EVG in MDR1-transfected cells was ≥ 13.6, suggesting that

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EVG is a substrate of P-gp transporters.

The effect of EVG (0.3 to 30 μM) on the bidirectional permeability of [^{3}H] digoxin (1μM) across monolayers of LLC-PK1 porcine kidney cells, transfected with an expression vector for human MDR1 or with the empty control expression vector was evaluated (JTK303-AD-026). Table 29 shows the effect of EVG and positive control inhibitor on the transport of digoxin by LLC-PK1 cells.

Table 29: Effect of EVG and positive control inhibitor on the transport of digoxin by LLC-PK1 cells

| | Transcellular Flux (μL/mg protein/h) | | | | | |
| | Control Cells | | | MDR1-Expressing Cells | | |
Inhibitor	A-B	B-A	Ratio	A-B	B-A	Ratio
None	20.6 ± 2.9	44.8 ± 6.7	2.2	18.5 ± 5.8	183.6 ± 10.6	9.9
EVG 0.3 μM	53.4 ± 3.5	73.5 ± 8.6	1.4	19.6 ± 7.1	204.6 ± 19.2	10.4
EVG 1 μM	47.7 ± 4.4	75.7 ± 4.7	1.6	27.0 ± 4.6	213.8 ± 21.1	7.9
EVG 3 μM	28.1 ± 2.8	46.6 ± 3.2	1.7	19.2 ± 1.7	205.9 ± 13.5	10.7
EVG 10 μM	29.1 ± 1.8	33.9 ± 3.3	1.2	18.0 ± 1.0	176.1 ± 9.3	9.8
EVG 30 μM	24.6 ± 2.8	29.9 ± 2.3	1.2	27.5 ± 6.8	164.6 ± 11.4	6.0
Verapamil 10 μM	24.9 ± 1.1	25.4 ± 2.9	1.0	34.6 ± 14.6	110.0 ± 6.8	3.2

A-B = apical to basal; B-A = basal to apical; EVG = elvitegravir; MDR1 = P-glycoprotein

Based on the results, an IC_{50} value > 30 μM was determined. Hence, EVG does not have the potential to cause significant inhibition of p-gp transporters in vivo.

Cobi:

Cobi is a substrate of P-glycoprotein transport process.

The bidirectional permeability of cobi was determined through monolayers of P-gp and BCRP over expressing cells (AD-216-2103). In MDCKII-MDR1 cells, the efflux ratio was ≥ 2, and when cyclosporine, a P-gp inhibitor was administered, the efflux ratio decreased by 94%. In MDCKII-BCRP cells, the efflux ratio was ≥ 2, and when Ko134, a compound used by the applicant as a BCRP inhibitor was administered, the efflux ratio decreased by 76%. These results suggest that cobi is a P-gp and a BCRP substrate.

The effect of cobi on P-gp transport is described in section 2.4.2.5, below.

2.4.2.5   Are there other metabolic/transporter pathways that may be important?

EVG:

The potential for EVG (at concentrations up to 2 μM) to inhibit OATP1B1 or

OATP1B3 was evaluated using cell lines expressing these transporters (JTK303-AD-026 and AD-183-2030).

The positive control, rifampicin (50 µM) reduced OATP1B1 activity by 98.6% and OATP1B3 by 98.4%, confirming the sensitivity of the cells to OATP1B1 inhibition. EVG was a weak inhibitor of OATP1B1, with < 40% reduction in activity at the highest concentration tested (IC_{50} > 2 µM). EVG inhibited OATP1B3 with an IC_{50} of 0.44 µM.

Cobi:

The potential for cobi to inhibit various transporters was evaluated in cell lines or vesicles expressing individual recombinant protein.

Table 30 shows the effect of cobi and ritonavir on the activities of human transporters.

Table 30: Effect of cobi and ritonavir on the activities of human transporters

Transporter	Cell line	Substrate (concentration)	IC_{50} (µM)		Tabulated Summary (Report)
			COBI	RTV	
MDR1	MDCK II	calcein AM (10 µM)	22.5 – 45.0[a]	10.0 – 20.0[a]	2.6.5.15.9 (AD-216-2030)
MRP1	MDCK II	calcein AM (10 µM)	45.0 – 90.0[a]	10.0 – 20.0[a]	
MRP2	MDCK II	calcein[b]	45.0 – 90.0[a]	> 20[d]	
MRP4	LLC-PK1[c]	DHEAS (0.02 µM)	20.7	> 20[d]	2.6.5.15.16 (AD-216-2105)
BCRP	MDCK II	Hoechst 33342 (10 µM)	59.0	> 20[d]	2.6.5.15.10 (AD-216-2099)
OAT1	CHO	p-aminohippurate (5 µM)	> 100[d]	> 20[d]	2.6.5.15.15 (AD-216-2105)
OAT3	HEK293	estrone 3-sulfate (0.2 µM)	> 100[d]	8.46	
OCT2	CHO	metformin (2 µM)	8.24	22.6	2.6.5.15.12 (AD-216-2093)
OCTN1	S2	tetraethylammonium (5 µM)	2.49	2.08	2.6.5.15.14 (AD-216-2098)
MATE1	HEK293	tetraethylammonium (5 µM)	1.87	1.34	2.6.5.15.13 (AD-216-2094)
MATE2-K	HEK293	tetraethylammonium (5 µM)	33.5	100	
OATP1B1	CHO	Fluo 3 (2 µM)	3.50	2.05	2.6.5.15.11 (AD-216-2100)
OATP1B3	CHO	Fluo 3 (2 µM)	1.88	1.83	

AM = acetomethoxy ester; BCRP = breast cancer resistance protein; COBI = cobicistat; DHEAS = 5-dehydroepiandrosterone sulfate; MATE1 = multidrug and toxin extrusion protein 1 (SLC47A1); MATE2-K = multidrug and toxin extrusion protein 2-K (SLC47A2); MDR1 = P-glycoprotein (multidrug resistance protein 1); MRP = multi-drug resistance-associated protein; OAT = organic anion transporter; OATP = organic anion transporting polypeptide; OCT2 = organic cation transporter 2; OCTN1 = organic cation transporter N1; RTV = ritonavir

a   Range of tested concentrations bracketing 50% inhibition (IC_{50} not calculated)

b   Generated from 10 µM calcein AM

c   Study performed with vesicles derived from the cell line

d   Maximum concentration tested

Cobi is not anticipated to inhibit OAT1, OAT3, or OCT2 and may inhibit OATP1B1 and OATP1B3. Of note, inhibition of OATP transporters observed *in vitro* is consistent with the results of trial GS-US-216-0123 (drug-drug interaction trial of EVG and Cobi given together and rosuvastatin) which showed an increase in exposure of co-administered rosuvastatin (AUC$_{0-\infty}$ increased 38 % compared to administration of rosuvastatin alone).

The potential for inhibition of intestinal MDR1 or BCRP by cobi was determined by assessing the effect of cobi on the bidirectional transport of digoxin and prazosin, respectively (AD-216-2072 and AD-216-2104).

Table 31 shows the bidirectional permeability of digoxin through Caco-2 cells in the presence of known MDR1 inhibitors and cobi.

Table 31: Bidirectional permeability of digoxin through Caco-2 cells in the presence of known MDR1 inhibitors and cobi

Inhibitor (Concentration)	Direction	Digoxin P$_{app}$ (10^{-6} cm/s)	Efflux Ratio
None	Cell-Free	38.5	
	Forward	1.30	7.72
	Reverse	10.0	
Cyclosporin A (10 µM)	Cell-Free	47.0	
	Forward	2.25	1.68
	Reverse	3.78	
RTV (20 µM)	Cell-Free	45.6	
	Forward	3.17	1.84
	Reverse	5.81	
COBI (90 µM)	Cell-Free	51.1	
	Forward	2.24	1.69
	Reverse	3.80	

COBI = cobicistat; RTV = ritonavir

Table 32 shows the bidirectional permeability of prazosin through Caco-2 cells in the presence of ritonavir or cobi

Table 32: Bidirectional permeability of prazosin through Caco-2 cells in the presence of ritonavir or cobi

Inhibitor (Concentration)	Direction	Prazosin P$_{app}$ (10^{-6} cm/s)	Efflux Ratio
None	Cell-Free	36.94	
	Forward	2.50	5.1
	Reverse	12.78	
Fumitremorgin C (2 µM)	Cell-Free	46.75	
	Forward	4.44	2.6
	Reverse	11.58	
RTV (20 µM)	Cell-Free	39.87	
	Forward	4.00	2.8
	Reverse	11.26	
COBI (90 µM)	Cell-Free	38.33	
	Forward	4.74	2.4
	Reverse	11.20	

COBI = cobicistat; RTV = ritonavir

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At a concentration of 90 µM, Cobi reduced the efflux of digoxin and prazosin, suggesting that higher concentrations of cobi in the intestinal lumen can inhibit intestinal MDR1 and BCRP. However, based on concentrations achieved in plasma, cobi is expected to be a weak inhibitor of both P-gp and BCRP. The results of clinical trial GS-US-216-0112 corroborate the *in vitro* findings: cobi increased the C_{max} of digoxin by 41 %, however the increase in AUC_{0-inf} was 8 %, suggesting that the p-gp inhibitory effect of cobi may be predominantly on the intestinal p-gp.

2.4.2.6 Does the label specify co-administration of another drug and, if so, has the interaction potential between these drugs been evaluated?

FDC is intended to be a complete regimen for HIV-1 infected treatment naïve patients and hence, will not be combined with other antiretroviral drugs.

HIV-1 infected patients, while receiving FDC, may also receive non antiretroviral drugs for treatment of comorbidities. Refer to section 2.4.2.7 and 2.4.2.8 for further details.

2.4.2.7 What other co-medications are likely to be administered to the target patient population?

HIV-1 infected patients may receive a variety of concurrent medications for treatment or prevention of comorbidities. These include antihyperlipidemics, selective serotonin reuptake inhibitors (SSRIs), antibacterials, phosphodiesterase 5-inhibitors, drugs used to treat opioid dependence, drugs used to treat tuberculosis, oral contraceptives, and drugs that alter gastric pH.

2.4.2.8 Are there any in vivo drug-drug interaction studies that indicate the exposure alone and/or exposure-response relationships are different when drugs are co-administered?

The applicant conducted several drug-drug interactions trials to evaluate the effect of co-administered drug on the pharmacokinetics of the individual components of the FDC (or EVG and Cobi in trials where EVG and Cobi were administered together) and the effect of FDC (or co-administration of EVG and Cobi) on the pharmacokinetics of the co-administered drug(s). The results obtained from drug-drug interaction trials in which EVG and Cobi were administered together are applicable to the FDC tablet because the results of trial GS-US-236-0110 showed that the mean systemic exposures of EVG and Cobi after administration of the FDC tablet were similar to the mean systemic exposures of EVG and Cobi when administered together.

The clinical recommendations proposed by the applicant are based on either:

- Results of a drug-drug interaction trial (with either FDC or EVG and Cobi

administered together; results shown in tables 34 and 35) OR

- Expected change in the concentration of EVG, Cobi, and the co-administered drug based on the metabolic properties of the individual drugs. As Cobi, similar to ritonavir, is a mechanism based inhibitor of CYP3A, clinical recommendations based on expected interactions with FDC are similar to expected interactions when ritonavir (or a protease inhibitor/ritonavir combination) is co-administered with non-antiretroviral drugs.

Table 33 shows the list of drugs that are contraindicated with FDC tablets.

Table 33: Drugs that are contraindicated with FDC

Drug Class	Drugs within class that are contraindicated with STRIBILD	Clinical Comment
Alpha 1-Adrenoreceptor Antagonist:	Alfuzosin	Potential for increased alfuzosin concentrations, which can result in hypotension.
Antimycobacterial	Rifampin	Rifampin is a potent inducer of CYP450 metabolism. STRIBILD should not be used in combination with rifampin, as this may cause significant decrease in the plasma concentration of elvitegravir and cobicistat. This may result in loss of therapeutic effect to STRIBILD.
Ergot Derivatives	Dihydroergotamine Ergotamine Methylergonovine	Potential for serious and/or life-threatening events such as acute ergot toxicity characterized by peripheral vasospasm and ischemia of the extremities and other tissues.
GI Motility Agent	Cisapride	Potential for serious and/or life-threatening events such as cardiac arrhythmias
Herbal Products	St. John's wort (Hypericum perforatum)	Patients taking STRIBILD should not use products containing St. John's wort because co-administration may result in reduced plasma concentrations of elvitegravir and cobicistat. This may result in loss of therapeutic effect and development of resistance.
HMG CoA Reductase Inhibitors:	Lovastatin Simvastatin	Potential for serious reactions such as myopathy including rhabdomyolysis.
Neuroleptic	Pimozide	Potential for serious and/or life-threatening events such as cardiac arrhythmias
Phosphodiesterase-5 (PDE5) Inhibitor	Sildenafil when dosed as REVATIO® for the treatment of pulmonary arterial hypertension	A safe and effective dose in combination with STRIBILD has not been established for sildenafil (REVATIO®) when used for the treatment of pulmonary hypertension. There is increased potential for sildenafil-associated adverse events (which include visual disturbances, hypotension, priapism, and syncope).
Sedative/hypnotics:	Triazolam Orally administered midazolam	Triazolam and orally administered midazolam are extensively metabolized by CYP3A4. Co-administration of triazolam or orally administered midazolam with STRIBILD may cause large increases in the concentration of these benzodiazepines. The potential exists for serious and/or life threatening events such as prolonged or increased sedation or respiratory depression.

Clinical Recommendations based on results of DDI Trials:

- GS-US-216-0120 (DDI Trial of EVG plus cobi with H_2 Receptor Antagonist [H_2RA] or Proton Pump Inhibitor[PPI]):
  - Omeprazole can either be administered 2 hours before- or staggered by 12 hours when given with FDC.
  - Famotidine should be staggered by 12 hours when given with FDC.

- GS-US-216-0122 (DDI of EVG plus cobi Trial with H_2 Receptor Antagonist):
  - Famotidine can be co-administered with FDC without any dose adjustments.

- GS-US-183-0119 (DDI Trial with Omeprazole or Antacid):
  - Coadministration of ritonavir-boosted elvitegravir and antacid should be separated by at least 2 hours.
  - Omeprazole can be co-administered with ritonavir-boosted elvitegravir without any dose adjustments.

The clinical recommendations based on the results of trial GS-US-216-0120 and GS-US-216-0122 can be extrapolated to other PPIs and H_2RAs, respectively.

- GS-US-216-0123: DDI Trial of EVG plus cobi with Atazanavir (ATV), Rosuvastatin (ROS), or Rifabutin (RIF) :

  - Initiate with lowest starting rosuvastatin dose and titrate carefully while monitoring for safety.
  - FDC and Rifabutin should not be co-administered due to the potential for significant reduction in EVG C_{tau}. Further, FDC should not be co-administered with other strong CYP3A inducers due to potential for significant decrease in EVG C_{tau}.
  - No recommendation provided for combination of FDC and atazanavir as FDC will not be combined with other antiretroviral drugs.

- GS-US-216-112:DDI "cocktail" trial with desipramine, digoxin,or efavirenz coadministered with cobicistat:

  - Cobicistat increases the C_{max}, $AUC_{(0-last)}$, and $AUC_{(0-inf)}$ of desipramine, a sensitive CYP 2D6 substrate, by 24%, 58% and 65%, respectively. Based on the magnitude of increase in desipramine exposure, cobicistat is a weak inhibitor of CYP 2D6.

  - Cobicistat increases digoxin C_{max}, $AUC_{(0-last)}$, and $AUC_{(0-inf)}$ of digoxin, a P-gp substrate, by 41%, 20% and 8%, respectively. The

90% confidence interval for the increase in $AUC_{(0\text{-}inf)}$ of digoxin was within 80%-125%. Overall, cobicistat's inhibitory effects on P-gp are not clinically significant. However, for subjects concurrently receiving digoxin with antiretroviral HIV-1 regimens that include cobicistat, the potential for increased digoxin exposure should be considered when monitoring digoxin concentrations.

- o When all subjects were analyzed, cobicistat decreased the C_{max}, $AUC_{(0\text{-}last)}$, and $AUC_{(0\text{-}inf)}$ of efavirenz, a sensitive CYP 2B6 substrate, by 13%, 7%, and 7%. The 90% confidence intervals for the decreases in $AUC_{(0\text{-}last)}$ and $AUC_{(0\text{-}inf)}$ were within 80%-125%.

- GS-US-216-0106: DDI Trial with Oral Contraceptives (Ortho Tri-Cyclen® Lo)

  - The mean C_{max}, C_{tau}, and AUC_{tau} of NGMN (norgestromin; the primary and pharmacologically active metabolite of norgestimate, also known as 17-desacetyl norgestimate) increased by 108 %, 167 %, and 126 % after co-administration of NGM/EE with EVG/Cobi/FTC/TDF as compared with when NGM/EE was administered alone.
  - The mean C_{tau}, and AUC_{tau} of EE decreased by 43% and 25 % after co-administration of NGM/EE with EVG/Cobi/FTC/TDF as compared with when NGM/EE was administered alone. There was no significant change in the C_{max} of EE.

The clinical relevance of increased NGMN exposures and decreased EE exposures is further discussed in section 2.4.2.10.

Table 34 shows established and other potentially significant drug interactions based on which alteration in dose or regimen may be recommended. The "*" proceeding the name of the drug indicates that a drug-drug interaction trial was conducted.

Table 34: Established and other potentially significant drug interactions based on which alteration in dose or regimen may be recommended (* indicates that a drug-drug interaction trial was conducted)

Concomitant Drug Class: Drug Name	Effect on Concentration[b]	Clinical Comment
Acid Reducing Agents: Antacids* (for example aluminium and magnesium hydroxide)	↓ elvitegravir	Elvitegravir plasma concentrations are lower when STRIBILD is ⬚ (b) (4) with antacids. It is recommended to separate STRIBILD and antacid administration by at least 2 hours. Note: No dose adjustment is needed when STRIBILD is combined with either H₂ receptor

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		antagonists or proton pump inhibitors.
Antiarrhythmics: e.g: amiodarone bepridil disopyramide flecainide systemic lidocaine mexiletine propafenone quinidine digoxin*	↑ antiarrhythmics ↑ digoxin	Concentrations of these antiarrhythmic drugs may be increased when coadministered with STRIBILD. Caution is warranted and therapeutic concentration monitoring, if available, is recommended for antiarrhythmics when co-administered with STRIBILD. (b) (4)
Antibacterials: clarithromycin telithromycin	↑ clarithromycin ↑ telithromycin ↑ cobicistat	Concentrations of clarithromycin and/or cobicistat may be altered when clarithromycin is coadministered with STRIBILD. Patients with CLcr greater than or equal to 60 mL/min: No dose adjustment of clarithromycin is required . Patients with CLcr between 50 mL/min and 60 mL/min The dose of clarithromycin should be reduced by 50%. Concentrations of telithromycin and/or cobicistat may be increased when telithromycin is coadministered with STRIBILD.
Anticoagulants: warfarin	(b) (4)	Concentrations of warfarin may be (b) (4) upon coadministration with STRIBILD. It is recommended that the international normalized ratio (INR) be monitored upon coadministration with STRIBILD.
Anticonvulsants: e.g.: carbamazepine oxcarbazepine phenobarbital phenytoin clonazepam ethosuximide	 ↑ carbamazepine ↓ elvitegravir ↓ cobicistat ↑ clonazepam ↑ ethosuximide	Coadministration (b) (4) with STRIBILD may significantly decrease cobicistat and elvitegravir plasma concentrations, which may result in loss of therapeutic effect and development of resistance. Alternative anticonvulsants should be considered. Concentrations of clonazepam and ethosuximide may be increased when administered with STRIBILD. Clinical monitoring is recommended upon co-administration with STRIBILD.
Antidepressants: Selective Serotonin Reuptake Inhibitors	↑ SSRIs ↑ TCAs ↑ trazodone	Concentrations of these antidepressant agents may be increased when coadministered with STRIBILD. Careful dose titration of the

(SSRIs) e.g. Paroxetine Tricyclic Antidepressants (TCAs) e.g. amitryptyline desipramine imipramine nortryptaline buproprione trazodone		antidepressant and monitoring for antidepressant response is recommended,
Antifungals: itraconazole ketoconazole* voriconazole	↑ elvitegravir ↑ cobicistat ↑ itraconazole ↑ ketoconazole ↑voriconazole	Concentrations of ketoconazole, itraconazole and voriconazole may increase upon coadministration with STRIBILD. When administering with STRIBILD, the maximum daily dose of ketoconazole or itraconazole should not exceed 200 mg per day.
Anti-gout: colchicine	↑ colchicine	STRIBILD should not be coadministered with colchicine to patients with renal or hepatic impairment. Treatment of gout-flares – coadministration of colchicine in patients receiving STRIBILD: 0.6 mg (1 tablet) x 1 dose, followed by 0.3 mg (half tablet) 1 hour later. Treatment course to be repeated no earlier than 3 days. Prophylaxis of gout-flares – coadministration of colchicine in patients receiving STRIBILD: If the original regimen was 0.6 mg twice a day, the regimen should be adjusted to 0.3 mg once a day. If the original regimen was 0.6 mg once a day, the regimen should be adjusted to 0.3 mg once every other day. Treatment of familial Mediterranean fever – coadministration of colchicine in patients receiving STRIBILD: Maximum daily dose of 0.6 mg (may be given as 0.3 mg twice a day).
Antimycobacterial: rifabutin* rifapentine	↓ elvitegravir ↓ cobicistat	Coadministration of rifabutin and rifapentine with STRIBILD may significantly decrease elvitegravir and cobicistat plasma concentrations, which may result in loss of therapeutic effect and development of resistance. Coadministration of STRIBILD with rifabutin or rifapentine is not recommended.
Beta-Blockers: e.g.	↑ beta-blockers	Concentrations of beta-blockers may be increased when coadministered with STRIBILD. Clinical

		monitoring is recommended and a dose decrease of the beta blocker may be necessary when these agents are coadministered with STRIBILD.
metoprolol timolol		
Calcium Channel Blockers: e.g. amlodipine diltiazem felodipine nicardipine nifedipine verapamil	↑ calcium channel blockers	Concentrations of calcium channel blockers may be increased when coadministered with STRIBILD. Caution is warranted and clinical monitoring is recommended upon coadministration with STRIBILD.
Corticosteroid: Systemic: Dexamethasone	↓ elvitegravir ↓ cobicistat	Systemic dexamethasone, a CYP3A inducer, may significantly decrease elvitegravir and cobicistat plasma concentrations, which may result in loss of therapeutic effect and development of resistance.
Corticosteroid: Inhaled/Nasal: Fluticasone	↑ fluticasone	Concomitant use of inhaled fluticasone and STRIBILD may increase plasma concentrations of fluticasone, resulting in reduced serum cortisol concentrations. Alternative corticosteroids should be considered, particularly for long term use.
Endothelin Receptor Antagonists: bosentan	↑ bosentan	Coadministration of bosentan in patients on STRIBILD: In patients who have been receiving STRIBILD for at least 10 days, start bosentan at 62.5 mg once daily or every other day based upon individual tolerability. Coadministration of STRIBILD in patients on bosentan: Discontinue use of bosentan at least 36 hours prior to initiation of STRIBILD. After at least 10 days following the initiation of STRIBILD, resume bosentan at 62.5 mg once daily or every other day based upon individual tolerability.
HMG CoA Reductase Inhibitors: Atorvastatin (b) (4)	↑ HMG-CoA reductase inhibitors	Initiate with the lowest starting dose of _____ (b) (4) _____ and titrate carefully while monitoring for safety.
Hormonal Contraceptives: norgestimate/ethinyl estradiol	↑ norgestimate ↓ ethinyl estradiol	(b) (4)

		(b) (4)
Immuno-suppressants: e.g. cyclosporine ^{(b) (4)} sirolimus tacrolimus	↑ immunosuppressants	Concentrations of immunosuppressive agents may be increased when coadministered with STRIBILD. Therapeutic monitoring of the immunosuppressive agents is recommended upon coadministration with STRIBILD.
Inhaled Beta Agonist: salmeterol	↑ salmeterol	Coadministration of salmeterol and STRIBILD is not recommended. Coadministration of salmeterol with STRIBILD may result in increased risk of cardiovascular adverse events associated with salmeterol, including QT prolongation, palpitations, and sinus tachycardia.
Neuroleptics: e.g. perphenazine risperidone thioridazine	↑ neuroleptics	A decrease in dose of the neuroleptic may be needed when coadministered with STRIBILD.
Phosphodiesterase-5 (PDE5) Inhibitors: sildenafil tadalafil vardenafil	↑ PDE5 inhibitors	Co-administration with STRIBILD may result in an increase in PDE-5 inhibitor associated adverse events, including hypotension, syncope, visual disturbances, and priapism. Use of PDE-5 inhibitors for pulmonary arterial hypertension (PAH): Use of sildenafil is contraindicated when used for the treatment of pulmonary arterial hypertension (PAH) The following dose adjustments are recommended for the use of tadalafil with STRIBILD: Co-administration of tadalafil in patients on STRIBILD: In patients receiving STRIBILD for at least 1 week, start tadalafil at 20 mg once daily. Increase tadalafil dose to 40 mg once daily based upon individual tolerability. Co-administration of STRIBILD in patients on tadalafil: Avoid use of tadalafil during the initiation of STRIBILD. Stop tadalafil at least 24 hours prior to starting STRIBILD. After at least one week following initiation of STRIBILD, resume tadalafil at 20 mg once daily. Increase tadalafil dose to 40 mg once daily based upon individual tolerability.

		Use of PDE-5 inhibitors for erectile dysfunction: Sildenafil at a single dose not exceeding 25 mg in 48 hours, Vardenafil at a single dose not exceeding 2.5 mg in 72 hours, or Tadalafil at a single dose not exceeding 10 mg in 72 hours can be used with increased monitoring for PDE-5 inhibitor associated with adverse events.
Sedative/hypnotics: Benzodiazepines: e.g. Parenterally administered midazolam clorazepate diazepam estazolam flurazepam buspirone zolpidem	↑ sedatives/hypnotics	Concomitant use of parenteral midazolam with STRIBILD may increase plasma concentrations of midazolam. Co-administration should be done in a setting that ensures close clinical monitoring and appropriate medical management in case of respiratory depression and/or prolonged sedation. Dosage reduction for midazolam should be considered, especially if more than a single dose of midazolam is administered. Co-administration of oral midazolam with STRIBILD is CONTRAINDICATED. With other sedative/hypnotics, dose reduction may be necessary and (b) (4) monitoring is recommended.

a.   This table is not all inclusive.

b.   ↑ = increase, ↓ = decrease

*: Indicates that a drug-drug interaction trial was conducted.

As the majority of the clinical recommendations pertaining to concomitant use of FDC and non-antiretroviral drugs were based on mechanism based extrapolations, the validity of these extrapolations was evaluated during the review process.

<u>Drugs that are contraindicated with CYP3A inhibitors:</u>

The following drug classes (drugs within the class) are highly dependant on CYP3A for clearance, hence, increased concentration in the presence of CYP3A inhibitors are associated with serious and/or life threatening events:

- Alpha-1 adrenoreceptor antagonist (Alfuzosin)
- Ergot Derivatives (Dihydroergotamine, ergotamine, methylergonovine
- Neuroleptics (Pimozide)
- GI Motility Agents (Cisapride)
- HMG-CoA Reductase Inhibitors [Statins] (simvastatin and lovastatin)
- PDE-5 Inhibitors for treatment of pulmonary arterial hypertension: sildenafil.
- Sedatives/hypnotics: Orally administered Midazolam, Triazolam

The applicant has proposed that co-administration of FDC and the drugs listed

above be contraindicated. As Cobi is an inhibitor of CYP3A, concomitant administration of the drugs listed above with FDC can lead to significantly higher concentrations of the co-administered drug. Hence, clinical recommendations proposed by the applicant are acceptable.

In addition to the CYP3A substrates listed above, FDC and rifampin are contraindicated. Rifampin is a potent CYP3A inducer that may significantly decrease plasma concentrations of EVG and cobi and lead to loss of therapeutic activity.

<u>Drugs that are routinely monitored in the clinical setting and for which concentrations are expected to increase when combined with CYP3A inhibitors</u>

- Antiarrhythmics (Amiodarone, Bepridil, Digoxin, Disopyramide, Flecainide Systemic lidocaine, Mexiletine, Propafenone, Quinidine)
- Anticonvulsants (clonazepam and ethosuximide)
- Beta blockers (metoprolol, timolol)
- Calcium Channel Blockers (Amlodipine, Diltiazem, Felodipine, Nicardipine, Nifedipine, Verapamil)
- Immunosuppressants (cyclosporine,   (b) (4)sirolimus, tacrolimus)

The applicant has proposed a general recommendation of exercising caution and therapeutic concentration monitoring. The applicant's proposal (with incorporation of suggested edits to the prescribing information section) is acceptable because Cobi is expected to increase the plasma concentration of all the drugs listed above (due to CYP3A inhibition by Cobi) and plasma concentrations of the drugs listed above are monitored in the clinical setting.

<u>Drugs that are NOT monitored in the clinical setting and for which concentrations are expected to increase when combined with CYP3A inhibitors</u>

- Inhaled beta agonist (Salmeterol)- The applicant indicates co-administration of salmeterol and STRIBILD is not recommended due to increased plasma concentration of salemeterol, which is associated with the potential for serious and/or life threatening reactions. Co-administration of salmeterol and STRIBILD is not recommended. The applicant's proposal is acceptable. Please refer to the labeling recommendation section for suggested edits to the prescribing information.
- Fluticasone: The applicant proposes that co-administration of fluticasone propionate and STRIBILD is not recommended unless the potential benefit to the patient outweighs the risks of systemic corticosteroid side effects. Fluticasone is a CYP3A substrate and the plasma concentration of fluticasone is expected to increase when concomitantly administered with FDC. In general, the applicant's proposed recommendation is acceptable. Please refer to the labeling recommendation section for suggested edits to

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the prescribing information.

Drugs that may decrease the concentration of EVG and Cobi due to CYP3A induction

- Anticonvulsants (carbamazepine, oxcarbazepine, phenobarbital, phenytoin)
- Antimycobacterial (rifabutin, rifapentine)
- Dexamethasone
- Herbal Products (St. John's wort [hypericum perforatum]-(will be contraindicated because St. John's wort can decrease EVG and Cobi concentrations due to CYP3A induction).

The applicant proposes that co-administration of FDC and the drugs listed above can lead to significant decrease in the concentrations of EVG and Cobi which may result in loss of therapeutic effect and development of resistance. Further, in some cases (for example anticonvulsants and modafinil), the clinical recommendation suggests considering alternatives.

In general, the applicant's proposed recommendations are acceptable. Please refer to the labeling recommendation section for suggested edits to the prescribing information.

Drugs for which dose adjustment is recommended when combined with strong CYP3A inhibitors

- Anti-gout (colchicine)
- Statins (Atorvastatin)
- Trazodone
- Neuroleptics (perphenazine, risperidone, thioridazine)
- Sildenafil and Tadalafil

All the drugs listed above are substrates of CYP3A, hence, the plasma concentrations are expected to increase when co-administered with FDC. The applicant's proposed recommendations regarding dose adjustment/reduction are similar to the clinical recommendations when the drugs are co-administered with ritonavir, a CYP3A inhibitor. The applicant's proposed recommendations are acceptable. Please refer to the labeling recommendation section for suggested edits to the prescribing information.

Additional Drug-Drug Interactions:

- Clarithromycin: The applicant proposes (b)(4)

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Because STRIBILD will be discontinued in patients with CLcr less than 50 mL/min, the following recommendation will be included in the prescribing information: No dose adjustment of clarithromycin is required in patients with CLcr greater than 60 ml/min. In patients with CLcr between 50 mL/min and 60 mL/min, the dose of clarithromycin should be reduced by 50 %.

- Warfarin: Warfarin is a racemic mixture of S-warfarin (primarily metabolized by CYP2C9) and R- warfarin (primarily metabolized by CYP1A2 and CYP3A4). The results of *in vitro* studies suggest that Cobi is not expected to significantly alter the activity of CYP2C9 (and hence the concentration of S-warfarin). On the other hand, the concentration of R-warfarin is expected to increase because of CYP3A inhibition by Cobi. The applicant proposes that "concentrations of warfarin may be affected upon co-administration with STRIBILD. It is recommended that international normalized ratio (INR) be monitored upon co-administration with STRIBILD". The applicant's proposal is acceptable because INR ratio is routinely monitored to guide warfarin therapy. Please refer to the labeling recommendation section for suggested edits to the applicant's proposed wording.

- Antifungal Agents

  o Ketoconazole is a CYP3A substrate, hence the plasma concentrations of ketoconazole are expected to increase when given concomitantly with FDC. The applicant's proposal to limit the maximum daily dose of ketoconazole to 200 mg is similar to the clinical recommendation when ketoconazole is combined with other CYP3A inhibitors such as ritonavir and boosted protease inhibitors.

  o Itraconazole is a CYP3A substrate, hence the plasma concentration of itraconazole is expected to increase when given concomitantly with FDC. The applicant has proposed ⟨(b) (4)⟩ when itraconazole is combined with STRIBILD. The applicant's proposal is different from the clinical recommendation when itraconazole is combined with other CYP3A inhibitors such as ritonavir and boosted protease inhibitors (clinical recommendation when itraconazole is combined with other CYP3A inhibitors such as ritonavir and boosted protease inhibitors is to limit the maximum daily dose of itraconazole to 200 mg). Please refer to the labeling recommendation section for suggested edits to the applicant's proposed wording.

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Table 35 shows the effect of co-administered drug on the pharmacokinetics of EVG (based on studies submitted in the NDA; see recommendations in table 33)

Table 35: Effect of co-administered drug on the pharmacokinetics of EVG (based on studies submitted in the NDA; see recommendations in table 33)

Co-administered Drug	Dose of Co-administered Drug (mg)	Elvitegravir Dose (mg)	Cobicistat or Ritonavir Booster Dose	N	% Change of Elvitegravir Pharmacokinetic Parameters[b] (90% CI)		
					C_{max}	AUC	C_{min}
Antacids	20 mL single dose ± 2 hours or ± 4 hours from elvitegravir administration	50 single dose	Ritonavir 100 single dose	39	⇔	⇔	⇔
Ketoconazole	200 twice daily	150 once daily	Ritonavir 100 once daily	18	⇔	↑48 (↑36 to ↑62)	↑67 (↑48 to ↑88)
Omeprazole	40 mg once daily 2 hours before elvitegravir administration x 5 days	50 once daily x 5 days	Ritonavir 100 once daily x 5 days	9	⇔	⇔	⇔
Rifabutin	150 once every other day	150 once daily	Cobicistat 150 once daily	12	⇔	↓21 (↓26 to ↓15)	↓67 (↓73 to ↓60)
Rosuvastatin	10 single dose	150 once daily	Cobicistat 150 once daily	10	⇔	⇔	⇔

a. All interaction studies conducted in healthy volunteers

b. ↑ = Increase; ↓ = Decrease; ⇔ = No Effect; NA = Not Applicable

Table 36A shows the effect of FDC (or EVG and Cobi given together) on the pharmacokinetics of the co-administered drug (based on studies submitted in the NDA; see recommendations in table 33) and Table 36B hows the effect of cobicistat on the pharmacokinetics of the co-administered drug. (based on a trial submitted in the NDA; see recommendations in table 33)

Table 36A: Effect of FDC (or EVG and Cobi given together) on the pharmacokinetics of the co-administered drug[a] (based on studies submitted in the NDA; see recommendations in table 33)

Co-administered Drug	Dose of Co-administered Drug (mg)	Elvitegravir Dose (mg)	Cobicistat or Ritonavir Booster Dose	N	% Change of Co-administered Drug Pharmacokinetic Parameters[b] (90% CI)		
					C_{max}	AUC	C_{min}
Norgestimate/ ethinyl estradiol	0.180/0.215/0.250 norgestimate once daily	150 once daily[c]	Cobicistat 150 once daily[c]	13	↑108 (↑100 to ↑117)	↑126 (↑115 to ↑137)	↑167 (↑143 to ↑192)
	0.025 ethinyl estradiol once daily				⇔	↓25 (↓31 to ↓19)	↓44 (↓48 to ↓39)
Rifabutin	150 once every other day	150 once daily	Cobicistat 150 once daily	12	⇔	⇔	⇔
25-O-desacetyl-rifabutin				12	↑384 (↑309 to ↑474)	↑525 (↑408 to ↑669)	↑394 (↑304 to ↑504)
Rosuvastatin	10 single dose	150 single dose	Cobicistat 150 single dose	10	↑89 (↑48 to ↑142)	↑38 (↑13 to ↑67)	N/A
a. All interaction studies conducted in healthy volunteers							
b. ↑ = Increase; ↓ = Decrease; ⇔ = No Effect; NA = Not Applicable							
c. Study conducted with STRIBILD							

Table 36B: Effect of cobicistat on the pharmacokinetics of the co-administered drug[a, b] (see recommendations in table 33)

Co-administered Drug	Dose of Co-administered Drug	Cobicistat Dose	N	% Change of Co-administered Drug Pharmacokinetic Parameters[c] (90% CI)		
				C_{max}	AUC	C_{min}
Desipramine	50 mg single dose	Cobicistat 150 mg once daily	8	↑24 (↑8 to ↑44)	↑65 (↑36 to ↑102)	NA
Digoxin	0.5 mg single dose	Cobicistat 150 mg once daily	22	↑41 (↑29 to ↑55)	↑8 (↑0 to ↑17)	NA
a. Trial conducted in healthy volunteers (GS-US-216-112)						
b. The efavirenz pharmacokinetic data will not be included in the STRIBILD prescribing information and is not displayed						
c. ↑ = Increase; ↓ = Decrease; NA = Not Applicable						

2.4.2.9   Is there a known mechanistic basis for pharmacodynamic drug-drug interactions, if any?

There are no pharmacodynamic drug-drug interactions for the FDC tablet.

2.4.2.10   Are there any unresolved questions related to metabolism, active metabolites, metabolic drug interactions, or protein binding?

Unresolved Issue # 1

- The safety and efficacy of the concomitant use of FDC and combined oral contraceptive (COC) is insufficiently characterized.   The applicant conducted a drug-drug interaction trial to assess the effect of FDC on the pharmacokinetics of individual components (norgestimate [NGM] and ethinyl estradiol [EE]) of a representative hormonal contraceptive medication, Ortho Tri-Cyclen® Lo (GS-US-236-0106).   The results of the trial showed that:

  - The mean C_{max}, C_{tau}, and AUC_{tau} of norgestromin (NGMN) increased by 108 %, 167 %, and 126 % after co-administration of NGM/EE with EVG/COBI/FTC/TDF as compared with when NGM/EE was administered alone.
  - The mean C_{tau}, and AUC_{tau} of EE decreased by 43% and 25 % after co-administration of NGM/EE with EVG/COBI/FTC/TDF as compared with when NGM/EE was administered alone.   There was no significant change (< 10 %) in the C_{max} of EE.

The clinical significance of the increased NGMN exposures and the benefit-vs-risk assessment of using FDC with oral contraceptives in general was discussed with the Division of Reproductive and Urology Products (DRUP). The response to the formal consult request and follow up discussions indicate that there is a potential risk of rare thromboembolic events which may occur due to higher NGMN exposures; however, the rarity of these thromboembolic events makes it challenging to quantify the risk. On the other hand, Ortho Tricyclen Lo is a widely used oral contraceptive and is a convenient option for contraception. Further, the results of the trial indicate that the efficacy of Ortho Tricyclen Lo is not expected to decrease when given with FDC because the progestin component is considered more important (than the estrogen component) for contraceptive efficacy. Based on the aforementioned considerations, it was determined that a) describing the PK changes of NGMN and EE in the package insert, b) indicating that DDIs between FDC and oral contraceptives containing other progestins has not been conducted and cannot be predicted based on the available data, c) not recommending an increase in the dose of EE to 35 μg   (b) (4)
         and d) suggesting healthcare professionals may consult with experts in

the area of pregnancy and women's health concerns before co-administration of FDC and Ortho Tricyclen Lo will convey sufficient benefit-to-risk information regarding use of FDC and Ortho Tri Cyclen® Lo.

Unresolved Issue # 2

Drug-drug interaction information related to the effect of FDC on methadone and buprenorphine/naloxone is currently unavailable (trial is ongoing). Although methadone is partially metabolized by CYP3A4 enzymes, previous drug-drug interaction trials with ritonavir-boosted PIs have shown an unexpected decrease in methadone exposures. Buprenorphine/naloxone are substrates of CYP3A4 and Cobi is a CYP3A4 inhibitor, therefore, there is potential for prolonged therapeutic/adverse effects of buprenorphine/naloxone when administered with FDC.

Unresolved Issue # 3

The applicant did not perform a drug-drug interaction trial to assess the effect of FDC on anti HCV direct acting antivirals [   ] (b) (4) and telaprevir. Both [   ] (b) (4) and telaprevir are metabolized by CYP3A enzymes, hence, there is a potential for increased concentrations of [   ] (b) (4) and telaprevir when either drug is co-administered with FDC.

2.4.3   What issues related to dose, dosing regimens, or administration are unresolved and represent significant omissions?

The proposed dosing regimen of FDC (150/150/200/300 mg administered once daily with food) is appropriate.

2.5   General Biopharmaceutics

2.5.1   Based on the biopharmaceutics classification system (BCS) principles, in what class is this drug and formulation? What solubility, permeability, and dissolution data support this classification?

Please refer to ONDQA review.

2.5.2   What is the relative bioavailability of the proposed to-be-marketed formulation to the pivotal clinical trial?

The applicant used the to-be-marketed formulation in the clinical trials. Therefore, no relative bioavailability study was needed.

2.5.2.1   What data support or do not support a waiver of in vivo BE data?

Not applicable

2.5.3   What is the effect of food on the bioavailability (BA) of the drug from the dosage form? What dosing recommendation should be made, if any, regarding administration of the product in relation to meals or meal types?

The effect of food on the pharmacokinetics of EVG, Cobi, FTC, and TDF was evaluated in trial GS-US-236-0105. The trial was designed to evaluate the effect of food (high-calorie/high-fat meal or light meal) on the individual components of the fixed dose combination tablet (EVG/Cobi/FTC/TDF).

Subjects were equally randomized to receive the following three treatments:

Treatment A: Single oral FDC tablet administered in the morning under **fasted conditions**.

Treatment B: Single oral FDC tablet administered in the morning under fed conditions **(light meal; 373 kcal, 20 % fat [8.29gms])**.

Treatment C: Single oral FDC tablet administered in the morning under fed conditions **(high calorie, high fat meal; 800 kcal, 50 % fat [44.4 gms])**.

Table 37 shows the statistical comparison of the mean pharmacokinetic parameters of EVG after single dose administration of the FDC tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal.

Table 37: Statistical comparison of the mean pharmacokinetic parameters of EVG after single dose administration of the FDC tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal

Treatment Condition N=24	EVG PK Parameters		
	C_{max} (ng/mL)	AUC_{inf} (ng•h/mL)	AUC_{last} (ng•h/mL)
HC/HF Meal GLSM	2151.74	28159.64	27338.54
Light Meal GLSM	1678.32	20269.50	19505.47
Fasted GLSM	1376.72	15095.22	14298.38
HC/HF Meal vs. Fasted GLSM ratio (90% CI), %	156.29 (138.44, 176.45)	186.55 (165.56, 210.20)	191.20 (169.56, 215.61)
Light Meal vs. Fasted GLSM ratio (90% CI), %	121.91 (107.98, 137.63)	134.28 (119.17, 151.30)	136.42 (120.97, 153.83)
HC/HF Meal vs. Light Meal GLSM ratio (90% CI), %	128.21 (113.56, 144.74)	138.93 (123.29, 156.54)	140.16 (124.29, 158.05)

CI=confidence interval, GLSM=geometric least-squares mean, HC=high calorie, HF=high fat

GLSMs were obtained using a mixed-effects model.

Table 38 shows the statistical comparison of the mean pharmacokinetic parameters of Cobi after single dose administration of the FDC tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal.

Table 38: Statistical comparison of the mean pharmacokinetic parameters of Cobi after single dose administration of the FDC tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal

Treatment Condition N=24	GS-9350 PK Parameters[a]		
	C_{max} (ng/mL)	AUC_{inf} (ng•h/mL)	AUC_{last} (ng•h/mL)
HC/HF Meal GLSM	845.51	5816.23	5727.63
Light Meal GLSM	1156.80	7207.17	7138.74
Fasted GLSM	1117.47	7020.03	6951.03
HC/HF Meal vs. Fasted GLSM ratio (90% CI), %	75.66 (68.44, 83.64)	82.85 (72.52, 94.65)	82.40 (71.92, 94.40)
Light Meal vs. Fasted GLSM ratio (90% CI), %	103.52 (93.64, 114.44)	102.67 (89.87, 117.29)	102.70 (89.64, 117.66)
HC/HF Meal vs. Light Meal GLSM ratio (90% CI), %	73.09 (66.12, 80.80)	80.70 (70.64, 92.19)	80.23 (70.03, 91.92)

CI=confidence interval, GLSM=geometric least squares mean, HC=high calorie, HF=high fat

GLSMs were obtained using a mixed-effects model.

FTC:

There was no significant change (all changes < 10 %) in either the C_{max} or AUC_{inf} of FTC under light meal- and high fat conditions as compared with fasting conditions.

TDF:

The mean C_{max} and AUC_{inf} of tenofovir increased by 20 % and 24 %, respectively, under light meal conditions as compared with fasting conditions. Under high fat conditions, the mean mean C_{max} of tenofovir was not significantly changed (< 10 %); however, the mean AUC_{inf} of tenofovir was increased by 23 %, respectively.

Recommendation: FDC tablets should be taken with food due to the increase in systemic exposure of EVG. The increase in systemic exposure (AUC_{inf}) of EVG and decrease in systemic exposure of Cobi when FDC was administered with a high fat meal (800kcal) as compared with when FDC was administered with a light meal (373 kcal) is not expected to be clinically relevant.

Of note, the FDC tablet was given with food in pivotal Phase III trials in which the safety and efficacy of the FDC tablets were determined.

2.5.4   When would a fed BE study be appropriate and was one conducted?

This question is not applicable to the FDC NDA submission.

2.6   Analytical section

2.6.1   How are the active moieties identified and measured in the plasma in the clinical pharmacology and biopharmaceutics studies?

The active moieties were identified in the plasma using validated LC/MS/MS methods.

2.6.2   Which metabolites have been selected for analysis and why?

The applicant did not monitor the metabolites except in the mass balance trial of EVG and Cobi.

2.6.3   For all moieties measured, is free, bound, or total measured? What is the basis for that decision, if any, and is it appropriate?

The analytical methods measured the total concentration of the individual components. It is appropriate to measure total concentrations of the individual components.

2.6.4   What bioanalytical methods are used to assess concentrations?

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Please refer to individual trial reviews for detailed bioanalytical information. Overall, the bioanalytical methods were found to be acceptable.

17 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

4. APPENDICES

4.1 INDIVIDUAL TRIAL REVIEWS

In Vivo Trials of Elvitegravir

Trial GS-US-183-0101

A Double-Blind, Randomized, Placebo-controlled Phase 1/2 Study of the Safety, Pharmacokinetics, and Antiviral Activity of GS-9137 Following Oral Administration in Subjects Infected with HIV-1

Trial Period
1 June 2005 to 19 December 2005
Final report date: 29 June 2006 (submitted to IND (b) (4))

Trial Sites
Orlando Immunology Center, Florida, USA (lead investigator)
Community Research Initiative of New England, Boston, Massachusetts, USA
NorthStar Medical Center, Chicago, Illinois, USA
The Rockefeller University Hospital, New York, New York, USA
Southwest C.A.R.E., Santa Fe, New Mexico, USA
C.A.R.E.-ID, Washington, District of Columbia, USA
AHF Research Center, Beverly Hills and Los Angeles, California, USA

Trial Rationale
GS-9137 (elvitegravir, EVG) is an inhibitor of the human immunodeficiency virus (HIV) integrase, currently under development for the treatment of HIV infection. Results from *in vitro* studies have demonstrated potent anti-HIV activity, including activity against viruses that are resistant to nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PI). Ritonavir (RTV) is an HIV PI indicated for use in combination with other antiretroviral drugs for the treatment of HIV infection. Coadministration of antiretroviral drugs that are cytochrome P450 (CYP) isoform 3A substrates with the potent CYP3A inhibitor RTV increases the systemic exposure of the substrate drugs. This was a proof-of-concept trial to assess the safety, tolerability, and antiviral activity, as well as the plasma and peripheral blood mononuclear cell (PBMC) pharmacokinetics, of GS-9137. A goal of this trial was to elucidate the GS-9137 dose-response relationship by varying the dose, dosing interval, and concurrent dosing of RTV, in order to optimize the dose and dosing regimen for future Phase 2 studies.

Trial Objectives
The primary objectives of the trial were to:

- investigate the safety, tolerability, and antiviral activity of GS-9137 administered as 10 consecutive daily oral doses (twice-daily for Cohorts 1, 2, and 4; once-daily for Cohorts 3 and 5) in subjects chronically infected with HIV-1 and not currently receiving antiretroviral therapy
- investigate the pharmacokinetics/pharmacodynamics of GS-9137

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Trial Design

This was a double-blind, sequential-cohort, dose-ranging study of orally administered GS-9137 as 50 or 200 mg tablets, or matching placebo tablets, for 10 days. The safety, tolerability, pharmacokinetics, and anti-HIV activity of GS-9137 were evaluated over 21 days. The study schema is shown in Figure 1.

Figure 1: Study schema (source: Study Report Figure 5-1)
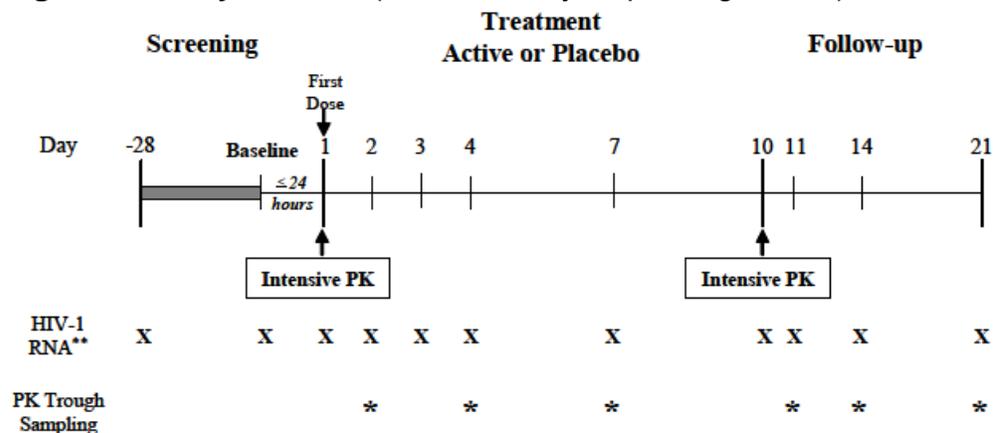
** Baseline and post-baseline HIV-1 RNA levels were not reported back to investigational centers until the study completion.

Five cohorts with eight subjects each (6 active and 2 placebo) were enrolled in this trial. The active treatments for each cohort are listed below:

Cohort 1: GS-9137 400 mg BID
Cohort 2: GS-9137 800 mg BID
Cohort 3: GS-9137 800 mg QD
Cohort 4: GS-9137 200 mg BID
Cohort 5: GS-9137/r 50/100 mg QD

The safety assessments for Cohort 1 (10 days after the last study drug administration) were to be complete before the dose was escalated in Cohort 2. Concurrent dosing of Cohorts 2 through 5 was permitted. If two or more subjects receiving GS-9137 in any cohort discontinued study drug due to treatment-related adverse events, enrollment in that cohort and cohorts with higher doses were suspended pending additional safety assessments.

Rationale for Dose Selection

The starting dose of 400 mg BID (800 mg/day) was selected based on nonclinical studies performed in rats and dogs (the rat and dog NOAELs exceeded the human starting dose by at least 10x), as well as on Phase 1 studies in human subjects (XAX1-1 and XAX1-2) conducted by Japan Tobacco Company (single doses of 800 mg GS-9137 was well-tolerated in healthy adult males).

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Dosing of GS-9137 was limited to 10 days in order to reduce the risk of the development of viral resistance during anti-HIV monotherapy.

Investigational Product
GS-9137 50 mg (Lot 071-4) and 200 mg (Lot 070-4) tablets were administered during active treatment periods. Matching placebo tablets (Lot 072-4) were identical in appearance to GS-9137 50 or 200 mg strength tablets and consisted of the inactive ingredients in the GS-9137 tablets. Ritonavir (NORVIR®, Abbott Laboratories, Lot 247022E21) 100 mg soft gelatin capsules were administered to Cohort 5.

Drug Administration
Subjects were instructed to take the study drug with food at 8:00 AM (all cohorts) and at 8:00 PM (Cohorts 1, 2, and 4). When subjects were at the clinic (Days 1, 2, 3, 4, 7, and 10), study drug was administered with 240 mL of water within 5 minutes of consuming a meal provided by the clinic. On Days 1 and 10 (during which intensive PK sampling was conducted), subjects fasted from midnight until study drug administration, water intake was limited to an additional 240 mL from two hours before until two hours after dosing, and subjects were instructed not to eat until four hours after dosing. Study sites were requested to provide meals of at least 550 calories on the mornings of Days 1 and 10 and meals of at least 350 calories at all other dosing times.

Dosing that was not observed by study staff was assessed by daily follow-up phone calls to study subjects on Days 2 through 9.

Key Inclusion and Exclusion Criteria
Subjects were HIV-1-infected males and nonpregnant females with plasma HIV-1 RNA levels between 10,000 and 300,000 (inclusive) copies/mL and CD4+ cell counts of 200 cells/mm^3 or higher. Subjects were between the ages of 18 and 60 years, inclusive, weighed 50 kg or more, and had a normal QT$_c$ interval.

Potential subjects were excluded from enrollment if they had prior treatment with any antiretroviral therapy within 90 days of baseline, or if they had any prior treatment with an antiretroviral integrase inhibitor. Potential subjects were also excluded if they received an immunization within 30 days of baseline, or if they were diagnosed with a new AIDS-defining condition within 30 days of baseline. Potential subjects were also excluded if they had a serious active infection that required parenteral antibiotic or antifungal activity within 30 days or baseline, or if they were participating in any other clinical trial.

Concomitant Medications
The following medications were disallowed while subjects were participating in the study:

- any medication with anti-HIV-1 activity, with the exception of RTV for Cohort 5 only
- ongoing therapy with any of the following medications: bepridil, amiodarone, flecainide, quinidine, simvastatin, lovastatin, rifampin, rifapentine, astemizole, terfenadine, cisapride, pimozide, midazolam, triazolam, dihydroergotamine, sildenafil, ergonovine, tadalafil, vardenafil, methylergonovine, ergotamine, St. John's Wort, systemic chemotherapeutic agents, systemic corticosteroids, interleukin-2, methadone/opiates, rifabutin, propafenone, fluticasone, or voriconazole
- other investigational agents
- certain citrus fruits and citrus fruit products (from 1 week prior to Day 1 until study completion)
- antacids that contain calcium, magnesium, or aluminum; sucralfate; or vitamin or mineral supplements that contain calcium, iron, or zinc (from at least 6 hours before until at least 6 hours after study drug administration)

Sample Collection

Blood was collected for pharmacokinetic analysis of GS-9137 in plasma at each of the following timepoints (in hours after dosing):

Days 1 and 10	0:00 (predose), 0:30, 1:00, 1:30, 2:00, 3:00, 4:00, 6:00, 8:00, 10:00, and 12:00 [and 24:00 for once-daily Cohorts 3 and 5]
Days 2, 4, 7, 11, 14, and 21	0:00 (predose)

PBMC samples were collected for intracellular pharmacokinetic analysis at the following timepoints (in hours after dosing):

Days 1 and 10	0:00 (predose), 2:00, 6:00, and 12:00
Days 2, 4, 7, 11, 14, and 21	0:00 (predose)

To assess efficacy, the following measurements were collected at baseline (Day 0) and on the specified days (pre-dose, if study treatment was administered):

Plasma HIV-1 RNA	Days 1, 2, 3, 4, 7, 10, 11, 14, and 21
CD4+/CD8+ count	Days 2, 4, 7, 11, 14, and 21

Analytical Plan

Pharmacokinetic data

The pharmacokinetic parameters C_{max}, T_{max}, C_{last}, T_{last}, C_{tau}, λ_z, $t_{1/2}$, AUC_{0-last}, $AUC_{0-\infty}$, %AUC_{exp}, AUC_{tau}, CL/F, and V_z/F were estimated for GS-9137 in plasma. These parameters were estimated using a nonlinear model derived using standard noncompartmental methods (WinNonlin® Professional Edition, Pharsight Corporation, Mountain View, California, USA). Pharmacokinetic

parameters that depend on an accurate estimation of the terminal elimination phase ($AUC_{0-\infty}$, CL/F, V_z/F, λ_z, $t_{1/2}$) were reported when %AUC_{exp} is <30% or if the pharmacokineticist deemed the data evaluable after examination.

The time to reach GS-9137 steady-state in plasma was evaluated using SAS® PROC MIXED (SAS Institute, Cary, North Carolina, USA) for each GS-9137 dose level. An evaluation of the dose-proportionality of GS-9137 pharmacokinetics was also performed. In addition, the geometric mean ratios (GMRs) comparing the $AUC_{0-\infty}$ and C_{max} values estimated on Day 1 and Day 10 were calculated in the context of an analysis of variance (ANOVA) model.

GS-9137 concentrations in PBMCs were normalized to cell counts. Similarly to plasma concentration analyses, the GMRs for PK parameters on Days 1 and 10 were calculated. GS-9137 concentrations that were below the limit of quantitation (BLQ) were assigned a value of zero.

Pharmacodynamic data
The correlation between GS-9137 pharmacokinetic parameters on Day 10 (C_{max}, AUC_{tau}, and C_{tau}) and dose level and maximum reduction in plasma HIV-1 RNA were examined for significance.

Trial Results
Bioanalytical methods
Concentrations of GS-9137 in plasma samples were measured by LC-MS/MS by Gilead Sciences [(b) (4)] ([(b) (4)] Report S-183-04). Frozen plasma samples were received between 17 Aug and 29 Dec 2005 and stored at -80°C. Analysis was performed between 26 Aug 2005 and 12 Jan 2006. The first day of sample collection was 21 June 2005, so the maximum storage sample time was 205 days, which is longer than the validated long-term frozen stability duration of 90 days for GS-9137 (this potential discrepancy is acceptable since the results of this trial will not be included in the product labeling). The LC-MS/MS method M-GS-9137-16407 (Versions 1-4) was used.

The calibration standards for GS-9137 ranged from 1.0-1000 ng/mL and the quality control (QC) concentrations were 1.0, 3.0, 250, and 1000 ng/mL. All inter-assay accuracy and precision estimates (% CV, displayed in Table 1) were within the acceptable range (≤20% deviation from nominal at the LLoQ concentration, and ≤15% from nominal at all other concentrations).

Table 1: Bioanalytical assay validation for GS-9137 in human plasma
(source: Study Report Table 5-3)

	GS-9137 in Plasma
Linear Range (ng/mL)	1.0 to 1000.0
LLQ (ng/mL)	1.0
Inter-Assay Precision Range[a]	4.6 to 20
Inter-Assay Accuracy Range[b]	−3.3 to 0.8
Stability in Frozen Matrix (days)	90

a   Relative standard deviation
b   Difference from nominal concentrations
LLQ = lower limit of quantitation
Source: Appendix 15

Concentrations of GS-9137 in PBMC samples were measured by LC-MS/MS by Gilead Sciences   (b) (4)   This objective was considered exploratory and a non-validated, research-grade assay (M-GS-9137-16484 Versions 1 and 2) was used. Most of the PBMC samples were received as PBMC pellets in 50 mL conical tubes (with approximately 100-200 uL of residual liquid). Analysis was performed between 8 Mar and 30 Mar 2006. The calibration standards for GS-9137 in PBMCs ranged from 0.6-300 ng/mL and the QC concentrations were 1.8, 50.0, and 240 ng/mL. Inter-assay precision and accuracy statistics are not available because the assay was not evaluated for performance over multiple assay runs. Data were reported as pg/10^6 cells.

Trial population

A total of 48 HIV-infected subjects were randomized on Day 1 of the study. Forty subjects received study drug, and all 40 subjects completed the study. The eight subjects who discontinued the study prematurely never received study drug. Reasons for discontinuation were withdrawal of consent (n=5), diagnosis of syphilis and treatment with a parenteral antibiotic at the baseline visit (n=1), prolonged QTc interval at baseline (n=1), and consumption of calcium-fortified orange juice within 1 week of baseline (n=1). The efficacy and safety analysis sets contained the 40 subjects who were dosed with placebo or GS-9137. The Day 1 PK analysis set contained 28 of the 30 subjects who receieved GS-9137: Subjects 1101 (high outlier) and 1301 (dosed incorrectly on Day 1) were excluded. The Day 10 PK analysis set contained all 30 subjects who received GS-9137.

The majority of subjects in the safety analysis set were white and male (88 and 93%, respectively). Median HIV RNA was 4.76 $\log_{10}$ copies/mL and median CD4+ cell count was 383 cells/uL. Other baseline demographic and disease characteristics were similar between the treatment groups. Most subjects (63% overall; 45% with an NNRTI-based regimen and 38% with a PI-based regimen) had been treated with antiretroviral drugs before they entered the study.

Results of efficacy analyses

The primary efficacy endpoint in this study was the maximum reduction from baseline in HIV-1 RNA between Days 2 and 11. The mean change in HIV-1 RNA is shown by treatment group in Figure 2. HIV-1 RNA levels declined in all GS-9137 treatment groups during GS-9137 administration (Days 1-10) and reached a nadir on Days 10-11 for all subjects except for Subject 14, who reached a nadir on Day 14. The maximum reduction in HIV-1 RNA at all GS-9137 dose levels was significantly greater than placebo based on a pairwise comparison using a two-sided Wilcoxon rank sum test (p=0.0007).

Figure 2: Mean change in HIV-1 RNA through Day 21 (efficacy analysis set; mean ± SD; semilog scale; source: Study Report Figure 7-1)
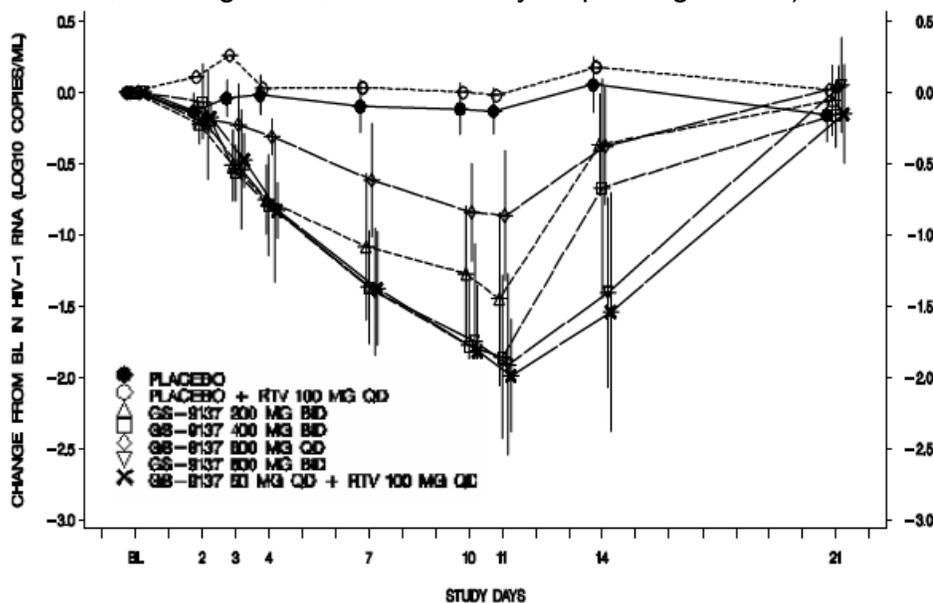
Source: Section 11.1, Figure 2.2

A summary of the antiviral activity of GS-9137 in various dosing regimens is displayed in Table 2. The greatest mean decline in HIV-1 RNA was observed in the GS-9137/r 50/100 mg QD treatment group. Subjects in the GS-9137 200 mg BID, 400 mg BID, 800 mg QD, and 800 mg BID treatment groups experienced similar declines in HIV-1 RNA.

Table 2: Antiviral activity of GS-9137 (efficacy analysis set; source: Study Report Table 7-1)

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Parameter	Placebo QD or BID (N = 8)	Placebo + 100 mg ritonavir QD (N = 2)	GS-9137 200 mg BID (N = 6)	GS-9137 400 mg BID (N = 6)	GS-9137 800 mg QD (N = 6)	GS-9137 800 mg BID (N = 6)	GS-9137 50 mg + 100 mg ritonavir QD (N = 6)
Maximum Reduction in HIV-1 RNA ($\log_{10}$ copies/mL)[a]							
Mean (SD)	−0.25 (0.15)	−0.05 (0.14)	−1.48 (0.55)[b]	−1.94 (0.52)[b]	−0.98 (0.37)[b,c]	−1.91 (0.60)[b]	−1.99 (0.38)[b,d]
Median (max, min)	−0.26 (−0.48, 0.01)	−0.05 (−0.15, 0.05)	−1.48 (−2.10, −0.87)	−2.03 (−2.44, −1.04)	−0.96 (−1.41, −0.56)	−1.78 (−2.67, −1.27)	−2.03 (−2.38, −1.54)
DAVG in HIV-1 RNA Baseline to Week 2 ($\log_{10}$ copies/mL)							
Mean (SD)	−0.09 (0.13)	0.05 (0.13)	−0.96 (0.33)[b]	−1.20 (0.29)[b]	−0.55 (0.22)[e]	−1.19 (0.49)[b]	−1.22 (0.27)[b]
Median (max, min)	−0.09 (−0.28, 0.14)	0.05 (−0.04, 0.14)	−1.02 (−1.32, −0.50)	−1.26 (−1.47, −0.69)	−0.56 (−0.89, −0.25)	−1.04 (−1.90, −0.67)	−1.22 (−1.53, −0.91)
Subjects Achieving HIV-1 RNA < 50 copies/mL After Baseline							
n (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (33%)	0 (0%)
Subjects Achieving HIV-1 RNA < 400 copies/mL After Baseline							
n (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (50%)	2 (33%)

a   The maximum reduction in HIV-1 RNA was defined as the maximum decrease from baseline in $\log_{10}$ copies/mL between Days 2 and 11. HIV-1 RNA values below the limit of quantitation (50 copies/mL) were entered as 49 copies/mL.

b   p = 0.0007 for the dose level versus placebo based on pairwise p-values for continuous data calculated by a 2-sided Wilcoxon rank sum exact test.

c   p = 0.0152 for the 800 mg of GS-9137 once-daily dose level versus 800 mg of GS-9137 twice-daily and versus 400 mg of GS-9137 twice-daily based on pairwise p-values for continuous data calculated by a 2-sided Wilcoxon rank sum exact test.

d   The 50 mg of GS-9137 + ritonavir once-daily dose level versus placebo + ritonavir, p = 0.0714; versus 200 mg twice-daily, p = 0.1797; versus 400 mg twice-daily, p = 0.9372; versus 800 mg once-daily, p = 0.0022; versus 800 mg twice-daily, p = 0.8182.

e   At the 800 mg once-daily dose level, p = 0.0027 versus placebo at Week 1 and p = 0.0013 at Week 2.

DAVG = Time-weighted average change from baseline in HIV-1 RNA through Week 2 ($\log_{10}$ copies/mL), QD = once-daily, BID = twice daily

Source: Section 11, Tables 9.1, 9.2, 11.1, 11.2; Appendix 14, Listing 7

Secondary efficacy endpoints included time-weighted average change in HIV-1 RNA from baseline through Week 2 (DAVG$_2$), which demonstrated significant viral load reductions at all dose levels compared to placebo; HIV-1 RNA <50 and <400 copies/mL (two subjects achieved the former and five the latter); and change in CD4+ or CD8+ cell counts or percentages from baseline. No clinically relevant or statistically significant changes in CD4+ or CD8+ cell counts were observed between baseline and Day 11 at any of the GS-9137 doses administered compared to placebo.

Results of pharmacokinetic analyses
Pharmacokinetic parameters of GS-9137 after a single dose and 10 consecutive days of dosing were evaluated for 200 mg BID, 400 mg BID, 800 mg BID, 800 mg QD, and GS-9137/r 50/100 mg QD. The mean GS-9137 plasma concentration-time profiles on Day 1 and Day 10 are shown in Figures 3 and 4, respectively.

Figure 3: GS-9137 plasma concentration-time profile on Day 1 (PK analysis set; mean ± SD; semilog scale; source: Study Report Figure 7-2)

Figure 4: GS-9137 plasma concentration-time profile on Day 10 (PK analysis set; mean ± SD; semilog scale; source: Study Report Figure 7-2)

Plasma concentrations of GS-9137 after administration alone were lower on Day 10 than on Day 1, suggesting autoinduction of metabolism. However, GS-9137/r

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administration resulted in higher GS-9137 plasma concentrations (50.2% increase in AUC_{tau}) on Day 10 compared to Day 1, indicating accumulation resulting from RTV-mediated CYP3A inhibition (i.e. the CYP3A inhibition caused by RTV overcome CYP3A induction caused by GS-9137). The longer half-life (8.86 h, compared to 2-3 h for unboosted GS-9137) implies attainment of steady-state within 10 days of GS-9137/r dosing. Concurrent administration of GS-9137 and RTV resulted in higher GS-9137 exposures, including trough concentrations (which were associated with efficacy in this study), than any of the other dosing regimens tested. The pharmacokinetic parameters of GS-9137 after administration of a single dose or multiple doses of the GS-9137 regimens tested in this study are shown in Tables 3 and 4, respectively.

Table 3: Summary of plasma GS-9137 pharmacokinetic parameters on Day 1 (PK analysis set; source: Study Report Table 7-3)

GS-9137 Plasma PK Parameter	GS-9137 200 mg BID (N = 6)	GS-9137 400 mg BID (N = 5)[a]	GS-9137 800 mg QD (N = 5)[b,c]	GS-9137 800 mg BID (N = 6)	GS-9137 50 mg + 100 mg ritonavir QD (N = 6)[c]
C_{max} (ng/mL), Mean (%CV)	727.82 (23.31)	523.94 (41.22)	1388.80 (31.13)	1011.90 (35.81)	397.97 (38.17)
T_{max} (h), Median (Min, Max)	1.75 (0.97, 3.05)	3.12 (2.00, 4.00)	2.00 (1.00, 3.02)	2.98 (1.00, 4.00)	3.00 (1.00, 4.00)
C_{last} (ng/mL), Mean (% CV)	41.83 (32.91)	65.40 (50.67)	12.90 (63.70)	197.92 (38.80)	102.40 (60.81)
T_{last} (h), Median (Min, Max)	11.99 (11.93, 12.03)	11.95 (11.72, 12.00)	24.00 (24.00, 24.00)	11.97 (11.90, 12.00)	24.00 (24.00, 24.00)
AUC_{0-last} (ng•h/mL), Mean (% CV)	2646.25 (36.29)	2747.80 (33.46)	6406.10 (28.85)	5848.57 (27.70)	4615.32 (44.71)
AUC_{inf} (ng•h/mL), Mean (% CV)	2823.67 (35.57)	3047.56 (32.86)	6476.90 (29.11)	7414.87 (20.91)	6588.42 (49.04)
% AUC_{exp} Mean (% CV)	6.47 (20.95)	9.52 (63.12)	1.02 (50.66)	21.25 (69.88)	27.33 (38.94)
$T_{1/2}$ (h), Median (Min, Max)	2.91 (2.60, 3.48)[d]	2.75 (2.18, 4.42)[d]	3.58 (3.34, 4.01)	4.50 (2.74, 8.44)[d]	10.92 (9.09, 18.57)
Vz/F (L) Mean (% CV)	341.26 (40.09)	583.45 (27.46)	705.00 (34.33)	764.73 (43.73)	173.54 (62.21)
CL/F (L/h) Mean (% CV)	78.19 (33.12)	142.69 (31.90)	136.04 (40.38)	111.47 (18.64)	10.62 (81.99)

a   Subject 1101 was excluded from summary statistics for the 400 mg twice-daily group because he was identified as a high outlier (see Section 5.7.2.1).

b   Subject 1301 was excluded from summary statistics for the 800 mg once-daily group because he was incorrectly administered 1600 mg of GS 9137 rather than 800 mg on Day 1 (see Section 5.7.2.1).

c   The predose concentration of GS-9137 on Day 2 was used for the 24-hour time point.

d   Sampling for half-life was limited to 12 hours post-dose.

QD = once-daily, BID = twice daily

Source: Section 11.1, Table 19.1, 19.2

Table 4: Summary of plasma GS-9137 pharmacokinetic parameters on Day 10 (PK analysis set; source: Study Report Table 7-4)

GS-9137 Plasma PK Parameter	GS-9137 200 mg BID (N = 6)	GS-9137 400 mg BID (N = 6)	GS-9137 800 mg QD (N = 6)[a]	GS-9137 800 mg BID (N = 6)	GS-9137 50 mg + 100 mg ritonavir QD (N = 6)[a]
C_{max} (ng/mL), Mean (% CV)	479.03 (42.58)	606.87 (77.58)	939.92 (54.31)	835.53 (48.20)	744.65 (20.40)
T_{max} (h), Median (min, max)	1.53 (1.00, 2.02)	1.53 (1.00, 4.00)	2.53 (1.00, 5.98)	2.00 (1.05, 2.02)	2.75 (1.00, 4.03)
C_{tau} (ng/mL), Mean (% CV)	30.73 (39.98)	48.68 (64.84)	13.62 (68.64)	47.98 (32.65)	135.00 (36.55)
T_{last} (h), Median (min, max)	12.00 (12.00, 12.00)[b]	12.00 (12.00, 12.00)[b]	24.00 (24.00, 24.00)[b]	12.00 (12.00, 12.00)[b]	24.00 (24.00, 24.00)[b]
AUC_{tau} (ng•h/mL), Mean (% CV)	1954.65 (46.35)	2335.30 (54.52)	5512.87 (53.59)	3566.35 (36.83)	8843.50 (25.46)
$T_{1/2}$ (h), Median (min, max)	2.82 (2.51, 4.75)[b]	3.08 (2.48, 5.02)[b]	3.80 (3.02, 4.60)[b]	2.53 (2.14, 3.03)[b]	8.86 (6.10, 10.91)[b]
V_z/F (L) Mean (% CV)	818.94 (134.38)	980.51 (47.44)	1022.71 (51.33)	946.64 (40.47)	73.23 (29.35)
CL/F (L/h) Mean (% CV)	152.84 (95.44)	204.75 (38.69)	178.35 (42.67)	251.20 (35.74)	5.93 (22.64)

a   Trough concentration on Day 11 was used as the 24-hour time point.

b   12- and 24-hour pharmacokinetic samples collected on Day 10 and 11, respectively, were assigned nominal times of 12 or 24 hours, respectively.

QD = once-daily, BID = twice daily

Source: Section 11.1, Table 19.3

An analysis of dose proportionality of a single unboosted dose of GS-9137 in the 200, 400, and 800 mg dosing groups (800 mg QD and BID groups combined) indicated that increases in GS-9137 AUC_{inf} and C_{max} were less than dose-proportional, suggesting that GS-9137 was incompletely absorbed.

An evaluation of plasma protein binding indicated that the free fraction of GS-9137 was 1.19% ± 0.21% independent of concentration, over the range of 1 ng/mL to 1552 ng/mL.

PBMC GS-9137 concentrations were substantially higher than plasma concentrations. Across all treatment groups, the Day 1 C_{max} values were 9.9 to 20.5-fold higher and the C_{last} values were 9.4 to 43.3-fold higher for PBMC compared to plasma. The Day 10 C_{max} values were 6.3 to 12.2-fold higher and the C_{tau} values were 9.8 to 18.4-fold higher in PBMC compared to plasma. T_{max} and $t_{1/2}$ values were similar for plasma and PBMC.

Results of pharmacodynamic analysis

GS-9137 C_{tau} values exhibited a strong exposure-reponse relationship and fit well to a simple E_{max} model (Hill coefficient = 1; see Figure 5). Based on the model, the maximum effect (E_{max}) was 2.32 log_{10} reduction in viral load from baseline and an EC_{50} of 14.4 ng/mL. The mean GS-9137 C_{tau} for GS-9137/r was greater than the EC_{90} predicted by the model. The estimated inhibitory quotient (mean C_{tau}/protein binding-adjusted in vitro IC_{95}) was 18.8 for GS-9137/r 50/100 mg.

Figure 5: GS-9137 pharmacokinetic/pharmacodynamics dose-response relationship (PK analysis set; source: Study Report Figure 7-4)

$E_{max} = 2.32 \log_{10}$
$EC_{50} = 14.4$ ng/mL
$R^2 = 0.779$
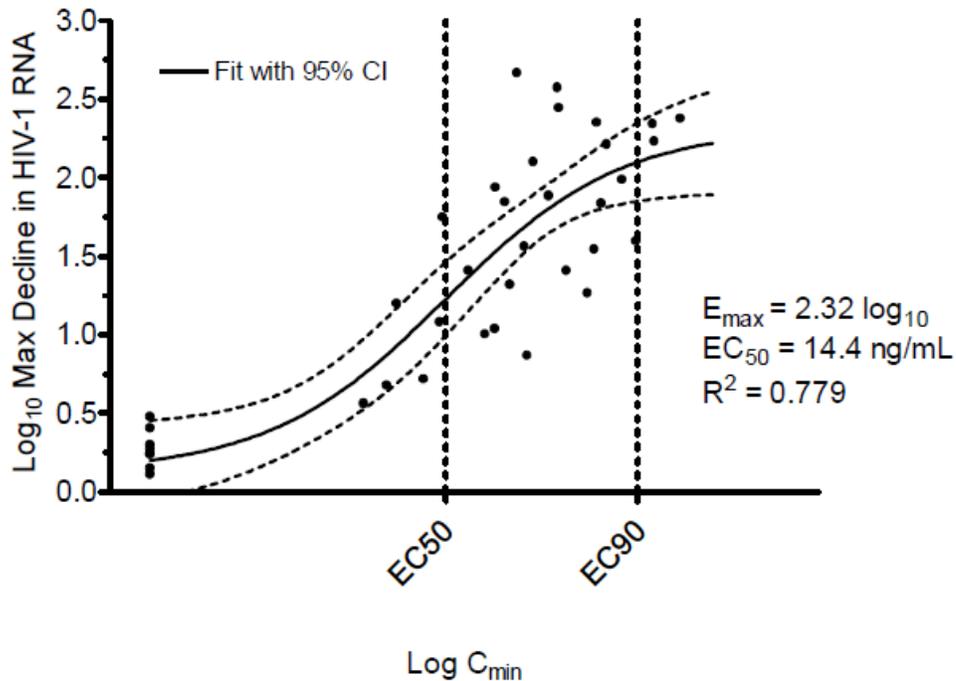
Source: Reference No. {8905}

Results of safety analysis

The safety analysis set contained all 40 subjects who received at least one dose of study drug. None of the subjects in the safety analysis set discontinued the study prematurely. Treatment-emergent adverse events were common in all treatment groups. Most were mild to moderate (Grade 1 to 2) in severity and resolved with therapy; no adverse events with severity greater than Grade 4 occurred. Two Grade 3 treatment-emergent adverse events were reported (sternocleidomastoid spasm in Subject 1209 and elevated triglyceride level in Subject 1106) but neither was considered by the investigator to be related to study drug. The only Grade 2 treatment-emergent adverse event that occurred in more than one subject within a treatment group (i.e. 800 mg QD) was headache.

Treatment-related adverse events occurred more frequently in placebo groups than treatment groups for all GS-9137 dose levels except for 200 mg BID. Three treatment-related adverse events occurred in multiple subjects: diarrhea was reported by two subjects in the placebo group, nausea was reported by two subjects in the placebo group and two subjects in the GS-9137 200 mg BID treatment group, and fatigue was reported by two subjects in the GS-9137 200 mg BID treatment group.

Five subjects had treatment-emergent Grade 3 or 4 laboratory abnormalities: two in the placebo group and one each in the placebo/r, GS-9137 400 mg BID, and GS-9137/r 50/100 mg QD. The two subjects who received GS-9137 and had laboratory abnormalities had abnormal values at baseline.

Trial Summary

GS-9137 demonstrated significant antiviral activity over a range of doses after ten days of treatment compared to placebo or placebo/r, with mean reductions in HIV-1 RNA nearing $-2 \log_{10}$ copies/mL by Day 11. Coadministration of GS-9137 and ritonavir 100/50 mg QD resulted in trough concentrations that exceeded those observed after GS-9137 800 mg BID dosing, as well as a terminal half-life that supports once-daily dosing. An analysis of the pharmacokinetic/pharmacodynamics relationship suggests that antiviral activity is dependent on maintaining therapeutic plasma drug concentrations through the dosing interval (i.e. $C_{tau} > IC_{95}$, where the protein binding-adjusted in vitro IC_{95} is 44.9 ng/mL [100 nM]).

GS-9137 was generally well-tolerated. No subjects who received study drug discontinued prematurely. There were no serious adverse events during the study. Only headache (50% of the GS-9137 800 mg QD and GS-9137/r 50/100 mg QD groups) and diarrhea (38% of the placebo group) were experienced in multiple subjects within a group. The incidence of treatment-emergent adverse events in GS-9137-treated groups was similar to or lower than the placebo groups, and the classes of adverse events were similar.

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Trial GS-US-183-0102
A Phase 1, Pilot Pharmacokinetic Study to Evaluate the Effect of Ritonavir Boosting on the Pharmacokinetics of GS-9137

Trial Period
21 July 2005 to 17 August 2005
Final report date: 2 June 2006 (submitted to IND (b) (4))

Trial Site
MDS Pharma Services, Phoenix, Arizona, USA

Trial Rationale
GS-9137 (elvitegravir, EVG) is an inhibitor of the human immunodeficiency virus (HIV) integrase, currently under development for the treatment of HIV infection. Results from *in vitro* studies have demonstrated potent anti-HIV activity, including activity against viruses that are resistant to nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PI). Ritonavir (RTV) is an HIV PI indicated for use in combination with other antiretroviral drugs for the treatment of HIV infection. It is used primarily at subtherapeutic doses to improve the pharmacokinetic (PK) profiles of HIV drugs that are metabolized by cytochrome P450 (CYP450) isoform 3A, which it inhibits. The goal of this trial was to determine whether or not RTV would similarly improve the pharmacokinetic profile of GS-9137 upon coadministration.

Trial Objectives
The primary objective of the trial was to:

- determine the effect of RTV 100 mg twice daily coadministration on the steady-state pharmacokinetics of GS-9137

The secondary objectives of the trial were to:

- evaluate the single-dose and multiple-dose pharmacokinetics of GS-9137
- assess the multiple-dose safety of GS-9137 administered alone and with RTV (GS-1937/r)

Trial Design
This trial had an open-label, fixed-sequence design. There were two treatment periods of 10 days each, with follow-up contact on Day 27. The study schema is shown in Figure 1.

Treatment 1: GS-9137 100 mg BID
Treatment 2: GS-9137/r 100/100 mg BID

Figure 1: Study schema (source: Study Report Figure 5-1)
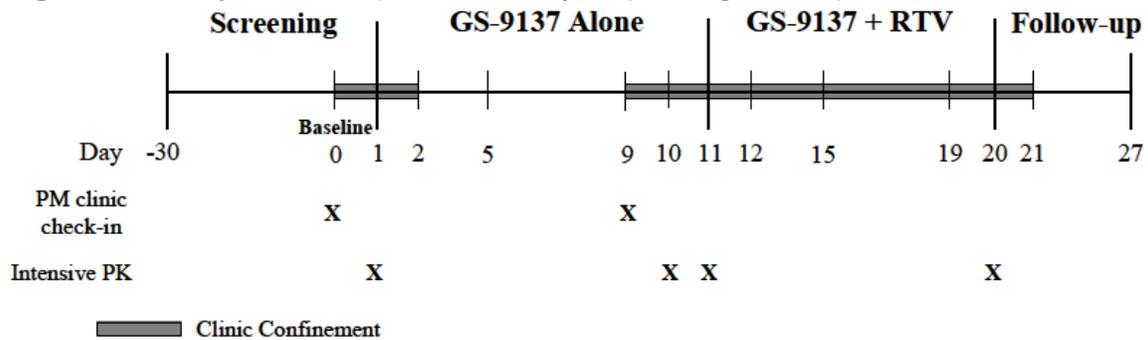
Rationale for Dose Selection

The GS-9137 dose of 100 mg BID was selected because it was expected to yield detectable plasma concentrations of GS-9137 when administered without RTV, while staying within a desirable plasma concentration range when administered with RTV. The RTV dose of 100 mg BID is the dose currently indicated to boost HIV antiretroviral drugs in the treatment of HIV.

Drug Administration

Study drugs were administered orally with 240 mL of water at approximately 8:00 am and 8:00 pm, within 5 minutes of completion of a meal. The evening dose of study medication was not administered on Day 10 or Day 20. Meals at the study facility were standardized and contained approximately 400 kcal and 13 g fat. Doses taken while at the study facility were observed. On days when intensive PK sampling was performed, all subjects fasted until after the 4 h post-dose sample was collected.

Investigational Product

GS-9137 was administered as 50 mg tablets (Lot 071-4). Subjects also received ritonavir (NORVIR®, Abbott Laboratories, Lot 247022E21) 100 mg soft gelatin capsules during Treatment 2.

Key Inclusion and Exclusion Criteria

Subjects were healthy males and females between the ages of 18 and 45 years, inclusive, with body weight within 15% of the normal range and normal liver function tests (LFT) and electrocardiogram (ECG). Potential subjects were excluded from enrollment if they had any abnormal finding of clinical relevance during the medical examination or any diseases or infections of clinical relevance. Exclusion criteria also included the use of alcohol or illicit drugs, the use of any prescription or over-the-counter medications or herbal products within 30 days of study drug administration (with the exception of vitamins, acetaminophen, ibuprofen, and hormonal contraception), and the use of systemic steroids, immunosuppressant therapies, or chemotherapeutic drugs within 3 months of study screening.

Concomitant Medications

No concomitant medications, over-the-counter medications, or herbal products were permitted except for vitamins, acetaminophen, ibuprofen, and hormonal contraceptives. In the case of adverse events, symptomatic therapy was permitted if judged medically necessary by the investigator.

Restrictions were placed on the consumption (prior to and during the trial) of products containing calcium, magnesium, or aluminum (antacids) or calcium, iron, or zinc (vitamin and mineral supplements); the ulcer medicine sucralfate; and certain fruit juices and citrus fruits.

Sample Collection

Blood was collected for pharmacokinetic analysis at each of the following timepoints (in hours after dosing):

Days 1, 10, 11, and 20	0:00 (predose), 0:15, 0:30, 0:45, 1:00, 1:30, 2:00, 2:30, 3:00, 3:30, 4:00, 5:00, 6:00, 8:00, 10:00, and 12:00
Day 21	24:00
Days 5, 9, 15, and 19	0:00 (predose)

Urine was collected for pharmacokinetic analysis during the following time periods (in hours after dosing):

Days 10 and 20	0 (predose), 0-4, 4-8, and 8-12

Analytical Plan

Pharmacokinetic data

The primary pharmacokinetic endpoints for GS-9137 were AUC_{0-tau}, $AUC_{0-\infty}$, and C_{max}. These parameters were estimated using a nonlinear model derived using standard noncompartmental methods (WinNonlin® Professional Edition, Pharsight Corporation, Mountain View, California, USA). The untransformed and natural log-transformed parameters were analyzed using an analysis of variance (ANOVA), incorporating treatment period and day as fixed effects and subject as a random effect, using SAS® PROC MIXED (SAS Institute, Cary, North Carolina, USA). Differences between treatments were evaluated by calculating the 90% confidence intervals (CIs) for the ratio of the geometric least-squares means of each parameter.

Trial Results

Bioanalytical method

Quantitative determination of GS-9137

Plasma concentrations of GS-9137 were quantified using LC-MS/MS by the Gilead Sciences ⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛ (b) (4) The bioanalytical study report was not been included in the current submission.

The calibration standards for GS-9137 were 1.00, 3.00, 10.0, 50.0, 100, 250, and 1000 ng/mL and the quality control (QC) concentrations were 1.00, 3.00, 250, and 1000 ng/mL. The intra-assay accuracy and precision estimates are displayed in Table 1. No inter-assay accuracy and precision estimates are available because the method used in this study (Method D) was performed in a single day. All available accuracy and precision (%CV) values were within the acceptable range (≤20% deviation from nominal at the LLoQ concentration, and ≤15% from nominal at all other concentrations).

Quantitative determination of RTV
Plasma concentrations of RTV were quantified using LC-MS/MS by ⬛⬛ (b) (4) ⬛⬛⬛⬛⬛⬛⬛⬛ (⬛⬛⬛⬛⬛⬛⬛⬛ (b) (4) Project Number 60-0507). Frozen plasma samples were received on 24 Aug 2005 and were stored at -70˚C until analysis, which was performed between 7 Sep and 20 Sep 2005. The maximum sample storage time was 27 days, which is within the validated long-term frozen stability duration of 343 days.

The method used to measure RTV concentrations was ⬛⬛ (b) (4) 42-0316. The calibration standards for RTV were 1.00, 3.00, 10.0, 100, 300, and 1000 ng/mL and the QC concentrations were 3.00, 9.00, 200, and 800 ng/mL. The inter- and intra-assay accuracy and precision estimates are displayed in Table 1. All accuracy and precision (%CV) values were within the acceptable range (≤20% deviation from nominal at the LLoQ concentration, and ≤15% from nominal at all other concentrations).

Table 1: Bioanalytical assay validation for GS-9137 and RTV (source: Study Report Table 5-4)

	GS-9137	Ritonavir
Linear Range (ng/mL)	1 to 1000	1 to 1000
LLQ (ng/mL)	1	1
Inter-assay Precision Range (%CV)	NA[a]	6.4% to 7.4%
Inter-assay Accuracy Range[b]	NA[a]	−7.7% to 1.6%
Intra-assay Precision Range (%CV)	3.2% to 10.7%[c]	1.5% to 6.6%
Intra-assay Accuracy Range[b]	−6.7% to 4.9%[c]	−2.4% to 9.1%
Stability in Frozen Matrix	3 months	343 days

LLQ, lower limit of quantitation; CV, coefficient of variation

a   The analytical method used for study samples (Method D) was a minor modification of a method that was validated over 3 days for the range of 1.0 to 5000 ng/mL (Method B). Although similar to Method B, Method D was evaluated over only 1 day of precision and accuracy. For Method B, interassay precision ranged from 3.2% to 18.2% for precision and from −3.3% to 10.0% for accuracy. Details are described in the bioanalytical reports in Appendix 15.

b   Values shown are percent differences from nominal concentrations.

c   Values shown are for Method D, which was used for analysis of study samples.

Source: Appendix 15

Method D was preferred over Method B for analysis of GS-9137 concentrations in study samples because of improved the recovery and decreased assay run.

Trial population

A total of 12 healthy subjects between the ages of 18 and 43 (median age: 26) were enrolled in and completed the study. All subjects were included in the safety set and the pharmacokinetic analysis set. Seven subjects were male and five were female. The majority of subjects were Hispanic (n=11, 92%) and the remaining subject was white.

Pharmacokinetics of GS-9137

The mean (± SD) plasma concentration-time profiles after oral administration of GS-9137 as a single dose (100 mg; Day 1) or multiple doses (100 mg BID; Day 10) are shown in Figure 2 and the corresponding pharmacokinetic parameters are listed in Table 1. After multiple doses, plasma concentrations of GS-9137 were lower compared to after a single dose, suggesting some degree of autoinduction of metabolism (i.e. CYP3A, which is the primary route of metabolism when GS-9137 is dosed alone). There was no evidence of accumulation after multiple dosing.

Figure 2: Plasma GS-9137 concentration-time profile after a single dose or multiple doses of GS-9137 alone (mean ± SD; source: Study Report Figure 7-1)
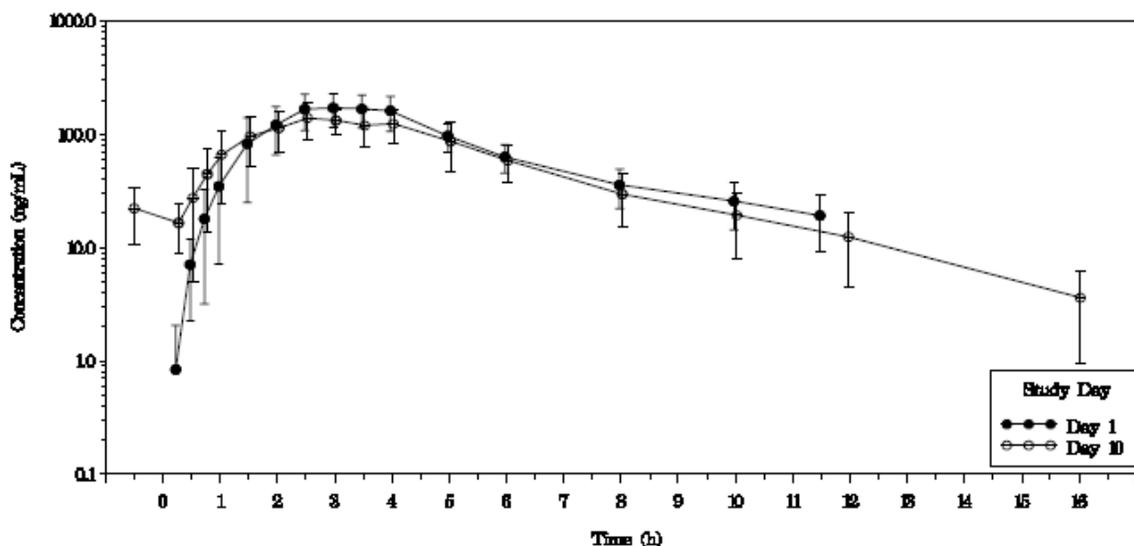
Table 1: Summary of GS-9137 pharmacokinetic parameters after a single dose or multiple doses of GS-9137 alone (source: Study Report Table 7-2)

GS-9137 PK Parameter	Single Dose (Day 1) (N = 12)	Multiple Doses (Day 10) (N = 12)
C_{max} (ng/mL), Mean (%CV)	200.1 (30.4)	164.1 (28.8)
T_{max} (h), Median (Min, Max)	3.00 (2.00, 4.00)	2.50 (1.00, 4.00)
C_{tau} (ng/mL), Mean (%CV)	19.2 (52.5)	12.4 (63.7)
T_{last} (h), Median (Min, Max)	11.5 (11.5, 11.6)	20.0 (16.0, 24.0)[a]
AUC_{tau} (ng·h/mL), Mean (%CV)	817.3 (25.1)	719.3 (26.2)
AUC_{inf} (ng·h/mL), Mean (%CV)	908.1 (28.3)	Not applicable
%AUC_{exp}, Mean (%CV)	10.1 (55.0)	Not applicable
$T_{\frac{1}{2}}$ (h), Median (Min, Max)	3.1 (2.2, 4.8)[b]	3.5 (2.2, 4.1)[a]
V_z/F (L), Mean, (%CV)	536.1 (23.8)	668.3 (41.1)
CL/F (L/h), Mean (%CV)	119.0 (30.1)	148.4 (27.8)

C_{tau} represents the concentration at the end of the dosing interval on Days 1, 10, 11, and 20.

On Day 1, $C_{tau} = C_{last}$ and $AUC_{tau} = AUC_{0-last}$.

a   The Day 10 evening dose was withheld, and the 16-hour and 24-hour (i.e., predose on Day 11) time points were used.

b   Sampling for half-life was limited to 12 hours postdose.

Source: Section 11.1, Table 5

The geometric least squares means for GS-9137 C_{max} and AUC_{tau} after multiple dosing were 17 and 20% lower, respectively, compared to single dosing. These values, along with the 90% confidence intervals (71.6 and 95.7% for C_{max}; 71.2 and 89.2% for AUC_{tau}) suggest that GS-9137 induces its own metabolism.

Pharmacokinetics of GS-9137 following ritonavir coadministration

The mean (± SD) GS-9137 plasma concentration-time profiles after oral administration of GS-9137/r as a single dose (100/100 mg; Day 11) or multiple doses (100/100 mg BID; Day 20) are shown on a semilog scale in Figure 3. As expected, plasma concentrations of GS-9137 were increased by coadministration of RTV.

Figure 3: Plasma GS-9137 steady-state concentration-time profile after coadministration of a single dose or multiple doses of ritonavir (mean ± SD; source: Study Report Figure 7-2)
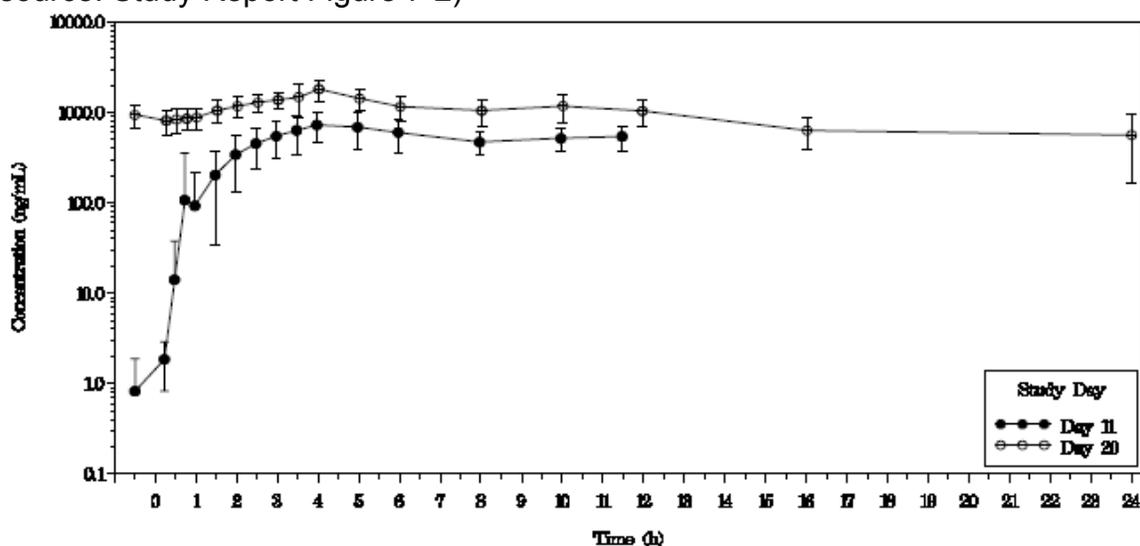
The pharmacokinetic parameters of GS-9137 after coadministration of a single dose or multiple doses of RTV are listed in Table 2. Note that GS-9137 was at steady-state by the beginning of this treatment period (i.e. Day 11), as it had been administered alone since Day 1, and that autoinduction of GS-9137 metabolism was already present; comparisons should therefore be made to GS-9137 pharmacokinetics on Day 10.

The pharmacokinetic parameters of GS-9137 following a single dose or multiple doses of GS-9137 are listed in Table 2. The mean systemic exposure after multiple doses of RTV (AUC_{0-tau}, Day 20) was higher than the mean exposures after a single dose of RTV (AUC_{0-tau}, Day 11) and after multiple doses of GS-9137 without RTV (AUC_{0-tau}, Day 10). Some accumulation was evident after multiple doses of RTV, indicating that, as expected, the primary effect of RTV was inhibition of metabolism rather than induction. Note that the estimation of

GS-9137 half-life after a single dose of RTV is approximate, since the post-dose sampling interval was limited to 12 h.

Table 2: Summary of GS-9137 pharmacokinetic parameters after a single dose or multiple doses of GS-9137 with RTV (source: Study Report Table 7-4)

GS-9137 PK Parameter	GS-9137 Alone (Day 10) (N = 12)	GS-9137 + Single-Dose RTV (Day 11) (N = 12)	GS-9137 + Multiple-Dose RTV (Day 20) (N = 12)
C_{max} (ng/mL), Mean (%CV)	164.1 (28.8)	795.3 (38.4)	1826.4 (26.4)
T_{max} (h), Median (Min, Max)	2.50 (1.00, 4.00)	4.00 (3.50, 11.58)	4.00 (3.50, 4.00)
C_{tau} (ng/mL), Mean (%CV)	12.4 (63.7)	543.3 (30.4)	1035.6 (32.0)
T_{last} (h), Median (Min, Max)	20.0 (16.0, 24.0)[a]	11.5 (11.5, 11.6)	24.1 (24.0, 24.1)[b]
AUC_{tau} (ng·h/mL), Mean (%CV)	719.3 (26.2)	6167.3 (29.1)	14302.1 (23.7)
$T_{1/2}$ (h), Median (Min, Max)	3.5 (2.2, 4.1)[a]	18.2 (9.0, 42.6)[c]	9.5 (5.9, 78.2)[b]
V_z/F (L), Mean (%CV)	668.3 (41.1)	517.7 (52.3)	161.0 (123.5)
CL/F (L/h), Mean (%CV)	148.4 (27.8)	17.4 (25.9)	7.4 (30.1)

a   The Day 10 evening dose was withheld, and the 16-hour and 24-hour (i.e., predose sample on Day 11) time points were used.

b   The Day 20 evening dose was withheld, and the 16-hour and 24-hour (i.e., Day 21 sample) time points were used.

c   This approximate value may not represent the terminal half-life, as the sampling time was limited to 12 hours postdose.

Source: Section 11.1, Table 5

The geometric least squares mean for GS-9137 AUC_{tau} after a single dose of RTV was 8.58-fold higher than GS-9137 administered without RTV. After multiple doses of RTV, systemic GS-9137 exposure increased even further (20-fold compared to GS-9137 alone), accompanied by an 11-fold increase in C_{max}. These increases in systemic GS-9137 exposure indicate that RTV coadministration enhances oral bioavailability of GS-9137 via a reduction in first-pass metabolism and also decreases GS-9137 elimination.

Pharmacokinetics of ritonavir
The mean (± SD) RTV plasma concentration-time profiles after oral administration of GS-9137/r as a single dose (100/100 mg; Day 11) or multiple doses (100/100 mg BID; Day 20) are shown on a semilog scale in Figure 4. Plasma concentrations of RTV were higher at steady-state than after a single dose, with an accumulation factor of approximately 2.3-fold.

Reference ID: 3153809

Figure 4: Plasma RTV concentration-time profile after a single dose or multiple doses coadministered with GS-9137[a] (mean ± SD; source: Study Report Figure 7-3)
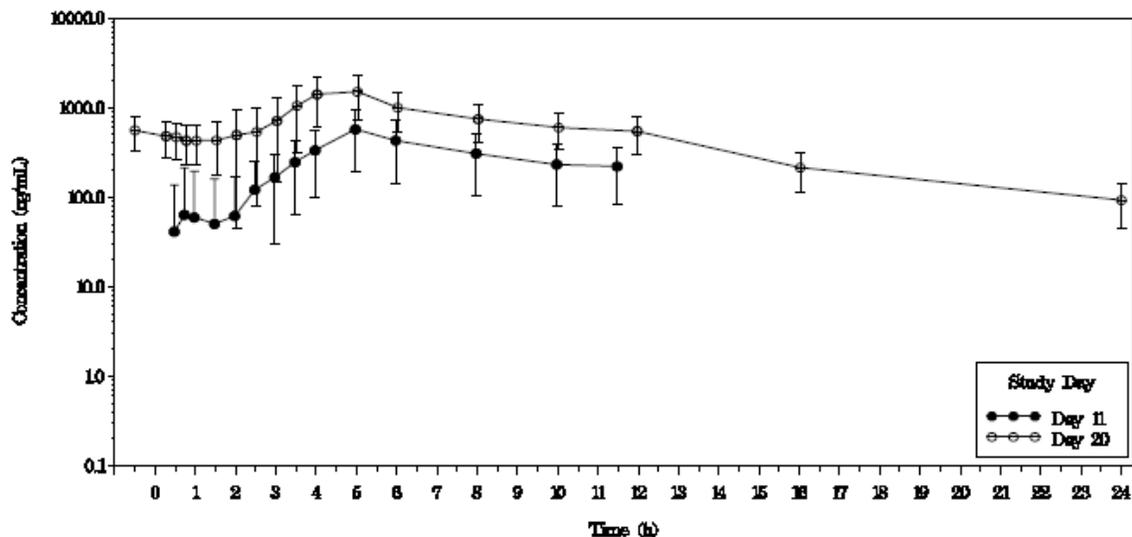
[a]GS-9137 is at steady-state

The pharmacokinetic parameters of RTV after of a single dose or multiple doses of GS-9137/r are listed in Table 3. Note that GS-9137 was at steady-state by the beginning of this treatment period (i.e. Day 11), as it had been administered alone since Day 1.

Table 3: Summary of RTV pharmacokinetic parameters after a single dose or multiple doses (source: Study Report Table 7-7)

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Reference ID: 3153809

Ritonavir PK Parameter	Steady-State GS-9137 + Single-Dose RTV (Day 11) (N = 12)	Steady-State GS-9137 + Multiple-Dose RTV (Day 20) (N = 12)
C_{max} (ng/mL), Mean (%CV)	616.3 (53.5)	1686.5 (46.5)
T_{max} (h), Median (Min, Max)	5.00 (1.02, 11.58)	5.00 (4.00, 11.98)
C_{tau} (ng/mL), Mean (%CV)	219.8 (61.8)	544.8 (44.3)
T_{last} (h), Median (Min, Max)	11.5 (11.5, 11.6)	24.1 (24.0, 24.1)[a]
AUC_{tau} (ng·h/mL), Mean (%CV)	2994.2 (56.8)	9402.5 (46.9)
AUC_{inf} (ng·h/mL), Mean (%CV)	4979.4 (57.8)	Not applicable
%AUC_{exp}, Mean (%CV)	32.2 (47.8)	Not applicable
$T_{½}$ (h), Median (Min, Max)	5.1 (2.2, 8.3)	4.8 (4.3, 6.9)[a]

On Day 1; $C_{tau} = C_{last}$ and $AUC_{tau} = AUC_{0-last}$

a   The evening dose on Day 20 was withheld, and the 16-hour and 24-hour (i.e., Day 21 sample) time points were used.

Source: Section 11.1, Table 5

The geometric least squares means for systemic RTV exposure (AUC_{tau}) and C_{max} after multiple dosing were 128 and 184% higher, respectively, compared to after a single dose. When dosed to steady-state, RTV exhibited nonlinear pharmacokinetics, likely due to its time-dependent, mechanism-based inhibition of CYP3A.

Results of safety analysis

Out of the 12 subjects enrolled in the study, 11 (45.8%) reported a treatment-emergent AE. Four of these experienced treatment-emergent AEs while receiving GS-9137 alone (five events) and the remaining seven subjects experienced 44 events while receiving GS-9137/r. No AE was reported in more than one subject on GS-9137 alone. The most common AE during administration of GS-9137/r was nausea, which was experienced by 4 subjects (33%).

Two AEs were Grade 2 and were considered unrelated to study drug by the investigator. The remainder of treatment-emergent AEs were Grade 1; most resolved with therapy. Seven subjects reported what the investigator considered to be treatment-related AEs: two subjects experienced two events while receiving GS-9137 alone, and five subjects experienced 21 events while receiving GS-9137/r. The treatment-related AEs reported in more than one subject were nausea, vomiting, headache, and pruritus.

No serious AEs occurred during the study and no subjects discontinued because
of an AE. Overall, oral doses of GS-9137 for up to 20 days were well-tolerated,
and concurrent administration of GS-9137 and RTV was generally well-tolerated.
Most AEs were reported after administration of GS-9137/r and are consistent
with those reported for RTV.

Trial Summary
The use of low-dose ritonavir as a pharmacokinetic booster of GS-9137 exposure
is supported by the results of this study. Coadministration of GS-9137 with
ritonavir at doses of 100/100 mg increased GS-9137 AUC_{tau} by 8.6-fold (a single
dose of RTV) and 19.9-fold (multiple doses of RTV) and prolonged GS-9137 half-
life, which may facilitate less frequent dosing. The elevation in exposure is likely
due to a decrease in presystemic GS-9137 metabolism caused by inhibition of
CYP3A by ritonavir. The increase in GS-9137 half-life suggests a simultaneous
decrease in systemic clearance. Treatment-related adverse events were more
frequent during GS-9137/r administration and included nausea, vomiting,
headache, and pruritus. In general, all AEs were mild and transient, and GS-
9137 with or without ritonavir was safe and well-tolerated.

Trial GS-US-183-0105

A Phase 2, Randomized Study of the Treatment of Antiretroviral Treatment-Experienced, HIV-1 Infected Subjects Comparing Ritonavir-Boosted GS-9137 (GS-9137/r) Versus a Comparator Ritonavir-Boosted Protease Inhibitor (CPI/r) in Combination with a Background Antiretroviral Therapy

Trial Period
21 Feb 2006 to 13 July 2007
Final report date: 14 January 2009 (submitted to IND $^{(b)\,(4)}$)

Trial Sites
75 sites in the USA (range: 0-16 subjects randomized)
3 sites in Puerto Rico (range: 3-6 subjects randomized)

Trial Rationale
GS-9137 (elvitegravir, EVG) is an inhibitor of the human immunodeficiency virus (HIV) integrase, currently under development for the treatment of HIV infection. Results from *in vitro* studies have demonstrated potent anti-HIV activity, including activity against viruses that are resistant to nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PI). Ritonavir (RTV) is an HIV PI indicated for use in combination with other antiretroviral (ARV) drugs for the treatment of HIV infection. Coadministration of ARVs that are cytochrome P450 (CYP) isoform 3A substrates with the potent CYP3A inhibitor RTV increases the systemic exposure of the substrate drugs. This study was designed to evaluate the noninferiority of RTV-boosted GS-9137 (GS-9137/r) compared to RTV-boosted comparator PIs (CPI/r), and to determine the optimum GS-9137/r dose to use in Phase 3 clinical trials.

Trial Objectives
The primary objective of the trial was to:

- assess noninferiority of GS-9137/r relative to CPI/r, both in combination with a background ARV regimen, as determined by the time-weighted average change from baseline in $\log_{10}$ HIV-1 RNA levels at Week 24 post-treatment ($DAVG_{24}$)

The secondary objectives of the trial were to:

- evaluate the safety and tolerability of GS-9137/r relative to CPI/r through 48 weeks of treatment
- assess noninferiority of GS-9137/r relative to CPI/r, both in combination with a background ARV regimen, as determined by the time-weighted average change from baseline in $\log_{10}$ HIV-1 RNA levels at Week 48 post-treatment ($DAVG_{48}$)

Trial Design

This was a randomized, partially blinded, multiple-dose, multicenter, 48-week study (including a pharmacokinetic substudy at Week 8 in a subset of subjects at selected sites) followed by 30 days of follow-up, with subsequent enrollment in an optional open-label rollover protocol (GS-US-183-0130) if desired. The CPI and background ARV regimens were selected by the investigator before study randomization based on each subjects's ARV history and viral resistance. The background ARV regimen consisted of at least two marketed ARV agents, not including a PI or NNRTI. The CPI regimen included one or two marketed PIs dosed in combination or coformulated with RTV.

Eligible subjects were randomized (1:1:1:1) to receive one of four treatments, detailed in Table 1.

Table 1: Treatments and treatment group populations

Treatment	Randomized (n)	Safety analysis set (n)	PK substudy (n)	PK analysis set (n)
CPI/r, dosed per labeling	73	63	n/a	n/a
GS-9137/r 20/100 mg QD	75	71	11	11
GS-9137/r 50/100 mg QD	75	71	12	12
GS-9137/r 125/100 mg QD	74	73	13	13

Randomization was stratified by the use of T-20 (enfuvirtide, Fuzeon™) in the background ARV regimen (a maximum of 50% of subjects were allowed to use T-20). Subjects were strongly discouraged from changing the composition of their CPI and ARV regimens for at least the first 16 weeks of the study. Changes were permitted only for toxicity management, after discussion with the study's medical monitor.

After Week 8, the GS-9137/r 20/100 mg group was discontinued due to higher rates of virologic rebound. Subjects in that treatment group were switched to open-label GS-9137/r 125/100 mg in combination with their background ARV regimens.

In the original protocol, subjects randomized to one of the GS-9137/r doses were not allowed to include a PI and an NNRTI as part of their background ARV regimens. After randomization, data became available indicating the lack of an interaction between GS-9137 and ritonavir-boosted darunavir (DRV) or tipranavir (TPV); thus, subjects were permitted to add DRV or TPV to their background

regimens. No subjects added DRV or TPV by Week 12, only four subjects elected to add DRV or TPV by Week 16, and an additional 23 subjects added DRV or TPV by the end of the study. The efficacy results at Week 16 are therefore the least confounded of the predefined efficacy timepoints (Weeks 16, 24, and 48). It should also be noted that the efficacy data from Weeks 24 and 48 are confounded because of changes to the RTV dose (to 200 mg BID) at Week 16 for subjects whose background regimens included TPV.

Key Inclusion and Exclusion Criteria

Subjects were HIV-1-infected males and females between the ages of 18 and 65 years, inclusive, with plasma HIV-1 RNA levels ≥ 1000 copies/mL and documentation of one or more of the protease gene mutations defined by the IAS-USA 2005 Guidelines. Subjects must have been on a stable ARV regimen for at least 30 days prior to screening, and must have remained on that regimen until the baseline visit. Subjects had to have a normal ECG and adequate renal function as defined by estimated creatinine clearance ≥ 60 mL/min. Screening laboratory evaluations must have fallen within normal ranges.

Potential subjects were excluded from enrollment if they had a new AIDS-defining condition diagnosed within 30 days of baseline. Exclusion criteria also included previous exposure to any HIV-1 integrase inhibitor (unless the subject participated in GS-US-183-0101), or any investigational agents within 30 days of baseline. Potential subjects were excluded if they used alcohol or other substances in a way that could interfere with study compliance, if they had a malignancy other than cutaneous Kaposi sarcoma or basal cell carcinoma, or if they had an active, serious infection requiring parenteral antibiotic or antifungal therapy within 30 days of baseline.

Potential subjects were excluded for the use of specified medications from the following drug classes within 30 days of the baseline visit (refer to Study Report Table 5-1 for details): sedatives/hypnotics, GI motility agents, neuroleptic drugs, ergot derivatives, immunosuppressants, antibacterials, calcium channel blockers, antiarrythmics, antimycobacterials, antihistamines, anticonvulsants, herbal supplements, systemic chemotherapeutic (antineoplastic) agents, immunomodulators, medications not to be taken with RTV, systemic corticosteroids, HMG-CoA reductase inhibitors (statins), antifungals, anticoagulants, opiates, and phosphodiesterase-5 inhibitors.

Rationale for Dose Selection

A dose-response model based on results from a proof-of-concept study (GS-US-183-0101) demonstrated that GS-9137 antiviral activity was strongly correlated with steady-state C_{min}. GS-9137/r doses of 20/100, 50/100, and 125/100 mg QD were selected for this study because they were expected to yield steady-state C_{min} similar to or higher than GS-9137 400 and 800 mg BID, which were associated with potent antiviral activity in study GS-US-183-0101. The GS-9137/r 20/100 mg QD dose was expected to result in C_{min} levels approximately

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10-fold higher than the protein binding-adjusted IC_{50} (i.e. inhibitory quotient ≈10). Systemic GS-9137 exposures after GS-9137/r 125/100 mg QD dosing were expected to be two- to three-fold lower than the NOAEL determined in toxicology studies.

Investigational Product

Tablets containing 20, 50, or 125 mg of GS-9137 were manufactured by [(b) (4)] ; those used during the blinded portion of the study (20, 50, and 125 mg strengths) were from Lot 183-0105/03, while the 125 mg strength tablet used during the open-label part of the study were from Lot 183-0105/06.

The Sponsor provided ritonavir (NORVIR®, Abbott Laboratories) 100 mg soft gelatin capsules; subjects also may have obtained ritonavir from a pharmacy (e.g. for dosing with darunavir and tipranavir). The Sponsor provided commercial fixed-dose emtricitabine/tenofovir disoproxil fumarate combination tablets (TRUVADA®) for subjects whose background regimens included TRUVADA®. All other components of the CPI and/or ARV regimens were dispensed in open-label fashion as prescribed by the investigator, and administered according to the prescribing information for each particular product.

Drug Administration

GS-9137 was administered orally with one tablet of ritonavir (100 mg) once daily with food, at approximately the same time each day. Subjects enrolled in the pharmacokinetic substudy were required to take GS-9137/r with food at the same time each morning for the first 8 weeks, until the PK substudy was completed.

Subjects in the pharmacokinetic substudy arrived at the clinic after an 8 h fast and were given a standardized breakfast. Their assigned dose of GS-9137/r was administered with 240 mL of water under staff supervision. Subjects also received their ARV regimens according to their dosing schedule. Food was restricted until after the 4 h blood draw, and water was restricted from 1 h before until 2 h after dosing.

Concomitant Medications

In addition to the medications detailed in the "Key Inclusion and Exclusion Criteria" section above, subjects were instructed not to use any drug with antiretroviral activity that was not part of their prescribed CPI or ARV regimens. The following were also prohibited 4 h before and 4 h after any dose of GS-9137/r: antacids containing calcium, magnesium, or aluminum; sucralfate; and vitamin or mineral supplements containing calcium, iron, or zinc.

Sample Collection

Blood was collected for the analysis of GS-9137, its metabolites GS-9202 and GS-9200, and RTV plasma levels at the end of Weeks 2, 4, 8, 12, 16, 20, 24, 28,

32, 36, 40, 44, and 48 at an unspecified time with regard to last administered dose. If possible, blood samples were to be collected pre-dose at Weeks 8, 16, 24, and 48 to determine trough concentrations.

In addition, blood was collected at selected study sites for intensive pharmacokinetic analysis of GS-9137, GS-9202, GS-9200, and RTV in plasma at each of the following timepoints (in hours after dosing):

Week 8   0:00 (predose), 0:30, 1:00, 2:00, 2:30, 3:00, 3:30, 4:00, 4:30, 5:00, 6:00, 8:00, 10:00, 12:00, and 24:00

Blood was collected for quantification of HIV-1 RNA in plasma at the screening and baseline (Day 1), and 30-day follow-up visits, as well as at the end of Weeks 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48.

Analytical Plan
Pharmacokinetic data
The pharmacokinetic parameters AUC_{tau}, C_{max}, T_{max}, C_{tau}, C_{last}, T_{last}, λ_z, $t_{1/2}$ were estimated by dose group for GS-9137, GS-9200 (M4), GS-9202 (M1), and RTV in plasma. The geometric means were also calculated for AUC_{tau}, C_{tau}, C_{max}, and C_{last}. All pharmacokinetic parameters were estimated using a nonlinear model derived using standard noncompartmental methods (WinNonlin® Professional Edition, Pharsight Corporation, Mountain View, California, USA). Pharmacokinetic parameters that depend on an accurate estimation of the terminal elimination phase (λ_z and $t_{1/2}$) were reported when the pharmacokineticist deemed the data evaluable after examination.

Trial Results
Bioanalytical methods
Concentrations of GS-9137, its metabolites GS-9200 and GS-9202, and RTV in plasma samples were determined using LC-MS/MS by Gilead Sciences

(b) (4) Frozen plasma samples were received between 24 Aug 2006 and 30 Aug 2007 and stored at -80°C. Analysis was performed between 6 Sept 2006 and 7 Nov 2007. The first day of sample collection was 12 July 2006, so the maximum storage sample time was 483 days, which is not within the validated long-term frozen stability duration of 268 days for all compounds assayed (this is acceptable since the results of this trial will not be included in the product labeling). The LC-MS/MS method M-GS-9137-16511 was used for all samples; if GS-9137 concentrations were below the quantitation limit, the LC-MS/MS method M-GS-9137-16553 was used.

The GS-9137 calibration standards ranged from 20-10,000 ng/mL (M-GS-9137-16511) and 0.5-50 ng/mL (M-GS-9137-16553) and the quality control (QC) concentrations were 50.0, 750, and 7500 ng/mL (M-GS-9137-16511) and 1.5, 10, and 40 ng/mL (M-GS-9137-16553). For GS-9200 and GS-9202, the calibration

standards ranged from 20-1000 ng/mL and the QC concentrations were 50.0, 150, and 750 ng/mL. For RTV, the calibration standards ranged from 5 to 5000 ng/mL and the QC concentrations were 15.0, 750, and 4000 ng/mL. All inter-assay accuracy and precision estimates (displayed in Table 2 for M-GS-9137-16511; for M-GS-9137-16553, the inter-assay accuracy range was 0.0-6.7% and the inter-assay precision range was 7.5-20.0%) were within the acceptable range (≤20% deviation from nominal at the LLoQ concentration, and ≤15% from nominal at all other concentrations).

Table 2: Bioanalytical assay validation for GS-9137, GS-9200, GS-9202, and RTV in human plasma (source: Study Report Table 5-4)

Parameter	GS-9137	GS-9200	GS-9202	Ritonavir
Linear Range (ng/mL)	20 to 10,000	20 to 1000	20 to 1000	5 to 5000
LLQ (ng/mL)	20	20	20	5
Inter-Assay Precision Range[a]	2.1% to 6.3%	4.7% to 14.7%	3.5% to 10.9%	8.0% to 11.6%
Inter-Assay Accuracy Range[b]	−13.0% to −2.4%	−4.5% to 1.5%	−5.1% to −3.3%	−2.0% to 9.4%
Stability in Frozen Matrix (days)	268	268	268	268

LLQ = lower limit of quantitation

a   % coefficient of variation for quality control samples

b   % bias (mean percent difference from nominal concentration) for quality control samples

Source: Appendix 10

Trial population

A total of 297 subjects were randomized in the study: 73 in the CPI arm, 75 in the GS-9137/r 20/100 mg arm, 75 in the GS-9137/r 50/100 mg arm, and 74 in the GS-9137/r 125/100 mg arm. Nineteen subjects were never dosed; the remaining 278 subjects received at least one dose of study drug and were included in the intent-to-treat and safety analysis sets. Sixty-seven subjects (24%) discontinued the study prematurely: 31 because of safety, tolerability, or efficacy reasons; 15 at the discretion of the investigator; 11 withdrew consent; 7 were lost to follow-up; and 3 for protocol violations. Approximately 64% of subjects (n=179) discontinued the originally assigned study drug, primarily to switch to open-label GS-9137/r 125/100 mg (n=122). Of these 122 subjects, 60 were originally in the GS-9137/r 20/100 mg treatment group and switched when that group was discontinued, and 15 and 17 subjects switched from the blinded GS-9137/r 50/100 mg and 125/100 mg treatment groups, respectively, because of virologic failure.

Subjects in the safety analysis set (n=278) were largely white (73%) and male (90%), with a mean age of 45 years (range: 19-65 years). There were statistically significant differences between treatment groups for race, gender, body mass index, and baseline CD4+ cell count (p-values were 0.0140, 0.0110,

0.0293, and 0.0444, respectively). The median number of resistance mutations in HIV-1 protease was 11 (range: 1-18). T-20 was included in the background ARV regimens of approximately 20% of subjects.

The per protocol (PP) analysis set included the 278 subjects in the intent-to-treat (ITT) set, but excluded data collected after subjects switched from CPI/r to GS-9137, from double-blind to open-label GS-9137, or added a protease inhibitor (darunavir/r or tipranavir/r) to the background ARV regimen.

Results of pharmacokinetic analyses
A total of 36 subjects participated in the pharmacokinetic substudy at Week 8; 35 subjects (11 in the GS-9137/r 20/100 mg treatment group, 12 in the 50/100 mg group, and 12 in the 125/100 mg group) had evaluable pharmacokinetic data (PK analysis set). The pharmacokinetics of GS-9137, GS-9200, and ritonavir in plasma were assessed; however, in the presence of ritonavir, plasma concentrations of the CYP3A metabolite GS-9202 were below the limit of quantitation, precluding the evaluation of GS-9202 pharmacokinetics in this study.

In addition, a single blood draw was performed for all subjects in the GS-9137 treatment groups at Weeks 2, 4, 8 (predose), 12, 16 (predose), 20, 24 (predose), 28, 32, 36, 40, 44, and 48 (predose).

The GS-9137 PK parameters estimated for each of the dose groups (GS-9137/r 20/100, 50/100, and 125/100 mg) after 8 weeks of treatment are listed in Table 3. GS-9137 exposure increased in a more than dose-proportional manner between 20 and 50 mg and a less than dose-proportional manner between 50 and 125 mg. Similar results were observed with the GS-9200 metabolite (results not shown; refer to Study Report Table 7-30). The ratio of the mean AUC_{tau} values for GS-9200 compared to GS-9137 ranged from 14% to 24% in the three GS-9137/r treatment arms. Ritonavir PK parameters were similar between the GS-9137/r 50/100 mg and 125/100 mg treatment groups (mean AUC_{tau}: 5634.93 ng·h/mL and 5261.05 ng·h/mL, respectively), but the presence of an outlier in the GS-9137/r 20/100 mg treatment group led to a modest increase in AUC_{tau} (8549.06 ng·h/mL; refer to Study Report Table 7-31).

Table 3: Summary of GS-9137 pharmacokinetic parameters at Week 8 (PK analysis set; source: Study Report Table 7-29)

GS-9137 Plasma PK Parameters[a]	GS-9137/r 20/100 mg (N = 11)	GS-9137/r 50/100 mg (N = 12)	GS-9137/r 125/100 mg (N = 12)
C_{max} (ng/mL) Mean (%CV)	265.79 (72.77)	753.71 (30.10)	1442.20 (33.97)
AUC_{tau} (ng•h/mL) Mean (%CV)	3029.25 (84.58)	8701.86 (40.84)	16,789.54 (33.06)
C_{tau} (ng/mL) Mean (%CV)	67.28 (176.31)	211.03 (77.51)	262.99 (52.13)
T_{max} (hours) Median (Q1, Q3)	3.98 (3.00, 5.75)	4.00 (2.38, 4.75)	4.01 (2.96, 4.87)
$T_{1/2}$ (hours) Median (Q1, Q3)	6.88 (6.14, 7.47)[b]	9.14 (8.62, 12.28)[b]	8.11 (7.31, 10.07)

CV = coefficient of variation; GS-9137/r = ritonavir-boosted GS-9137; PK = pharmacokinetic; Q1 = first quartile; Q3 = third quartile

a   The PK analysis set excludes Subject 0433-2003 (GS-9137/r 125/100 mg) who did not have evaluable PK profiles at Week 8.

b   N = 10

Source: Section 11.1, Table 54

Results of efficacy analyses

Efficacy data are summarized through the end of study treatment (Week 48), although the data are confounded after Week 16 because of three changes to the study protocol: re-randomization of subjects in the CPI/r treatment arm to GS-9137, discontinuation of the GS-9137/r 20/100 mg group, and the addition of a protease inhibitor (darunavir/r or tipranavir/r) to the background ARV regimens of a subset of subjects. Week 16 is therefore the latest timepoint in the study at which prespecified comparisons between GS-9137/r and CPI/r may be made without being overly influenced by the confounding factors mentioned previously.

The lowest dose of ritonavir-boosted GS-9137, 20 mg, resulted in GS-9137 exposures that were lower than predicted, corresponding to unacceptably high rates of virologic rebound (mean change from baseline in HIV-1 RNA was -1.54 $\log_{10}$ copies/mL and -1.07 $\log_{10}$ copies/mL at Weeks 2 and 8, respectively). At Week 16, 60 of the 71 subjects randomized to GS-9137/r 20/100 mg switched to open-label GS-9137/r 125/100 mg (34 of whom also added a protease inhibitor); the remaining 11 subjects discontinued the study. By Week 24, the subjects who were originally randomized to GS-9137/r 20/100 mg were indistinguishable from the subjects who were originally randomized to GS-9137/r 125/100 mg in terms of efficacy (mean change from baseline in HIV-1 RNA was -1.70 $\log_{10}$ copies/mL). No efficacy analyses for the GS-9137/r 20/100 mg treatment group were performed.

The predefined primary and secondary efficacy endpoints for this trial were the time-weighted average changes from baseline in HIV-1 RNA (DAVG) at Weeks 24 ($DAVG_{24}$, primary endpoint) and 48 ($DAVG_{48}$, secondary endpoint). $DAVG_{16}$ was also calculated, since this was the last timepoint prior to the addition of

protease inhibitors. DAVG for Weeks 16, 24, and 48 are listed in Table 4. All three DAVG values for GS-9137/r 50/100 mg and 125/100 mg were noninferior to the comparable CPI/r DAVG values; moreover, $DAVG_{16}$ and $DAVG_{24}$ values for the GS-9137/r 125/100 mg treatment group were statistically superior to those for the CPI/r treatment group.

Table 4: Time-weighted average change from baseline in HIV-1 RNA (ITT analysis set; source: Study Report Table 7-1)

DAVG (log_{10} copies/mL)	CPI/r (N = 63)	GS-9137/r 50/100 mg (N = 71)	GS-9137/r 125/100 mg (N = 73)
$DAVG_{16}$			
Mean ± SD	−1.15 ± 1.21	−1.51 ± 1.07	−1.68 ± 1.17
Median	−1.09	−1.52	−1.59
P-value vs CPI/r		0.096	0.008
Difference (95% CI)		−0.30 (−0.66 to 0.05)	−0.48 (−0.83 to −0.13)
$DAVG_{24}$			
Mean ± SD	−1.19 ± 1.17	−1.44 ± 1.07	−1.66 ± 1.20
Median	−1.00	−1.34	−1.57
P-value vs CPI/r		0.29	0.021
Difference (95% CI)		−0.19 (−0.54 to 0.17)	−0.42 (−0.77 to −0.06)
$DAVG_{48}$			
Mean ± SD	−1.32 ± 1.12	−1.45 ± 1.03	−1.68 ± 1.21
Median	−1.33	−1.36	−1.71
P-value vs CPI/r		0.65	0.084
Difference (95% CI)		−0.08 (−0.44 to 0.27)	−0.31 (−0.67 to 0.04)

CI = confidence interval; CPI = comparator protease inhibitor; DAVG = time-weighted average change from baseline; ITT = intent-to-treat; /r = ritonavir-boosted; SD = standard deviation; T-20 = enfuvirtide

Note: P-values were obtained using analysis of variance contrast method and T-20 score was included in the model.

Differences were least square mean differences for the specified paired groups.

For ITT analysis, all available data were used, regardless of switching from CPI to GS-9137 or adding a protease inhibitor to a GS-9137-containing regimen.

Programming Details: /u/gs9137/s0105/wk_48/version6/prog/t_davghiv.sas v8.2 Output file: t_davghiv_itt.out 31OCT2008:14:34

Source: Section 11.1, Table 11

The presence of potent active background therapy (in this study, first use of T-20) during GS-9137/r administration was crucial for the maintenance of virologic suppression. A subset analysis demonstrated that subjects receiving GS-9137/r and a background ARV including T-20 had enhanced virologic response compared to those who were administered GS-9137/r without T-20 (mean $DAVG_{16}$ values were -2.80 and -1.34 in subjects who received GS-9137/r 125/100 mg with and without T-20, respectively; refer to Study Report Table 7-2).

The change from baseline in HIV-1 RNA at single timepoints was a predefined secondary endpoint for this trial (Figure 1). The change from baseline in HIV-1 RNA at Weeks 16, 24, and 48 was not significantly different for any of the GS-9137/r treatment groups compared to CPI/r (refer to Study Report Table 7-4). This lack of significant difference between any of the GS-9137/r treatment groups and CPI/r is primarily because the change from baseline in HIV-1 RNA parameter compares the average values at baseline versus the average values at subsequent timepoints. In comparison, the DAVG parameter (for which GS-9137/r 125/100 mg with T-20 in the background regimen was significantly different from CPI/r) incorporates an area-under-the-curve approach, thus capturing differences in the degree of early reductions in viral load.

Figure 1: Change from baseline in HIV-1 RNA by visit (ITT analysis set; source: Study Report Figure 7-1)
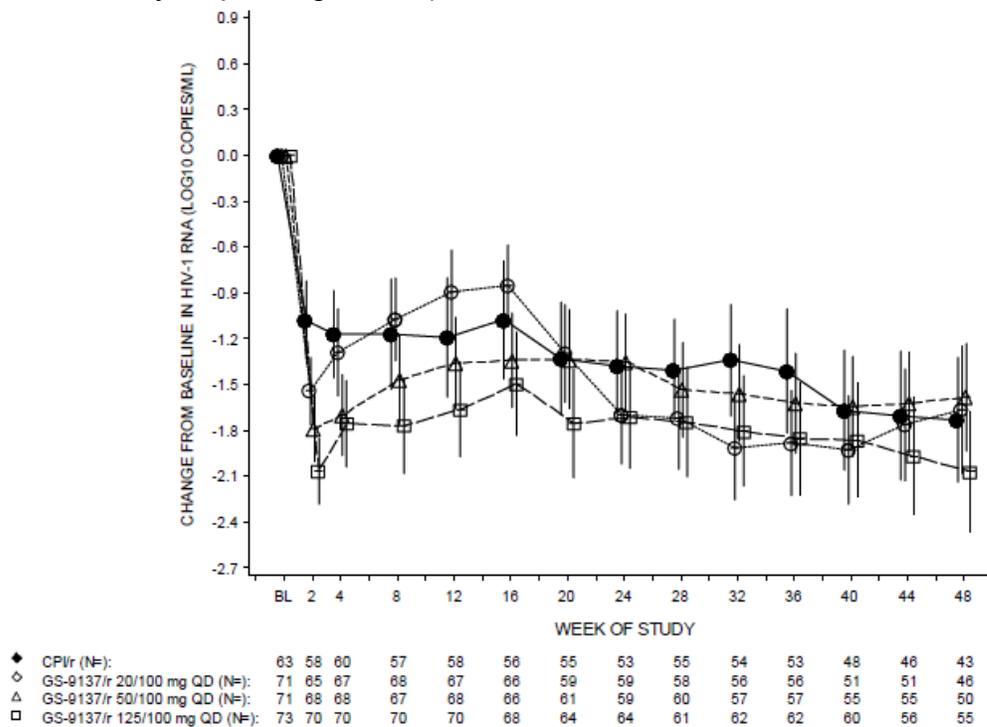

CPI = comparator protease inhibitor; ITT = intent-to-treat; QD = once daily; r/ = ritonavir-boosted
Source: Section 11.1, Figure 4

Similar to DAVG, the magnitude of the change from baseline in HIV-1 RNA was greater in subjects who were receiving T-20 as part of their ARV background regimen (shown in Figure 2; also refer to Study Report Tables 7-5, 7-6, and 7-7).

Figure 2: Impact of activity of background therapy on the mean change from baseline in HIV-1 RNA for the GS-9137/r 125/100 mg treatment group (PP analysis set; source: Study Report Figure 7-2)

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Legend:
- ‣■· No Active Drugs in OBT (n=26)
- ▸▲· ≥1 Active NRTI in OBT (n=30)
- ●— First Use of T-20 (n=17)

Y-axis: Change in HIV RNA from Baseline $\log_{10}$ copies/mL

X-axis: Week
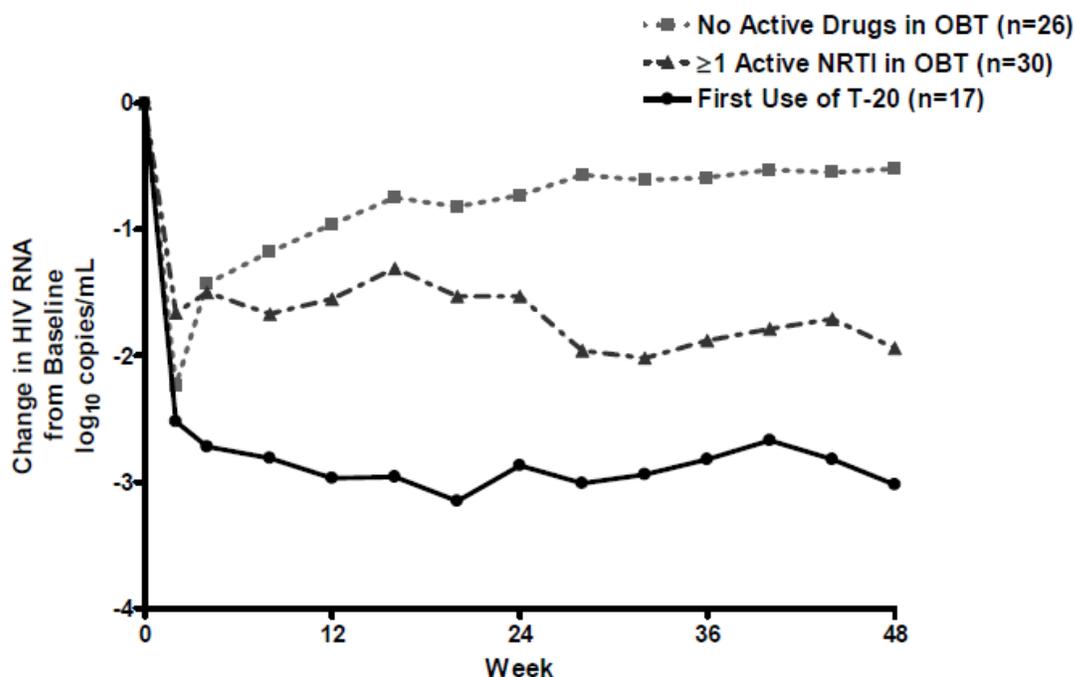
NRTI = nucleoside reverse transcriptase inhibitor; OBT = optimized background therapy; T-20 = enfuvirtide
Source: Section 11.1, Tables 23.1, 23.2, and 23.3

The majority of subjects who were treated with GS-9137/r 50/100 or 125/100 mg achieved at least a 1 $\log_{10}$ drop in HIV-1 RNA by Week 24 (91% and 92% of subjects, respectively). In the GS-9137/r 50/100 mg treatment group, 69% of subjects achieved at least a 2 $\log_{10}$ decrease in HIV-1 RNA by Week 24, while in the 125/100 mg treatment group, 76% of subjects met the same endpoint (refer to Study Report Table 7-8). For comparison, 62% and 53% of subjects receiving CPI/r achieved at least a 1 or 2 $\log_{10}$ decrease in HIV-1 RNA by Week 24. Statistical analyses indicated that GS-9137/r 50/100 and 125/100 mg had superior efficacy relative to CPI/r in terms of 1 and 2 $\log_{10}$ decreases in viral load at Week 24.

There were no statistically significant differences in time to virologic failure or virologic rebound between the GS-9137/r 50/100 mg and 125/100 mg treatment groups compared to the CPI/r treatment group.

Results of safety analysis
The safety analysis set included 278 subjects who received at least one dose of study drug. Eight subjects discontinued the study because of adverse events; the adverse events were considered treatment-related in three subjects (diarrhea, nausea, and oral hypoesthesia in one subject in the CPI/r arm; hypersensitivity in one subject in the GS-9137/r 20/100 mg arm; and convulsion in one subject in the GS-9137/r 50/100 mg arm). Three subjects died but none of the deaths were considered related to study drug. The most common (≥5%) treatment-related adverse events were diarrhea and nausea. Treatment-

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emergent serious adverse events were reported in 52 subjects, with a similar incidence across treatment groups.

Trial Summary

In this study, the pharmacokinetics of ritonavir-boosted GS-9137 (GS-9137/r 20/100, 50/100, and 125/100 mg) were assessed after 8 weeks of daily dosing. GS-9137 exposures increased in a more than dose-proportional manner between 25 and 50 mg but in a slightly less than dose-proportional manner between 50 and 125 mg. The GS-9200 metabolite exhibited a similar pharmacokinetic profile but approximately 14-24% of the exposure of GS-9137 (mean AUC_{tau}). Formation of the GS-9202 metabolite was inhibited by ritonavir; concentrations were below the limit of quantitation in all subjects. Ritonavir pharmacokinetics were comparable between treatment groups.

The results of this study demonstrated noninferiority of GS-9137/r 50/100 and 125/100 mg compared to ritonavir-boosted comparator PI in terms of the primary efficacy endpoint of time-weighted change from baseline in HIV-1 RNA at Week 24 ($DAVG_{24}$). The mean $DAVG_{16}$ and $DAVG_{24}$ values in the GS-9137/r 125/100 mg treatment group were statistically superior to CPI/r; however, an active background regimen was crucial for the maintainance of virologic suppression.

Diarrhea and nausea were the only treatment-related adverse events that occurred in at least 5% of subjects. Overall, ritonavir-boosted GS-9137 administered with a background antiretroviral regimen was well-tolerated in HIV-1-infected, treatment-experienced subjects.

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Trial GS-US-183-0113

A Phase 1, Pilot, Dose-ranging Pharmacokinetic Study to Evaluate the Effect of Ritonavir Doses on the Pharmacokinetics of GS-9137

Trial Period

27 June 2006 to 6 August 2006

Final report date: 21 April 2008 (submitted to IND   (b) (4))

Trial Site

Northwest Kinetics, Tacoma, Washington, USA

Trial Rationale

GS-9137 (elvitegravir, EVG) is an inhibitor of the human immunodeficiency virus (HIV) integrase, currently under development for the treatment of HIV infection. Results from *in vitro* studies have demonstrated potent anti-HIV activity, including activity against viruses that are resistant to nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PI). Ritonavir (RTV) is an HIV PI indicated for use in combination with other antiretroviral drugs for the treatment of HIV infection. Coadministration of antiretroviral drugs that are cytochrome P450 (CYP) isoform 3A substrates with the potent CYP3A inhibitor RTV increases the systemic exposure of the substrate drugs. The goal of this trial was to simultaneously determine the effect of a range of ritonavir doses on the pharmacokinetics of GS-9137 and on hepatic CYP3A activity using an intravenous dose of the CYP3A substrate midazolam.

Trial Objectives

The primary objectives of the trial were to:

- examine the effect of a range of RTV doses (20, 50, 100, and 200 mg QD) on the pharmacokinetics of GS-9137
- determine the effect of a range of RTV doses (20, 50, 100, and 200 mg QD) on hepatic CYP3A activity using a CYP3A substrate (midazolam)

The secondary objective of the trial was to:

- evaluate the safety and tolerability of a range of ritonavir doses in combination with GS-9137

Trial Design

This was a randomized, open-label, multiple-dose study designed to evaluate the pharmacokinetics of GS-9137 125 mg coadministered with RTV 20, 50, 100, and 200 mg (GS-9137/r) once daily and determine the effect of RTV on CYP3A activity using the probe substrate metabolism. A "leap-frog" design (in which one study group received RTV 20 and 100 mg and the other received RTV 50 and

200 mg) was used in order to shorten study duration and minimize the number of study drug exposures in subjects while still providing information about the RTV dose-response relationship. Subjects were required to take their doses of study drug at the clinic and were confined to the clinic during periods of intensive sampling. Adverse events were monitored for an additional seven days. The treatment sequence is shown in Table 1.

Table 1: Treatment sequence (Treatment A: GS-9137/r 125/20 mg QD; Treatment B: GS-9137/r 125/50 mg QD; Treatment C: GS-9137/r 125/100 mg QD; Treatment D: GS-9137/r 125 200 mg QD; source: Study Report Table 5-1)

Day	Treatment and/or Midazolam Probe	PK Assessment Conducted
Group 1		
1	Midazolam	Midazolam
2–10	A: in the morning	None
11	A: in the morning Midazolam: 6 hours postdose	GS-9137, Ritonavir, and Midazolam
12–20	C: in the morning	None
21	C: in the morning Midazolam: 6 hours postdose	GS-9137, Ritonavir, and Midazolam
Group 2		
1	Midazolam	Midazolam
2–10	B: in the morning	None
11	B: in the morning Midazolam: 6 hours postdose	GS-9137, Ritonavir, and Midazolam
12–20	D: in the morning	None
21	D: in the morning Midazolam: 6 hours postdose	GS-9137, Ritonavir, and Midazolam

Rationale for Dose Selection

The GS-9137/r dose of 125/100 mg QD was the highest dose in a Phase 2 study that was ongoing at the time of this study. This dose was expected to provide an upper limit of GS-9137 levels observed during planned clinical studies. Preliminary PK data from drug-drug interaction studies suggested that BID dosing of RTV did not have an additional boosting effect on GS-9137 exposure compared to RTV QD dosing. GS-9137/r was dosed for 10 days in each treatment arm in order to ensure steady-state pharmacokinetics.

The midazolam dose of 1 mg is subtherapeutic (RTV and oral midazolam are contraindicated at their therapeutic doses) and is the dose often used in pharmacokinetic studies.

Drug Administration

Ritonavir oral solution mixed with 25 mL of Ensure® nutritional supplement was administered to study subjects within 5 min of completion of a standardized meal (with approximately 400 kcal and 13 g fat), at approximately the same time every day. Immediately following the RTV dose, subjects were administered GS-9137 with water (240 mL, some of which was used to rinse the RTV dosing cup).

Consumption of water and food was restricted on Days 11 and 21. No water was allowed up to 1 h before and 2 h after study drug administration, and subjects fasted until the 4 h post-dose blood draw.

Midazolam was administered intravenously (slow IV push, up to 1 min in duration) to subjects in either a supine or sitting position, to the arm opposite the location of the blood draw site. Subjects were asked to remain in either a supine or sitting position for at least 2 h after administration for safety monitoring.

Investigational Product
GS-9137 125 mg tablets (Lot AJ603A1) and ritonavir (NORVIR®, Abbott Laboratories, Lot 39098AW21) 80 mg/mL oral solution were used in all treatments. Midazolam hydrochloride salt was acquired commercially ((b) (4)
    Lot 38-238-DK) as a sterile, nonpyrogenic parenteral dosage form for IV and IM injection.

Key Inclusion and Exclusion Criteria
Subjects were healthy nonsmoking males and females between the ages of 18 and 45 years, inclusive, with BMI between 19 and 30 kg/m^2, inclusive, and normal electrocardiogram (ECG).

Potential subjects were excluded from enrollment if they were pregnant or lactating, or if they had any abnormal finding of clinical relevance during the medical examination or any diseases or infections of clinical relevance. Exclusion criteria also included previous exposure to investigational drugs (within 30 days of study drug administration), the use of alcohol or illicit drugs, and the use of any of the following drugs within the specified timeframes:

- any prescription medications and over-the-counter medications including herbal products and antacids, but with the exception of vitamins, acetaminophen, ibuprofen, and/or hormonal contraceptives (within 30 days prior to study drug administration, or 60 days if the elimination half-life is longer than 10 days)
- contraindicated drugs (within 30 days prior to study screening): α_1-adrenoreceptor antagonist, antiarrhythmics, antihistamines, ergot derivatives, GI motility agent, neuroleptics, sedative/hypnotics, antineoplastics, herbal products, HMG-CoA reductase inhibitors, proton-pump inhibitors

- hepatotoxic drugs (within 3 months prior to study screening): e.g. anabolic steroids, itraconazole, isoniazid, ketoconazole, lovastatin, HIV protease inhibitors, rifabutin, rifampin, simvastin
- other drugs (within 3 months prior to study screening): systemic steroids, immunosuppressants, chemotherapeutic agents, immune- or cytokine-based therapies

Concomitant Medications

No concomitant medications, over-the-counter medications, or herbal products were permitted except for mild laxatives, acetaminophen, and ibuprofen.

Restrictions were placed on the consumption (prior to and during the trial) of products containing caffeine and xanthine; calcium, magnesium, or aluminum (antacids) or calcium, iron, or zinc (vitamin and mineral supplements); the ulcer medicine sucralfate; and certain fruit juices and citrus fruits.

Sample Collection

Blood was collected for pharmacokinetic analysis of GS-9137 and RTV at each of the following timepoints (in hours after dosing):

Days 1, 11, and 21 0:00 (predose), 0:30, 1:00, 1:30, 2:00, 3:00, 3:30, 4:00, 4:30, 5:00, 5:30, 6:00, 8:00, 10:00, 12:00, 14:00, 16:00, 18:00, and 24:00

Blood was collected for pharmacokinetic analysis of midazolam at each of the following timepoints (in hours after dosing):

Days 11 and 21   0:00 (predose), 0:05, 0:10, 0:15, 0:30, 1:00, 2:00, 4:00, 6:00, 8:00, 10:00, 12:00, and 18:00

One primary and one back-up sample were collected for each PK analysis, so that either two (GS-9137/r or midazolam) or four (GS-9137/r and midazolam) samples were collected at each timepoint.

Analytical Plan

Pharmacokinetic data

The pharmacokinetic parameters C_{max}, T_{max}, C_{last}, T_{last}, C_{tau}, λ_z, AUC_{tau}, $t_{1/2}$, CL/F, and V_z/F were estimated for GS-9137, M4, and RTV in plasma; and AUC_{0-last}, $AUC_{0-\infty}$, $\%AUC_{exp}$, C_{last}, T_{last}, λ_z, $t_{1/2}$, CL, and V_z were estimated for midazolam in plasma. The primary pharmacokinetic parameters were AUC_{tau}, C_{max}, and C_{tau} (GS-9137, M4, and RTV) and AUC_∞ and AUC_{0-last} (midazolam). These parameters were estimated using a nonlinear model derived using standard noncompartmental methods (WinNonlin® Professional Edition, Pharsight Corporation, Mountain View, California, USA). Pharmacokinetic parameters for N1 and 1'-OH midazolam were not conducted due to plasma

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concentrations below the limit of quantitation at most timepoints. Additionally, the study was only powered for the assessment of GS-9137 AUC_{tau} and C_{max}; therefore, this study may not be adequately powered to provide accurate estimates of any other pharmacokinetic parameters for GS-9137, M4, RTV, or midazolam.

Statistical comparisons of parameters between RTV dosing levels was assessed using a nonparametric Kruskal-Wallis test (overall treatment effect) or the nonparametric Wilcoxon signed rank (paired data) and rank sum (unpaired data) tests. Ad hoc parametric analyses using mixed effects models of clearance were also performed.

Trial Results
Bioanalytical methods
Quantitative determination of GS-9137, GS-9202, GS-9200, and RTV
Concentrations of GS-9137, its metabolites GS-9202 and GS-9200, and RTV in plasma samples were measured by LC-MS/MS by Gilead Sciences (b) (4) (b) (4) Frozen plasma samples were received between 20 July and 10 Aug 2006 and stored at -80˚C. Analysis was performed between 29 Aug and 5 Oct 2006. The first day of sample collection was 11 July 2006, so the maximum storage sample time was 86 days, which is within the validated long-term frozen stability duration of 93 days for GS-9137 and its metabolites and 102 days for RTV. The LC-MS/MS methods M-GS-9137-16511 (for quantification of GS-9137, GS-9202, GS-9200, and RTV 5-5000 ng/mL) and M-GS-9137-16553 (for quantification of RTV 0.5-50 ng/mL) were used.

The calibration standards for GS-9137 ranged from 20.0-10,000 ng/mL; for GS-9202 and GS-9200, calibration standards ranged from 20.0-1000 ng/mL. The quality control (QC) concentrations for GS-9137 were 50.0, 750.0, and 7500 ng/mL; for GS-9200 and GS-9202, QC concentrations were 50.0, 150, and 750 ng/mL. For higher concentrations of RTV, the calibration standards ranged from 5.0-5000 ng/mL and the QC concentrations were 15.0, 750, and 4000 ng/mL), while for lower concentrations of RTV, the calibration standards ranged from 0.5-50 ng/mL and the QC concentrations were 1.5, 10, and 40 ng/mL). All inter-assay accuracy and precision estimates (% CV, displayed in Table 2) were within the acceptable range (≤20% deviation from nominal at the LLoQ concentration, and ≤15% from nominal at all other concentrations).

Quantitative determination of midazolam
Midazolam concentrations in plasma samples were measured by LC-MS/MS by (b) (4) Frozen plasma samples were received between 18 July and 8 Aug 2006 and stored at -70˚C. Analysis was performed between 26 Jul and 20 Sep 2006. The maximum storage sample time was 70 days, which is within the validated long-term frozen

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stability duration of 135 days. The LC-MS/MS method [b] [4] 42-0624 was used to determine midazolam plasma concentrations.

The calibration standards for midazolam ranged from 0.1 to 100 ng/mL. The QC concentrations were 0.1, 0.3, 5, 30, and 75 ng/mL. The inter-assay accuracy and precision estimates (% CV, displayed in Table 2) were within the acceptable range (≤20% deviation from nominal at the LLoQ concentration, and ≤15% from nominal at all other concentrations).

Table 2: Bioanalytical assay validation for GS-9137, GS-9200, GS-9202, RTV, and midazolam (source: Study Report Table 5-7)

	GS-9137[a]		M4	M1	Ritonavir[a]		
	Low	High	(GS-9200)	(GS-9202)	Low	High	Midazolam
Linear Range (ng/mL)	0.5 to 50	20 to 10,000	20 to 1,000	20 to 1,000	0.5 to 50	5 to 5000	0.1 to 100
LLQ[b] (ng/mL)	0.5	20	20	20	0.5	5	0.1
Inter-Assay Precision Range[c]	7.5% to 20.0%	2.1% to 6.3%	4.7% to 14.7%	3.5% to 10.9%	0.0% to 6.7%	8.0% to 11.6%	2.4 to 7.1%
Inter-Assay Accuracy Range[d]	0.0% to 6.7%	−13.0% to −2.4%	−4.5% to 1.5%	−5.1% to −3.3%	−2.8% to 0.0%	−2.0% to 9.4%	−2.7% to −1.0%
Stability in Frozen Matrix (days)	—	93	93	93	—	102	135

a   A low range calibration curve for GS-9137 (0.5 to 50 ng/mL) and ritonavir (0.5 to 50 ng/mL) was used in addition to the 20 to 10,000 ng/mL (GS-9137) and 5 to 5,000 ng/mL (ritonavir) range.

b   LLQ, lower limit of quantitation

c   Relative standard deviation

d   Difference from nominal concentrations

Source: Appendix 10

Details about the bioanalytical sample analysis can be found in Appendix 10 of the Study Report (Report S-183-15 V2 for GS-9137, GS-9200, GS-9202, and RTV; [b] [4] Project 60-0623 Document 20009v1 for midazolam).

Trial population
A total of 24 healthy subjects were enrolled in the study (12 in each treatment sequence) and were included in the safety analysis set; 21 completed the study and were included in the pharmacokinetic analysis set. The majority of subjects in both analysis sets were white (63% and 67% in the safety and pharmacokinetic analysis sets, respectively) and male (63% and 67% in the safety and pharmacokinetic analysis sets, respectively). Other baseline

characteristics (such as age, BMI, and creatinine clearance) were similar between the two analysis sets.

Three subjects discontinued the study prematurely: Subject 1008 withdrew consent and discontinued study drug on Day 10, Subject 1023 discontinued on Day 20 due to a positive pregnancy test, and Subject 1024 discontinued on Day 10 due to drug-related hypertension.

Steady-state pharmacokinetics of GS-9137

The mean (± SD) plasma concentration-time profiles of GS-9137 after oral administration with increasing doses of RTV (20, 50, 100, and 200 mg) are shown in Figure 1. GS-9137 concentrations increased in a RTV-dose dependent manner; this increase was less than dose-proportional.

Figure 1: Plasma concentration-time profile of GS-9137 with increasing doses of RTV (PK analysis set; mean ± SD; semilog scale; source: Study Report Figure 7-1)
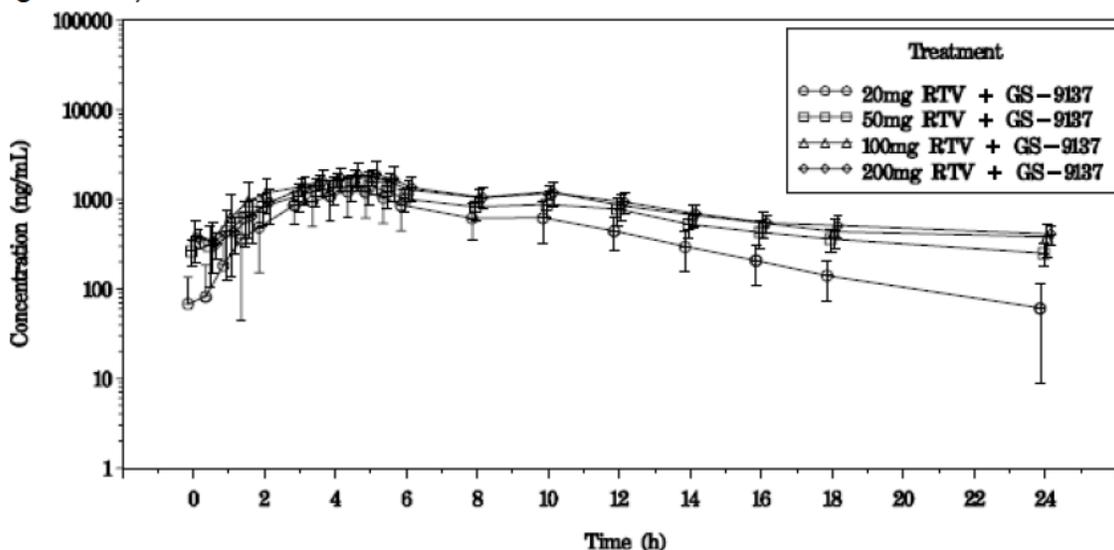
Source: Section 11.1, Figure 1.1 (semi-logarithmic scale)

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Steady-state pharmacokinetic parameters of GS-9137 in plasma after administration with RTV 20, 50, 100, and 200 mg are listed in Table 3. The median T_{max} was between 4-5 h for all RTV doses, but the median half-life ranged from 4.04 h to 13.25 h and increased with RTV dose. GS-9137 CL/F was inversely affected by RTV dose, decreasing as the dose of RTV increased, although this relationship reached a plateau by the 100 mg dose.

Table 3: Summary of pharmacokinetic parameters of GS-9137 with increasing doses of RTV (PK analysis set; source: Study Report Table 7-1)

GS-9137 Plasma PK Parameters[a]	GS-9137 + 20-mg RTV (N = 11)	GS-9137 + 50-mg RTV (N = 10)	GS-9137 + 100-mg RTV (N = 11)	GS-9137 + 200-mg RTV (N = 10)
C_{max} (ng/mL) Mean (%CV)	1369.9 (43.0)	1558.2 (36.4)	1830.1 (19.9)	2032.1 (37.4)
AUC_{tau} (ng·h/mL) Mean (%CV)	10,204.5 (36.4)	15,793.0 (24.5)	20,235.9 (24.6)	20,544.0 (24.2)
C_{tau} (ng/mL) Mean (%CV)	60.9 (85.6)	250.8 (27.7)	380.2 (39.9)	410.1 (26.0)
T_{max} (hours) Median (Q1, Q3)	4.00 (3.00, 5.00)	4.01 (3.50, 5.00)	5.00 (4.00, 5.00)	4.75 (4.00, 5.00)
$T_{\frac{1}{2}}$ (hours) Median (Q1, Q3)	4.04 (3.21, 4.70)	8.87 (8.02, 9.52)	9.94 (9.58, 12.65)	13.25 (10.62, 15.09)
T_{last} (hours) Median (Q1, Q3)	24.00 (21.80, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
CL/F (mL/h) Mean (%CV)	14,155.1 (43.7)	8407.9 (27.7)	6488.9 (22.0)	6468.9 (28.5)
CL/F ratio (%)[b] (95% CI)				
vs. 20-mg RTV	nc	62.16 (47.34, 81.60)	48.34 (39.13, 59.73)	47.78 (36.40, 62.73)
vs. 50-mg RTV	nc	nc	77.78 (59.24, 102.11)	76.88 (61.58, 95.97)
vs. 100-mg RTV	nc	nc	nc	98.84 (75.29, 129.76)

RTV, ritonavir; PK, pharmacokinetic; nc, not calculated

a   The pharmacokinetic analysis set excludes Subjects 1008, 1023, and 1024 because they did not have evaluable pharmacokinetic profiles for each treatment pair.

b   Mixed-model (ANOVA)–based ratios. CL/F of GS-9137 at each RTV dose vs. 20 mg RTV, $p \leq 0.0016$; RTV 100 mg vs. 50 mg, $p = 0.068$; RTV 200 mg vs. 50 mg, $p = 0.023$; RTV 200 mg vs. 100 mg, $p = 0.929$.

Source: Section 11.1, Table 5.1 and Table 6.4

When an E_{max} model was applied to the GS-9137 AUC_{tau} data and RTV dose, the ED_{50} was estimated to be approximately 25.9 mg RTV, reaching maximal effect (i.e. maximal inhibition of CYP3A) between 50 and 100 mg RTV. RTV doses above 100 mg did not result in any additional increase in GS-9137 exposure.

Statistical comparisons of GS-9137 PK parameters after administration of GS-9137 with increasing doses of RTV are listed in Table 4. The increase in GS-9137 exposure was dose-dependent but in a less than dose-proportional manner. Comparing the RTV-boosted GS-9137 exposures from this study to historical unboosted GS-9137 exposure data (GS-US-183-0102), changes in AUC_{tau} indicate substantial decreases in GS-9137 clearance (via inhibition of metabolism) at RTV doses as low as 20 mg, reaching maximal effect between 50 and 100 mg, with no additional effect at doses above 100 mg.

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Table 4: Statistical comparisons of pharmacokinetic parameters of GS-9137 with increasing doses of RTV (PK analysis set; source: Study Report Table 7-2)

Test Versus Reference Comparison of GS-9137 Plasma PK Parameters[a]	Geometric Least Squares Mean Ratio (GMR%, 90% CI)		
Test: GS-9137 +	**50-mg RTV**	**100-mg RTV**	**200-mg RTV**
Reference: GS-9137 + 20-mg RTV			
AUC_{tau} (ng·h/mL)	160.89 (128.49, 201.46)	206.85 (173.69, 246.34)	209.28 (167.14, 262.06)
C_{max} (ng/mL)	117.46 (90.01, 153.29)	143.20 (117.57, 174.40)	152.02 (116.49, 198.38)
C_{tau} (ng/mL)	400.21 (285.58, 560.84)	586.39 (444.40, 773.75)	658.03 (469.56, 922.15)
Test: GS-9137 +	**20-mg RTV**	**100-mg RTV**	**200-mg RTV**
Reference: GS-9137 + 50-mg RTV			
AUC_{tau} (ng·h/mL)	nc	128.57 (102.68, 160.99)	130.08 (108.30, 156.24)
C_{max} (ng/mL)	nc	121.91 (93.42, 159.09)	129.42 (105.24, 159.14)
C_{tau} (ng/mL)	nc	146.52 (106.13, 202.29)	164.42 (125.33, 215.71)
Test: GS-9137 +	**20-mg RTV**	**50-mg RTV**	**200-mg RTV**
Reference: GS-9137 + 100-mg RTV			
AUC_{tau} (ng·h/mL)	nc	nc	101.17 (80.80, 126.69)
C_{max} (ng/mL)	nc	nc	106.16 (81.35, 138.53)
C_{tau} (ng/mL)	nc	nc	112.22 (81.28, 154.93)

RTV, ritonavir; PK, pharmacokinetic; CI, confidence interval; nc, not calculated

a   The pharmacokinetic analysis set excludes Subjects 1008, 1023, and 1024 because they did not have evaluable pharmacokinetic profiles for each treatment pair.

Source: Section 11.1, Table 6.1

Steady-state pharmacokinetics of GS-9200 (M4)

The mean (± SD) plasma concentration-time profiles of the GS-9137 glucuronide metabolite M4 after oral administration of GS-9137 with increasing doses of RTV (20, 50, 100, and 200 mg) are shown in Figure 2. Similar to GS-9137 concentrations, concentrations of M4 increased in a RTV-dose dependent manner. M4 concentrations were BLoQ in most subjects by 12 h after administration of GS-9137/r.

Figure 2: Concentration-time profile of M4 in plasma after administration of GS-9137 with increasing doses of RTV (mean ± SD; semilog scale; PK analysis set; source: Study Report Figure 7-2)
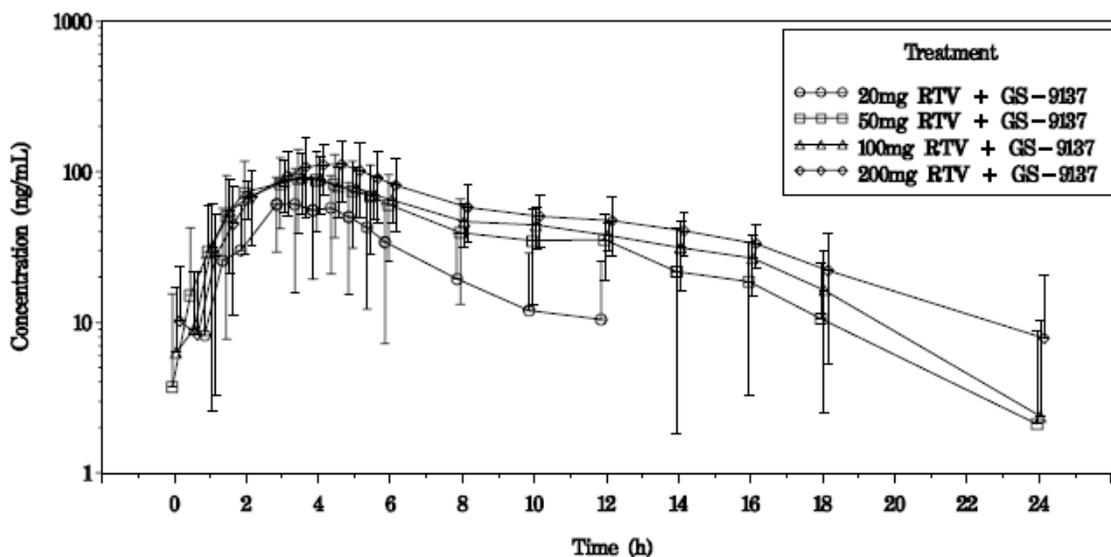
Source: Section 11.1, Figure 1.4 (semi-logarithmic scale)

Pharmacokinetic parameters of M4 in plasma after multiple doses of GS-9137 with 20, 50, 100, and 200 mg RTV are listed in Table 5. M4 exposure (i.e. AUC_{tau}) comprised <10% of the corresponding GS-9137 exposures. There was no change in the metabolite:parent ratio as RTV dose changed, indicating that M4 maintained linear pharmacokinetics based on systemic exposure.

Table 5: Summary of pharmacokinetic parameters for M4 following administration of GS-9137 with increasing doses of RTV (PK analysis set; source: Study Report Table 7-2)

M4 Plasma PK Parameters[a]	GS-9137 + 20-mg RTV (N = 11)	GS-9137 + 50-mg RTV (N = 10)	GS-9137 + 100-mg RTV (N = 11)	GS-9137 + 200-mg RTV (N = 10)
C_{max} (ng/mL) Mean (%CV)	81.6 (37.3)	110.2 (40.3)	106.8 (32.6)	122.5 (46.5)
AUC_{tau} (ng·h/mL) Mean (%CV)	450.1 (59.4)	890.4 (43.7)	965.5 (26.8)	1155.3 (37.4)
T_{max} (hours) Median (Q1, Q3)	3.00 (1.50, 3.50)	2.00 (2.00, 3.52)	3.00 (1.50, 3.50)	4.04 (4.00, 4.50)
$T_{1/2}$ (hours) Median (Q1, Q3)	3.27 (2.28, 6.39)	6.51 (5.68, 9.02)	8.23 (6.92, 10.53)	9.46 (8.39, 11.62)
T_{last} (hours) Median (Q1, Q3)	8.00 (5.50, 12.00)	16.02 (12.00, 18.00)	18.00 (16.05, 18.00)	18.00 (16.00, 24.00)

RTV, ritonavir; PK, pharmacokinetic

a   The pharmacokinetic analysis set excludes Subjects 1008, 1023, and 1024 because they did not have evaluable pharmacokinetic profiles for each treatment pair.

Source: Section 11.1, Table 5.4

Single dose pharmacokinetics of midazolam

The mean (± SD) concentration-time profile of midazolam after administration alone (Day 1) or after coadministration with steady-state GS-9137/r 125/20, 125/50, 125/100, or 125/200 mg (Days 11 and 21) is shown in Figure 3. Coadministration of GS-9137/r substantially increased mean midazolam plasma concentrations, presumably via RTV inhibition of CYP3A metabolism of midazolam. The increase in midazolam plasma concentrations was RTV dose-dependent but not dose-proportional.

Figure 3: Concentration-time profile of midazolam in plasma after administration of a single dose of midazolam and after coadministration with multiple doses of GS-9137 plus RTV (PK analysis set; mean ± SD; semilog scale; source: Study Report Figure 7-3)
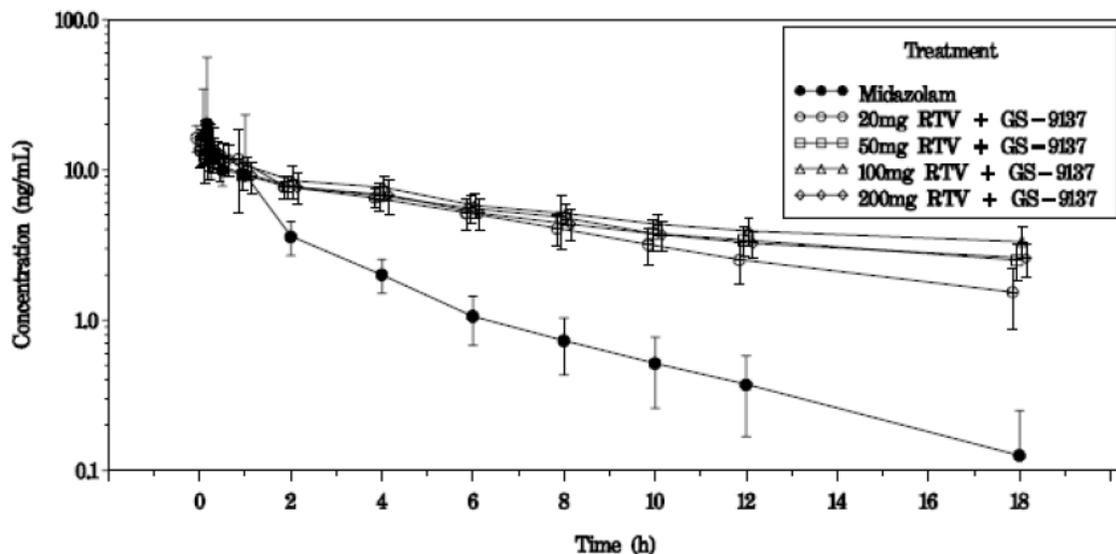
Source: Section 11.1, Figure 1.2 (semi-logarithmic scale)

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Pharmacokinetic parameters of a single dose of midazolam in plasma before and after coadministration with GS-9137/r are listed in Table 6. Reductions in midazolam clearance were less than proportional to RTV dose, with little difference in midazolam exposures with RTV 100 and 200 mg. Inhibition of hepatic CYP3A may be discerned because midazolam was administered intravenously; maximal inhibition of hepatic CYP3A occurred at RTV doses of 100 or 200 mg (82 or 85% reduction in CYP3A activity, respectively). The majority of the increase in midazolam exposure was achieved by the 20 mg dose (66% reduction in midazolam clearance).

Overall, the midazolam data appear to be in agreement with the GS-9137 results, which demonstrated that near-maximal inhibition of CYP3A occurred between RTV doses of 50 and 100 mg

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Table 6: Summary of pharmacokinetic parameters of midazolam after administration of midazolam alone or with GS-9137 plus RTV (PK analysis set; source: Study Report Table 7-4)

MDZ Plasma PK Parameters[a]	MDZ Alone (N = 21)	GS-9137 + 20-mg RTV (N = 11)	GS-9137 + 50-mg RTV (N = 10)	GS-9137 + 100-mg RTV (N = 11)	GS-9137 + 200-mg RTV (N = 10)
AUC_{inf} (ng•h/mL) Mean (%CV)	31.0 (31.0)	99.0 (22.8)	139.6 (25.4)	211.0 (37.6)	151.9 (24.4)
AUC_{last} (ng•h/mL) Mean (%CV)	29.8 (31.4)	80.9 (18.8)	89.6 (19.8)	101.4 (15.2)	87.6 (19.1)
% AUC_{exp} Mean (%CV)	3.9 (58.9)	17.4 (45.8)	33.9 (33.1)	48.1 (26.0)	40.1 (32.9)
C_{last} (ng/mL) Mean (%CV)	0.2 (40.2)	1.5 (42.4)	2.5 (26.7)	3.3 (23.1)	2.6 (24.9)
$T_{1/2}$ (hours) Median (Q1, Q3)	3.92 (2.83, 4.92)	6.91 (5.75, 9.83)	12.89 (11.18, 13.35)	16.85 (15.49, 23.69)	16.62 (11.61, 18.68)
T_{last} (hours) Median (Q1, Q3)	18.00 (12.00, 18.00)	18.00 (18.00, 18.00)	18.00 (18.00, 18.00)	18.00 (18.00, 18.00)	18.00 (18.00, 18.00)
CL (mL/h) Mean (%CV)	34,814.2 (27.7)	10,603.1 (23.5)	7653.6 (29.3)	5220.4 (29.2)	7040.5 (31.3)
CL ratio (%)[b] (95% CI)					
vs. 0-mg RTV	nc	34.47 (29.35, 40.49)	19.52 (16.49, 23.11)	16.64 (14.17, 19.54)	17.93 (15.14, 21.22)
vs. 20-mg RTV	nc	nc	56.62 (44.85, 71.49)	48.26 (41.09, 56.69)	52.00 (41.19, 65.65)
vs. 50-mg RTV	nc	nc	nc	85.24 (67.52, 107.61)	91.84 (77.58, 108.72)
vs. 100-mg RTV	nc	nc	nc	nc	107.74 (85.34, 136.03)

RTV, ritonavir; PK, pharmacokinetic; MDZ; midazolam; nc, not calculated

a   The pharmacokinetic analysis set excludes Subjects 1008, 1023, and 1024 because they did not have evaluable pharmacokinetic profiles for each treatment pair.

b   Mixed-model (ANOVA)–based ratios. MDZ CL at all RTV doses vs. 0 mg RTV, $p < 0.0001$; RTV 100 mg or 200 mg vs. 50 mg, $p = 0.173$ and 0.313, respectively; RTV 200 mg vs. 100 mg, $p = 0.521$.

Source: Section 11.1, Table 5.2 and Table 6.4

Statistical comparisons of midazolam PK parameters after administration of GS-9137 with increasing doses of RTV are listed in Table 7. The increase in midazolam exposure was dose-dependent but in a less than dose-proportional manner, reaching maximal effect between RTV doses of 50 and 100 mg.

Table 7: Statistical comparisons of pharmacokinetic parameters of midazolam with GS-9137/r (PK analysis set; source: Study Report Table 7-5)

Test Versus Reference Comparison of MDZ Plasma PK Parameters[a]	Geometric Least Squares Mean Ratio (GMR%, 90% CI)			
Test:	MDZ+GS-9137 + 20-mg RTV	MDZ+GS-9137 + 50-mg RTV	MDZ+GS-9137 + 100-mg RTV	MDZ+GS-9137 + 200-mg RTV
Reference: MDZ Alone				
AUC_{inf} (ng•h/mL)	304.56 (267.97, 346.16)	485.82 (425.11, 555.19)	631.05 (555.22, 717.23)	528.97 (462.87, 604.50)
AUC_{last} (ng•h/mL)	259.65 (234.86, 287.06)	330.97 (298.07, 367.50)	327.62 (296.33, 362.20)	323.73 (291.55, 359.47)

RTV, ritonavir; MDZ, midazolam; PK, pharmacokinetic; CI, confidence interval

a   The pharmacokinetic analysis set excludes Subjects 1008, 1023, and 1024 because they did not have evaluable pharmacokinetic profiles for each treatment pair.

Source: Section 11.1, Table 6.1

Steady-state pharmacokinetics of RTV

The mean (± SD) concentration-time profile of ritonavir after 10 days of oral administration of GS-9137 with RTV 20, 50, 100, or 200 mg is shown in Figure 4. RTV plasma concentrations exhibited a greater than dose-proportional increase.

Figure 4: Concentration-time profile of RTV in plasma after administration of multiple doses of GS-9137 plus increasing doses of RTV (PK analysis set; mean ± SD; semilog scale; source: Study Report Figure 7-4)
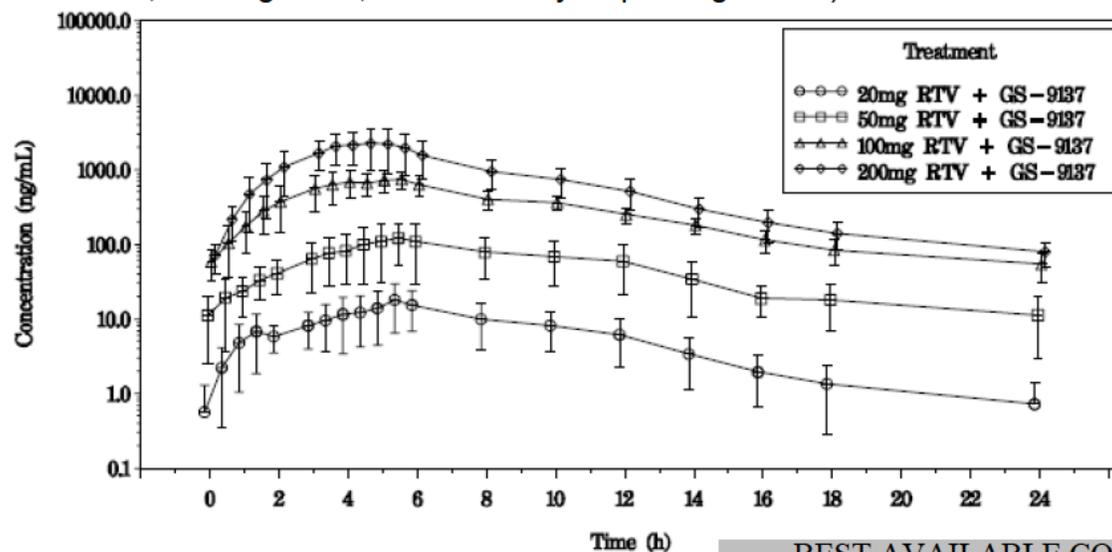

Source: Section 11.1, Figure 1.3 (semi-logarithmic scale)

The greater than dose-proportional increases in C_{max}, AUC_{tau}, and C_{tau}, along with reductions in interindividual variability, indicate non-linear PK. RTV clearance was significantly reduced with increasing dose, decreasing by nearly 70% when the dose was increased from 20 mg to 50 mg (CL/F decreased from

198,850 mL/h to 59,650 mL/h). At doses of 100 and 200 mg, RTV clearance reached a nadir of <10% of clearance after a 20 mg dose. Over a 10-fold range of RTV doses, AUC increased 119-fold (AUC_{tau} values for RTV doses of 20, 50, 100, and 200 mg were 134.4, 1118.8, 6528.4, and 15,960.4 ng·h/mL, respectively).

Ritonavir dose-response relationship for midazolam and GS-9137 clearance
Figure 5 shows an inhibitory dose-response curve that was fitted to weight-normalized substrate clearance data (GS-9137 CL/F and midazolam CL) and RTV doses (see Figure 5). Midazolam CL fit well to a simple inhibitor E_{max} model with an estimated ED_{50} (the estimated dose of RTV that will result in 50% inhibition of hepatic CYP3A) of 12.2 mg. Historical unboosted GS-9137 exposure data from GS-US-183-0102 was used to fit a simple E_{max} model for RTV dose-response. The estimated ED_{50} using GS-9137 AUC_{tau} was 25.9 mg RTV. The midazolam and GS-9137 analyses suggest that RTV doses between 20 and 50 mg substantially inhibit both gut-associated and hepatic CYP3A.

Figure 5: Dose-response relationship for midazolam and GS-9137 clearance
(source: Study Report Figure 7-5)
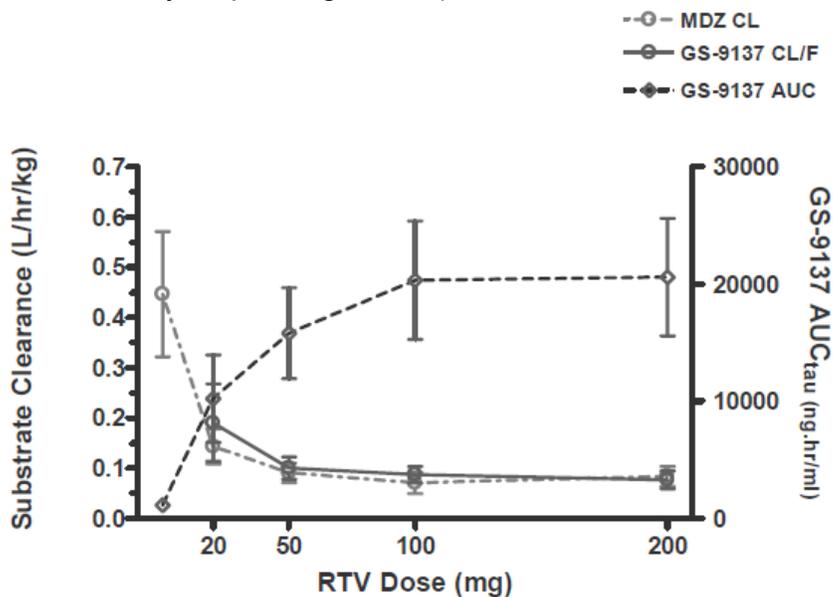
Source: Section 11.1, Table 5.1, Table 9.1 and Table 10.1

Results of safety analysis
The safety set contained all 24 enrolled subjects, since they all received at least one dose of study drug. The most common adverse events were headache, fatigue, nausea, and diarrhea. One subject in the GS-9137/r 125/50 mg experienced Grade 2 drug-related hypertension and subsequently discontinued study drug. One SAE occurred during the trial: a pregnancy that ended in an early-term miscarriage with "no clear-cut etiology."

Seven out of 12 subjects (58%) receiving GS-9137/r 125/20 mg reported a treatment-emergent adverse event; for the GS-9137/r 125/50 mg group, that number was 8 of 12 (75%); for the GS-9137/r 125/100 mg group, it was 7 of 11 (64%); and for the GS-9137/r 125/200 mg group, it was 5 of 11 (46%). There were no treatment-emergent Grade 3 or 4 AEs in this study. Overall, GS-9137/r was generally safe and well-tolerated at all four doses of RTV.

None of the subjects who received midazolam had an AE that was considered by the investigator to be drug-related. No serum chemistry, hematology, or urinalysis abnormalities were reported as AEs.

Trial Summary

Ritonavir increased exposure of GS-9137 and the CYP3A probe substrate midazolam by reducing CYP3A-mediated clearance in a non-linear dose-dependent manner. The increase in exposures reached a plateau around 100 mg doses of RTV. Exposures of the M4 (GS-9200) glucuronide metabolite comprised <10% of the corresponding GS-9137 exposure. The presence of RTV did not affect the parent:metabolite plasma concentration ratio, indicating that M4 formation and elimination was independent of CYP3A.

Based on a RTV dose-response curve fitted to midazolam clearance data, the ED50 for hepatic CYP3A was 12.2 mg, indicating that RTV substantially inhibits hepatic CYP3A at doses as low as 20 mg. Likewise, changes in GS-9137 CL/F indicate RTV-inhibition of first-pass effect, as well as a reduction in systemic clearance, at RTV doses as low as 20 mg. These data suggest that RTV doses of 50 to 100 mg are sufficient to boost GS-9137 exposure; doses above 100 mg are unlikely to result in further increases in GS-9137 plasma concentrations.

Trial GS-US-183-0119
A Phase 1, Open-Label, Randomized Pharmacokinetic Drug Interaction Study of GS-9137/r and Antacid or Omeprazole

Trial Period
14 Aug to 5 Oct 2006
Final report date: 30 May 2007 (submitted to IND ^(b) (4))

Trial Site
Northwest Kinetics, Inc., Tacoma, Washington, USA

Trial Rationale
GS-9137 (elvitegravir, EVG) is an inhibitor of the human immunodeficiency virus (HIV) integrase, currently under development for the treatment of HIV infection. Results from *in vitro* studies have demonstrated potent anti-HIV activity, including activity against viruses that are resistant to nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PI). Coadministration of GS-9137 with the HIV PI ritonavir (RTV; GS-9137/r) results in a 20-fold increase in GS-9137 exposures due to the inhibition of CYP3A activity by RTV. Integrase inhibitors such as GS-9137 are known to complex with divalent cations in the active site of the HIV integrase enzyme, leading to a local drug interaction in the GI tract. Study GS-US-183-0103 demonstrated a 45% decrease in GS-9137 AUC_{tau} following simultaneous administration of GS-9137/r and antacid. This study was conducted to evaluate the effect of staggered administration of antacids and other acid-reducing medications commonly used by patients infected with HIV-1 on the steady-state pharmacokinetics of GS-9137/r.

Trial Objectives
The primary objectives of the trial were to:

- evaluate whether the pharmacokinetics of GS-9137 are affected after staggered administration of GS-9137/r and antacid compared to administration of GS-9137/r alone
- evaluate whether the pharmacokinetics of GS-9137 are affected after coadministration of GS-9137/r and omeprazole compared to administration of GS-9137/r alone

The secondary objective of the trial was to:

- evaluate the safety and tolerability of GS-9137/r alone and in combination with antacid or omeprazole

Trial Design

This was a randomized open-label drug interaction study. All subjects received GS-9137/r 50/100 mg QD (Treatment A) for 10 days, followed by one of five randomly assigned treatment arms (Treatments B through F):

Treatment A GS-9137/r QD x 10 days (Days 1-10)
Treatment B antacid suspension followed by GS-9137/r 4 h later x 1 day (Day 11)
Treatment C GS-9137/r followed by antacid suspension 4 h later x 1 day (Day 11)
Treatment D antacid suspension followed by GS-9137/r 2 h later x 1 day (Day 11)
Treatment E GS-9137/r followed by antacid suspension 2 h later x 1 day (Day 11)
Treatment F omeprazole followed by GS-9137/r 2 h later, QD x 5 days (Days 11-15)

GS-9137/r was administered within 5 minutes of completion of a meal. Omeprazole was administered under fasted conditions. Study drug administration was observed on Days 1, 4, 9, and 10 (Treatment A), 11 (Treatments B-F), and 14 and 15 (Treatment F). The study schema is shown in Figure 1.

Figure 1: Study schema (source: Study Report Table 5-1)
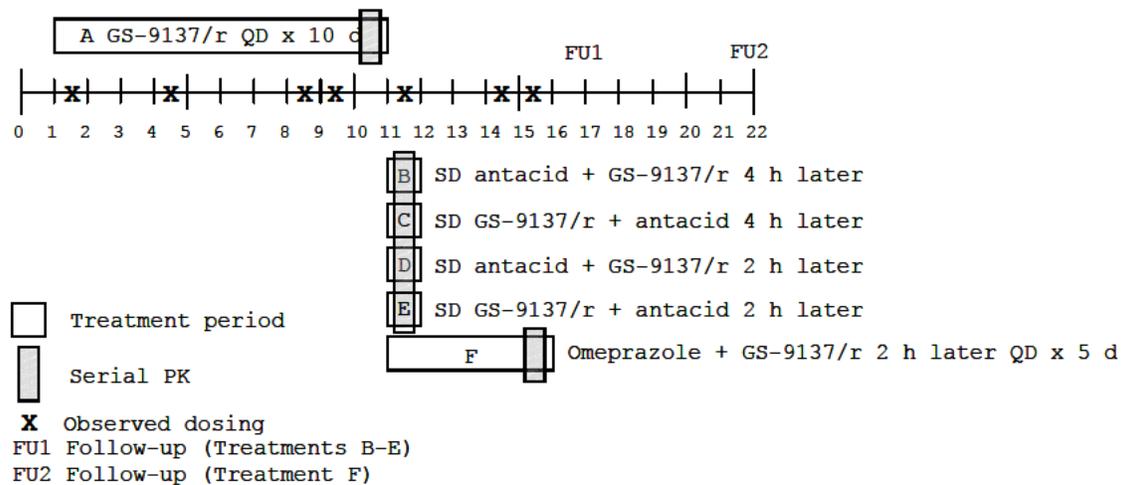
Rationale for Dose Selection

The GS-9137/r dose of 50/100 mg was selected for this study because it was considered to be a suitable dose to investigate potential drug interactions based on physiochemical processes (e.g. chelation, pH). Results from GS-US-183-0103 (which also investigated the effect of antacids on GS-9137/r pharmacokinetics) supported selection of the 50/100 mg dose. GS-9137/r exposures following GS-9137/r 50/100 mg were expected to be representative of exposures in safety and efficacy study.

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Four teaspoons (20 mL) of antacid suspension were administered in Treatments B through E; this is the maximum single dose recommended by the manufacturer. The omeprazole dose of 40 mg is the highest dose indicated for the treatment of gastric ulcers. Omeprazole was administered for five days because its maximum antisecretory effect is observed within that timeframe.

Investigational Product

Tablets containing 50 mg of GS-9137 were supplied by Gilead Sciences, Inc. (Foster City, California, USA; Lot AJ602A1) and were used for all treatments. Soft gelatin capsules containing 100 mg ritonavir (NORVIR®) were distributed by Abbott Laboratories (North Chicago, Illinois, USA; Lot 415132E21) and were used for all treatments. The antacid suspension (400 mg aluminum hydroxide, 400 mg magnesium hydroxide, and 40 mg simethicone per 5 mL teaspoon) Maalox Max® (Novartis Consumer Health, Inc., Parsippany, New Jersey, USA; Lot 10021627) was used in Treatments B, C, D, and E. Hard gelatin delayed-release capsules containing 40 mg omeprazole in the form of enteric-coated granules (PRILOSEC 40®) were distributed by AstraZeneca LP (Wilmington, Delaware, USA; Lot F2156).

Drug Administration

All subjects received Treatment A (GS-9137/r 50/100 mg) for 10 days and then received one of five randomized concomitant treatments (B through F). All doses of GS-9137/r were administered in the morning with 240 mL water within 5 minutes after completion of a standardized meal. All doses of antacid suspension were administered using a syringe and without water. All doses of omeprazole were administered in the morning with 240 mL water to fasted subjects, two hours before GS-9137/r administration.

Subjects were required to fast from midnight the evening before until breakfast on Days 1, 10, 11, and 15. On intensive sampling days, subjects fasted from the time of GS-9137/r dosing until after the 4-hour (Treatments A, B, D, E, and F) or 6-hour (Treatment C) blood draws, at which time a standardized meal was provided.

Water was allowed as desired except on Days 10, 11, and 15, when no water was allowed for 1 hour before and 2 hours after study drug administration, except for the 240 mL consumed with study drug administration.

Key Inclusion and Exclusion Criteria

Subjects were healthy nonsmoking males and females between the ages of 18 and 45 years, inclusive. Potential subjects were excluded if they were pregnant or lactating, or if they had taken any prescription or over-the-counter medication (including herbal products and antacids, with the exception of vitamins,

acetaminophen, ibuprofen, and hormonal contraceptives) within 30 days prior to study drug dosing.

Potential subjects were excluded for the use of specified medications from the following drug classes within 30 days of the baseline visit: alpha$_1$-adrenoreceptor antagonist, antiarrhythmics, anticoagulants, antihistamines, ergot derivatives, GI motility agents, neuroleptics, sedative/hypnotics, antineoplastics, herbal products, proton-pump inhibitors, or H$_2$-receptor antagonists. Potential subjects were also excluded if they had received hepatotoxic drugs, systemic steroids, immunosuppressants, or chemotherapeutic agents within 3 months prior to study screening.

Concomitant Medications
In addition to the medications detailed in the "Key Inclusion and Exclusion Criteria" section above, food or beverages containing caffeine, xanthine, or soda were not permitted starting 48 h before study drug administration and during all confinement periods. Alcohol was not permitted for the duration of the study. Consumption of certain citrus fruits, fruit and vegetable juices, antacids, and calcium channel blockers was restricted during the study.

Sample Collection
Blood was collected for the analysis of GS-9137, its metabolites GS-9202 (M1) and GS-9200 (M4), and RTV plasma levels on Day 10 (Treatment A), Day 11 (Treatments B, C, D, or E), and Day 15 (Treatment F) at the following timepoints in hours after dosing: 0:00 (predose), 1:00, 2:00, 3:00, 3:30, 4:00, 4:30, 5:00, 6:00, 8:00, 10:00, 12:00, 18:00, and 24:00. Blood samples taken after Treatment F were also analyzed for omeprazole.

Analytical Plan
Pharmacokinetic data

The pharmacokinetic parameters AUC$_{tau}$, C$_{max}$, T$_{max}$, C$_{tau}$, T$_{last}$, λ_z, t$_{1/2}$ were estimated for GS-9137, GS-9200 (M4), RTV, and omeprazole in plasma. The effects of antacid and omeprazole administration on GS-9137/r pharmacokinetics were investigated through comparisons of AUC$_{tau}$, C$_{max}$, and C$_{tau}$ after Treatment A (reference) and after each of the test treatments (B through F). All pharmacokinetic parameters were estimated using a nonlinear model derived using standard noncompartmental methods (WinNonlin® Professional Edition, Pharsight Corporation, Mountain View, California, USA). Pharmacokinetic parameters that depend on an accurate estimation of the terminal elimination phase (AUC$_{inf}$, λ_z, and t$_{1/2}$) were reported when the pharmacokineticist deemed the data evaluable after examination.

Trial Results
Bioanalytical methods

Concentrations of GS-9137, its metabolites GS-9200 and GS-9202, and RTV in plasma samples were determined using LC-MS/MS (Method M-GS-9137-16511 Version 4) by Gilead Sciences ▮▮▮▮▮▮▮▮▮▮ (b) (4) ▮▮▮▮ Frozen plasma samples were received between 19 Sept and 4 Oct 2006 and stored at -80˚C. Analysis was performed between 13 Oct and 1 Dec 2006. The first day of sample collection was 2 Sept 2006, so the maximum storage sample time was 90 days, which is within the validated long-term frozen stability duration of 93 days GS-9137, GS-9202, and GS-9200 and 102 days for RTV.

Concentrations of omeprazole were determined using LC-MS/MS (Project 60-0632) by ▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮ (b) (4) Frozen plasma samples were received between 19 Sept and 4 Oct 2006 and stored at -20˚C. Analysis was performed between 5 Oct and 20 Oct 2006. The first day of sample collection was 16 Sept 2006, so the maximum storage sample time was 34 days, which is within the validated long-term frozen (-20˚C) stability duration of 88 days.

The GS-9137 calibration standards ranged from 20-10,000 ng/mL and the quality control (QC) concentrations were 50.0, 750, and 7500 ng. For GS-9200 and GS-9202, the calibration standards ranged from 20-1000 ng/mL and the QC concentrations were 50.0, 150, and 750 ng/mL. For RTV, the calibration standards ranged from 5-5000 ng/mL and the QC concentrations were 15.0, 750, and 4000 ng/mL. For omeprazole, the calibration standards ranged from 20-2000 ng/mL and the QC concentrations were 50, 600, and 1800 ng/mL. All inter-assay accuracy and precision estimates (displayed in Table 1) were within the acceptable range (≤20% deviation from nominal at the LLoQ concentration, and ≤15% from nominal at all other concentrations).

Table 1: Bioanalytical assay validation for GS-9137, GS-9200, GS-9202, ritonavir, and omeprazole in human plasma (source: Study Report Table 5-4)

	GS-9137	M4	M1	Ritonavir	Omeprazole
Linear Range (ng/mL)	20 to 10,000	20 to 1000	20 to 1000	5 to 5000	20 to 2000
LLQ[a] (ng/mL)	20	20	20	5	20
Inter-Assay Precision Range[b]	4.9% to 8.0%	6.5% to 7.9%	5.3% to 7.3%	8.7% to 9.4%	5.1% to 12.5%
Inter-Assay Accuracy Range[c]	3.0% to 7.0%	−3.7% to 1.1%	3.3% to 6.5%	0.0% to 1.3%	−5.8% to 5.5%
Stability in Frozen Matrix (days)	93	93	93	102	88

a   LLQ, lower limit of quantitation
b   Percentage coefficient of variation
c   Difference from nominal concentrations
Source: Appendix 10

Trial population
A total of 60 subjects were randomized in the study and received study drug. Eleven subjects discontinued the study prematurely: 4 because of a protocol

violation, 4 due to reasons related to safety or tolerability, 2 withdrew consent, and 1 at the investigator's discretion.

Subjects in the safety analysis set (n=60) were largely white (63.3%) and male (65%), with a mean age of 29 years (range: 18-45 years). Twelve subjects did not have an evaluable GS-9137 pharmacokinetic profile pair and were excluded from the GS-9137 pharmacokinetic analysis set (n=48); eleven subjects did not have an evaluable ritonavir pharmacokinetic profile pair and were excluded from the RTV pharmacokinetic analysis set (n=49).

Results of pharmacokinetic analyses

The pharmacokinetic analysis sets for Treatment A (GS-9137/r alone) included 48 subjects; for Treatment B (GS-9137/r 4 h after antacid), 8 subjects; Treatment C (GS-9137/r 4 h before antacid), 10 subjects; Treatment D (GS-9137/r 2 h after antacid), 11 subjects; Treatment E (GS-9137/r 2 h before antacid), 10 subjects; and Treatment F (GS-9137/r 2 h after omeprazole), 9 subjects. Staggered administration of antacid or omeprazole did not affect the plasma concentration-time profile of GS-9137. The pharmacokinetic parameters for GS-9137 are listed by treatment in Table 2.

Table 2: Summary of steady-state pharmacokinetic parameters for GS-9137 by treatment (PK analysis set; source: Study Report Table 7-1)

GS-9137 PK Parameter[a]	GS-9137/r Alone (N = 48)	GS-9137/r 4 h after AA (N = 8)	GS-9137/r 4 h before AA (N = 10)	GS-9137/r 2 h after AA (N = 11)	GS-9137/r 2 h before AA (N = 10)	GS-9137/r + Omeprazole (N = 9)
C_{max} (ng/mL) Mean (%CV)	928.7 (31.2)	928.9 (12.8)	892.7 (50.1)	699.6 (17.5)	758.6 (15.5)	798.7 (20.0)
AUC_{tau} (ng·hr/mL) Mean (%CV)	10,666.7 (28.3)	11,507.2 (19.6)	9056.8 (44.8)	8584.6 (19.0)	9427.0 (12.4)	9756.0 (19.1)
C_{tau} (ng/mL) Mean (%CV)	211.4 (39.9)	258.7 (31.3)	181.4 (65.4)	181.2 (32.6)	179.7 (20.0)	201.6 (44.2)
$T_{1/2}$ (h) Median (Q1,Q3)	10.35 (9.22,12.42)	10.55 (10.37,13.19)	10.15 (8.78,11.01)	10.46 (9.08,11.35)	11.30 (10.46,12.26)	11.07 (9.58,12.04)
T_{max} (h) Median (Q1,Q3)	4.00 (4.00, 4.50)	4.25 (3.75, 4.76)	4.00 (3.50, 4.98)	4.50 (4.00, 4.50)	3.26 (3.00, 4.00)	4.00 (4.00, 4.50)

GS-9137/r, 50 mg GS-9137 + 100 mg ritonavir; AA, 20 mL antacid suspension; omeprazole, 40 mg omeprazole; %CV, percentage coefficient of variation; Q1, first quartile, Q3, third quartile

a The pharmacokinetic analysis set for GS-9137 includes 48 subjects who had evaluable pharmacokinetic profiles for the treatment pair.

Source: Section 11.1, Table 5.1

A statistical comparison of GS-9137 C_{max}, AUC_{tau}, and C_{tau} values following GS-9137/r alone (Treatment A) or staggered administration with antacid (Treatments B, C, D, and E) or omeprazole (Treatment F) are shown in Table 3. All of the 90% confidence interval values were within the Applicant's prespecified bounds of 70-143% that indicated no change in GS-9137 steady-state pharmacokinetics.

Upon further review of the pharmacokinetics of each treatment, it was noted that Treatment E (administration of GS-9137/r two hours before antacid) resulted in the largest decrease in GS-9137 plasma concentrations of all the treatments (GS-9137 C_{tau} and AUC_{tau} GMR values of 80.48 and 80.30 and corresponding lower bounds of the 90% CI at 72.90 and 74.63). In consultation with the Division of Pharmacometrics reviewer for this application and taking into account the lack of a GS-9137 exposure-response relationship, the decreases in GS-9137 C_{tau} and AUC_{tau} following administration of GS-9137/r and antacid staggered by two hours are unlikely to affect GS-9137 efficacy in a clinical setting.

Table 3: Statistical comparisons of PK parameters for GS-9137 between treatments (PK analysis set; source: Study Report Table 7-2)

Test Treatment	Geometric Least-Squares Means		Geometric Least-Squares Mean Ratio (%)	90% Confidence Interval
Pharmacokinetic Parameter[a]	Test (Mean)	Reference (Mean)		
GS-9137/r 4 h After AA (N = 8)				
C_{max} (ng/mL)	834.79	881.05	94.75	83.90, 107.00
AUC_{tau} (ng•h/mL)	9731.00	10,155.55	95.82	88.29, 103.99
C_{tau} (ng/mL)	200.92	192.63	104.30	93.39, 116.50
GS-9137/r 4 h Before AA (N = 10)				
C_{max} (ng/mL)	866.23	881.05	98.32	88.16, 109.65
AUC_{tau} (ng•h/mL)	9972.25	10,155.55	98.20	91.26, 105.66
C_{tau} (ng/mL)	192.60	192.63	99.98	90.14, 110.90
GS-9137/r 2 h After AA (N = 11)				
C_{max} (ng/mL)	724.51	881.05	82.23	74.10, 91.26
AUC_{tau} (ng•h/mL)	8608.06	10,155.55	84.76	79.04, 90.90
C_{tau} (ng/mL)	174.22	192.63	90.44	82.29, 99.39
GS-9137/r 2 h Before AA (N = 10)				
C_{max} (ng/mL)	694.80	881.05	78.86	70.71, 87.95
AUC_{tau} (ng•h/mL)	8155.20	10,155.55	80.30	74.63, 86.40
C_{tau} (ng/mL)	155.03	192.63	80.48	72.90, 88.85
GS-9137/r Plus Omeprazole (N = 9)				
C_{max} (ng/mL)	816.18	881.05	92.64	82.59, 103.91
AUC_{tau} (ng•h/mL)	10,012.21	10,155.55	98.59	91.27, 106.50
C_{tau} (ng/mL)	181.12	192.63	94.02	84.71, 104.36

GS-9137/r, 50 mg GS-9137 + 100 mg ritonavir; AA, 20 mL antacid suspension; omeprazole, 40 mg omeprazole

a   The pharmacokinetic analysis set for GS-9137 includes subjects who had evaluable pharmacokinetic profiles for the indicated treatment pair. The reference treatment for all analyses was GS-9137/r (Treatment A).

Source: Section 11.1, Table 6.1

The steady-state pharmacokinetic profiles of GS-9200 (the glucuronide metabolite M4) were similar across treatment groups (AUC_{tau} = 538.3, 688.1, 527.7, 544.4, 688.6, and 550.1 ng·h/mL for Treatments A, B, C, D, E, and F, respectively). Plasma GS-9200 concentrations were below the limit of quantitation in the majority of subjects by the end of the dosing interval. GS-9200 exposures were approximately 5-8% of the corresponding GS-9137 values.

Plasma concentrations of GS-9202 (M1, formed via CYP3A) were below the limit of quantitation in all subjects at all timepoints.

The steady-state pharmacokinetics of ritonavir were similar across treatment groups (AUC_{tau} = 5825.0, 5492.0, 7283.2, 5775.8, 6556.8, and 4801.6 ng·h/mL for Treatments A, B, C, D, E, and F, respectively) and all of the 90% confidence interval values were within the Applicant's prespecified no effect bounds of 70-143%, indicating that ritonavir steady-state pharmacokinetics were not affected by staggered administration of GS-9137/r and antacid or omeprazole.

Omeprazole pharmacokinetics were consistent with historical data. The estimated omeprazole half-life was 0.83 h (n=9). Systemic exposures were below quantifiable limits before the end of the dosing interval in all subjects.

Results of safety analysis
The safety analysis set included 60 subjects who received at least one dose of study drug. Four subjects discontinued the study because of adverse events; the adverse events were considered treatment-related in two subjects (mild elevation in CPK and AST in one subject and elevated GGT, ALT, and AST in one subject, both during GS-9137/r treatment alone). Treatment-related adverse events were only reported during treatment with GS-9137/r alone and included headache (4 subjects), diarrhea (3 subjects), elevated AST (2 subjects), and abdominal pain, dyspepsia, nausea, elevated ALT, elevated blood CPK, elevated GGT, and somnolence (each in one subject). No deaths or serious adverse events occurred during the study.

Trial Summary
This study was designed to detect alterations in the steady-state pharmacokinetics of ritonavir-boosted GS-9137 (GS-9137/r 50/100 mg) caused by staggered administration of antacid suspension (2 and 4 h before and after GS-9137/r administration) or omeprazole (2 h before GS-9137/r administration). Based on the statistical bounds for lack of interaction specified in the protocol, staggered administration of antacid by 2 or 4 hours or omeprazole by 2 hours did not influence the steady-state pharmacokinetics of GS-9137 or ritonavir.

Taking into consideration the drug interaction study GS-US-183-0103, which determined that simultaneous coadministration of GS-9137/r and antacid resulted in alterations in GS-9137 exposure, and this study, which found that staggering

the antacid and GS-9137/r doses by at least 2 hours had no effect on GS-9137 pharmacokinetics, the Applicant has recommended that coadministration of GS-9137/r and antacid should be separated by at least 2 hours. This recommendation is supported by the clinical pharmacology information generated by the GS-9137 development program to date.

Pharmacokinetic analysis of subjects who received Treatment F, a dose of the proton pump inhibitor omeprazole followed by a dose of GS-9137/r two hours later, indicated that GS-9137 exposures were unaffected by prior omeprazole administration. These findings suggest that GS-9137 absorption is not affected by gastric pH when GS-9137/r and omeprazole coadministration are staggered by two hours. In Table 4 ("Established and Other Potentially Significant Drug Interactions: Alteration in Dose or Regimen may be Recommended Based on Drug Interaction Studies or Predicted Interaction"), the Applicant states, (b) (4) The available clinical pharmacology information indicates that this is true when GS-9137/r is administered two hours after omeprazole. Although there are no pharmacokinetic data to support simultaneous administration of omeprazole and GS-9137, according to the approved omeprazole labeling, the maximum effect of inhibition of acid secretion (and therefore the time of highest potential for alterations in GS-9137 absorption) occurs two to six hours after omeprazole administration. Therefore, the absence of a drug interaction when GS-9137 and omeprazole administration are staggered by two hours suggests that simultaneous administration of omeprazole and GS-9137 is acceptable.

Trial GS-US-183-0126
A Phase 1 Study to Evaluate the Pharmacokinetics, Metabolism and Excretion of GS-9137 Following Administration of a Single, Oral Dose of Ritonavir-boosted [^{14}C]GS-9137

Trial Period
21 August 2006 to 19 October 2006
Final report date: 13 November 2008 (submitted to IND ⬛⬛⬛ (b) (4))

Trial Site
Covance Clinical Research Unit, Inc., Madison, Wisconsin, USA

Trial Rationale
GS-9137 (elvitegravir, EVG) is an inhibitor of the human immunodeficiency virus (HIV) integrase, currently under development for the treatment of HIV infection. Results from *in vitro* studies have demonstrated potent anti-HIV activity, including activity against viruses that are resistant to nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PI). Ritonavir (RTV) is an HIV PI indicated for use in combination with other antiretroviral drugs for the treatment of HIV infection. Coadministration of GS-9137 with a subtherapeutic dose of RTV (GS-9137/r) increases the systemic exposure of GS-9137 by approximately 20-fold (based on AUC). The goal of this trial was to characterize the in vivo metabolism and excretion of a single dose of [^{14}C]GS-9137 in healthy subjects, following the dosage recommendations likely to be recommended to patients.

Trial Objectives
The primary objective of the trial was to:

- determine the mass balance of GS-9137 following administration of a single, oral dose of ritonavir-boosted [^{14}C]GS-9137

The secondary objectives of the trial were to:

- evaluate the pharmacokinetics of GS-9137 and, where possible, its metabolites following administration of a single, oral dose of RTV-boosted [^{14}C]GS-9137
- determine the metabolite profile of GS-9137 in humans following administration of a single, oral dose of RTV-boosted [^{14}C]GS-9137

Trial Design
This was an open-label mass balance trial designed to evaluate the pharmacokinetics, metabolism, and excretion of a single oral dose of [^{14}C]GS-9137/r. Subjects were confined to the clinic during sample collection (at least 96 h but less than 22 days, depending on measured radioactivity) and adverse

events were monitored for an additional seven days after discharge from the clinic. The study schema is shown in Figure 1.

Figure 1: Study schema (source: Study Report Figure 5-1)
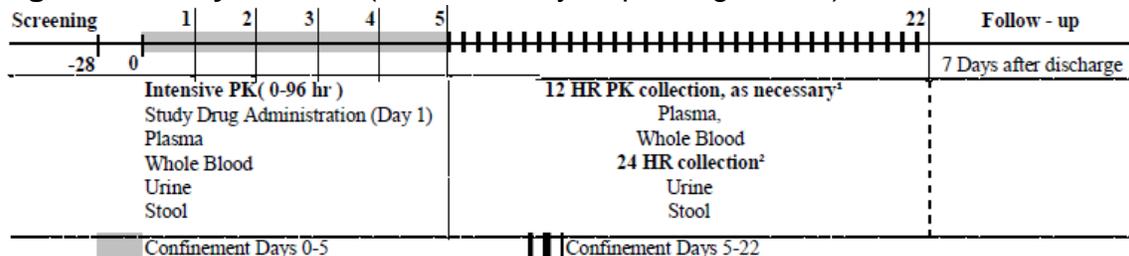
[1]Additional samples collected until assays indicated that the radioactivity levels in two consecutive samples had decreased to less than or equal to twice the level of background radioactivity, or both the urine and stool collections were discontinued, whichever came first

[2]Additional samples collected until assays indicated that the radioactivity levels in samples from two consecutive collection intervals were equal to or less than 1% of the administered dose and the cumulative ^{14}C radioactivity recovered in urine and stools over the sampling period was greater than 90% of the administered dose

Rationale for Dose Selection

The GS-9137 dose of 50 mg was selected because it was representative of the range of exposures under investigation (i.e. 20, 50, and 125 mg GS-9137 with 100 mg RTV). The dose of radioactivity was estimated to be 100 uCi and was expected to result in human whole body radioactivity exposures of <1% of the 3 rem limit specified by federal regulations in 21CFR361.1(b)(3)(i). The RTV dose of 100 mg is the dose currently indicated to boost HIV antiretroviral drugs in the treatment of HIV. This dose maximally increases GS-9137 exposures; coadministration of RTV 200 mg did not result in an additional increase in GS-9137 exposure compared to RTV 100 mg.

Drug Administration

One capsule of RTV and two capsules of [^{14}C]GS-9137 (in that order) were administered orally with 240 mL of water within 5 minutes of completion of a meal that was consumed over a period of 30 min or less. Water was withheld for 1 h before and 2 h after dosing. All subjects were required to remain upright for at least 2 h after dosing and to fast until after the 4 h blood draw, when a standardized meal (400 kcal, 13 g of fat) was provided. During confinement at the study facility, subjects were permitted light exercise only.

Investigational Product

Each subject received a single 50 mg dose of GS-9137 in the form of two 25 mg hard gelatin capsules containing a mixture of unlabeled GS-9137 and 50uCi of ^{14}C-labeled GS-9137 solubilized in a 50% mixture of propylene carbonate and d-alpha tocopheryl polyethylene glycol 1000 succinate.

Ritonavir (NORVIR®, Abbott Laboratories, Lot 247022E21) was administered as 100 mg soft gelatin capsules.

Key Inclusion and Exclusion Criteria
Subjects were healthy nonsmoking males between the ages of 18 and 50 years, inclusive, with BMI between 19 and 30 kg/m^2, inclusive, and normal electrocardiogram (ECG). Subjects also had to have a creatinine clearance (using the Cockcroft-Gault method) of 80 mL/min or greater.

Potential subjects were excluded from enrollment if they had any abnormal finding of clinical relevance during the medical examination, any diseases or infections of clinical relevance, or if they had a history of GI disorders, chronic liver disease or hepatic impairment, significant mental illness, drug sensitivity or allergy, or difficulty with blood donation. Exclusion criteria also included previous exposure to investigational drugs (within 30 days of study drug administration) or radioactivity (within 12 months of study entry), the use of alcohol within 48 h before study drug dosing or history of abuse of alcohol, the use of illicit drugs, the use of any medications within 30 days or five half-lives (whichever is longer) of study drug administration, the use of nonprescription medication or herbal products other than St John's wort within three days of Day 1, with the exception of acetaminophen, ibuprofen, and vitamins, or the use of any of the following drugs within the specified timeframes:

- contraindicated drugs (within 30 days prior to study screening): $_1$-adrenoreceptor antagonist, antiarrhythmics, antihistamines, ergot derivatives, GI motility agent, neuroleptics, sedative/hypnotics, antineoplastics, herbal products, HMG-CoA reductase inhibitors
- hepatotoxic drugs (within 3 months prior to study screening): e.g. anabolic steroids, itraconazole, isoniazid, ketoconazole, lovastatin, HIV protease inhibitors, rifabutin, rifampin, simvastin
- other drugs (within 3 months prior to study screening): systemic steroids, immunosuppressants, chemotherapeutic agents, immune- or cytokine-based therapies

Concomitant Medications
No concomitant medications, over-the-counter medications, or herbal products were permitted except for mild laxatives, acetaminophen, and ibuprofen. Restrictions were placed on the consumption (prior to and during the trial) of products containing calcium, magnesium, or aluminum (antacids) or calcium, iron, or zinc (vitamin and mineral supplements); the ulcer medicine sucralfate; and certain fruit juices and citrus fruits.

Sample Collection
Blood was collected for pharmacokinetic analysis at each of the following timepoints (in hours after dosing):

Day 1	0:00 (predose), 0:30, 1:00, 1:30, 2:00, 3:00, 3:30, 4:00, 4:30, 5:00, 5:30, 6:00, 8:00, 10:00, and 12:00
Days 2 through 4	18:00, 24:00, 28:00, 32:00, 36:00, 48:00, 60:00, 72:00, and 84:00
Days 5 through 22	96:00, then at 12 h intervals through Day 22, or until assays indicated that a) radioactivity levels in two consecutive samples were ≤ 2x background radioactivity, or b) both urine and stool collection were discontinued (see criteria below)

Urine was collected for pharmacokinetic analysis during the following time periods (in hours after dosing):

Day 1	0 (predose), 0-4, 4-8, and 8-12, 12-24
Days 2 through 4	24-48, 48-72, 72-96
Days 5 through 22	for 24 h intervals through Day 22, or until assays indicated that radioactivity levels in samples from two consecutive collection intervals were ≤ 1% of the administered dose, and that the cumulative ^{14}C recovered over the sampling period was ≥ 90% of the administered dose

Stools were collected beginning from the evening before GS-9137 administration (predose) and for 24 h collection intervals after dosing through Day 22, or until assays indicated that radioactivity levels in samples from two consecutive collection intervals were ≤ 1% of the administered dose, and that the cumulative ^{14}C recovered over the sampling period was ≥ 90% of the administered dose.

Analytical Plan
Pharmacokinetic data
The primary analysis in this study was to determine the mass balance of orally administered GS-9137 by measuring ^{14}C radioactivity in urine and stool samples after administration of GS-9137/r. Secondary analyses examined the pharmacokinetics of GS-9137 and its metabolites to determine the metabolic profile of GS-9137, as well as the pharmacokinetics of RTV. No statistical comparisons were performed.

Liquid scintillation counting (LSC) was used to measure ^{14}C radioactivity. ^{14}C-labeled GS-9137 and its ^{14}C-labeled metabolites were quantified in plasma, urine, and feces using HPLC. Plasma concentrations of unlabeled GS-9137, its metabolites, and RTV were determined using LC-MS/MS.

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The pharmacokinetic parameters C_{max}, T_{max}, C_{last}, T_{last}, λ_z, $AUC_{0\text{-last}}$, $AUC_{0\text{-}\infty}$, $\%AUC_{exp}$, $t_{1/2}$, CL/F, and V_z/F were estimated (when applicable) for plasma and whole blood [^{14}C]-radioactivity and for GS-9137, its metabolite GS-9200, and RTV in plasma. These parameters were estimated using a nonlinear model derived using standard noncompartmental methods (WinNonlin® Professional Edition, Pharsight Corporation, Mountain View, California, USA).

Trial Results
Bioanalytical methods
Radioanalysis
Levels of radioactivity in whole blood, plasma, urine, and fecal samples were quantified by liquid scintillation counting (LSC) performed by ▊▊▊ (b) (4) ▊▊▊▊ (▊▊▊▊▊▊ (b) (4) Study ID 6438-479). Samples were received between 14 Sep and 6 Oct 2006. Blood and plasma samples were stored at approximately 2 to 8°C before analysis and at -20°C after analysis; urine and fecal samples were stored at -20°C before and after analysis. The date of analysis is not specified, so sample stability cannot be evaluated for this non-pivotal study. All samples were analyzed in Model 2900TR liquid scintillation counters (Packard Instrument Company, Meriden, Connecticut, USA) for at least 5 min or 100,000 counts. Plasma, blood, and urine samples were analyzed in triplicate and reanalyzed if results differed by more than 10% of the mean.

Metabolite profiling
Evaluation of the metabolite profiles of [^{14}C]GS-9137 in plasma, urine, and feces was performed using HPLC followed by LC-MS or radioanalysis by ▊▊▊ (b) (4) ▊▊▊▊ (▊▊ (b) (4) Study 6438-529). Samples were transferred to this study from ▊▊ (b) (4) Study 6438-479 (radioanalysis, described above) after storage at -20°C. The dates of transfer and analysis are not specified, so sample stability cannot be evaluated for this non-pivotal study.

Quantitative determination of GS-9137, GS-9202, GS-9200, and RTV
Concentrations of GS-9137, its metabolites GS-9202 and GS-9200, and RTV in plasma, urine, and fecal samples were measured by LC-MS/MS by ▊▊▊ (b) (4) ▊▊▊ (▊▊▊▊▊▊▊▊▊▊ (b) (4) Study ▊▊ (b) (4) S06-191). Plasma samples were received on 26 Sep 2006 and 18 Oct 2006 (Applicant-requested repeats) and stored at -70°C. Analysis was performed between 27 Sep and 20 Oct 2006. The maximum storage sample time was 24 days, which is within the validated long-term frozen stability duration of 93 days for GS-9137 and its metabolites and 96 days for RTV. The LC-MS/MS methods ▊▊ (b) (4) M05194 (for quantification of GS-9137 and its metabolites GS-9202 and GS-9200) and ▊▊ (b) (4) M06121 (for quantification of RTV) were used.

The calibration standards for GS-9137, GS-9200, and GS-9202 were 20.0, 40.0, 120, 500, 2000, 5000, 8000, and 10,000 ng/mL and the quality control (QC) concentrations were 60.0, 3500, and 7500 ng/mL. For RTV, the calibration

standards were 5.00, 10.0, 50.0, 250, 750, 1250, 2100, and 2500 ng/mL and the QC concentrations were 15.0, 1000, and 2000 ng/mL). The inter-assay accuracy and precision estimates (displayed in Table 1) were similar for all four analytes. All accuracy and precision (%CV) values were within the acceptable range (≤20% deviation from nominal at the LLoQ concentration, and ≤15% from nominal at all other concentrations).

Table 1: Bioanalytical assay validation for GS-9137, GS-9200, GS-9202, and RTV (source: Study Report Table 5-3)

	GS-9137	GS-9200 (M4)	GS-9202 (M1)	Ritonavir
Linear Range (ng/mL)	20.0 to 10,000	20.0 to 10,000	20.0 to 10,000	5.00 to 2,500
LLQ (ng/mL)	20.0	20.0	20.0	5.00
Inter-Assay Precision Range[a]	3.1% to 5.6%	2.0% to 4.6%	2.0% to 9.5%	2.6% to 4.6%
Inter-Assay Accuracy Range[b]	−8.4% to 6.0%	−5.5% to 4.0%	−10.4% to 3.3%	−4.5% to 4.7%
Stability in Frozen Matrix (days)	93	93	93	96

LLQ = lower limit of quantitation

a   Percent coefficient of variation

b   Difference from nominal concentrations

Source: Appendix 10

Trial population

A total of 8 healthy males between the ages of 18 and 48 (median: 25) were enrolled in and completed the study. All subjects were included in the safety set and the pharmacokinetic analysis set. Six of the subjects were white, one was Asian, and one was black.

Pharmacokinetics of [^{14}C]GS-9137 in whole blood

The mean (± SD) concentration-time profile of [^{14}C]GS-9137 in whole blood after oral administration of a single dose of [^{14}C]GS-9137/r 50/100 mg is shown in Figure 1. Radioactivity was quantifiable up to 36 h post-dose; concentrations were BLQ at all timepoints thereafter.

Figure 1: Whole blood [^{14}C]GS-9137 concentration-time profile after a single oral dose of [^{14}C]GS-9137/r (mean ± SD; semilog scale; source: Study Report Figure 7-1)
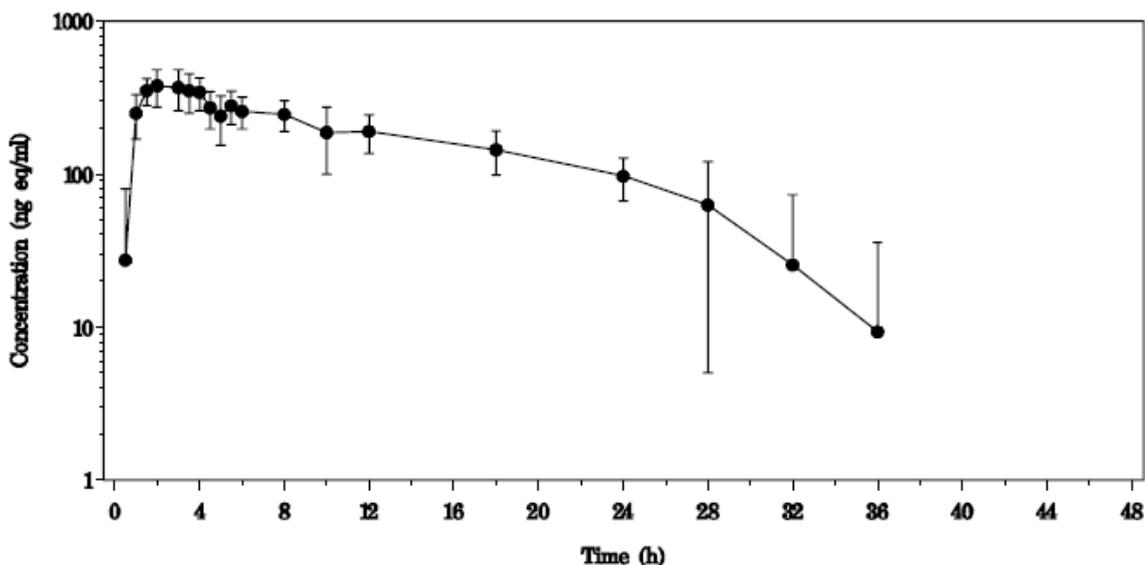
Pharmacokinetic parameters of total [^{14}C]-radioactivity in whole blood after a single dose of [^{14}C]GS-9137/r are listed in Table 2. The median T_{max} was 1.75 h and the median half-life was 11.82 h.

Table 2: Summary of total [^{14}C]-radioactivity pharmacokinetic parameters in whole blood by LSC (source: Study Report Table 7-1)

PK Parameter	Total [^{14}C]-Radioactivity in Whole Blood
C_{max} (ng eq/mL) Mean (%CV)	406.2 (25.6)
AUC_{last} (ng eq·h/mL) Mean (%CV)	5159.0 (30.0)
AUC_{inf} (ng eq·h/mL) Mean (%CV)	6492.6 (27.5)
%AUC_{exp} Mean (%CV)	20.9 (23.9)
T_{max} (h) Median (Q1, Q3)	1.75 (1.50, 2.52)
T_{last} (h) Median (Q1, Q3)	28.00 (24.00, 30.00)
$T_{½}$ (h) Median (Q1, Q3)	11.82 (9.67, 14.33)

%CV = percent coefficient of variation; Q1 = first quartile; Q3 = third quartile
Source: Section 11.1, Table 12

Pharmacokinetics of [^{14}C]GS-9137 in plasma
The mean (± SD) concentration-time profile of [^{14}C]GS-9137 in plasma after oral administration of a single dose of [^{14}C]GS-9137/r 50/100 mg is shown in Figure 2. Radioactivity was quantifiable up to 48 h post-dose; concentrations were BLQ at all timepoints thereafter.

Figure 2: Plasma [¹⁴C]GS-9137 concentration-time profile after a single oral dose of [¹⁴C]GS-9137/r (mean ± SD; semilog scale; source: Study Report Figure 7-2)
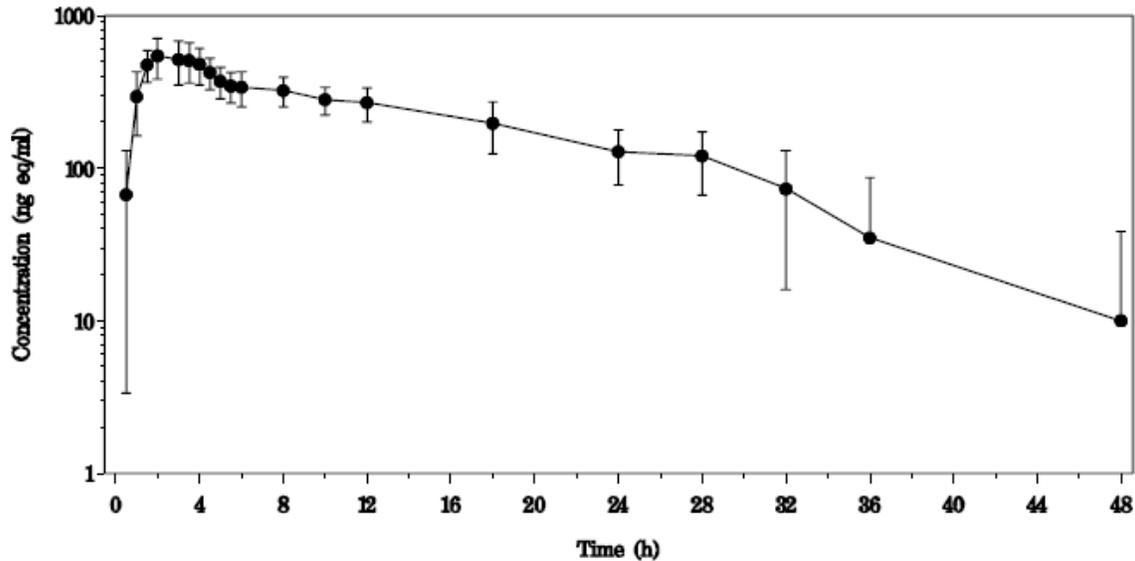
Pharmacokinetic parameters of total [¹⁴C]GS-9137 in plasma after a single dose of [¹⁴C]GS-9137/r are listed in Table 3. The median T_{max} was 2.00 h and the median half-life was 11.54 h.

Table 3: Summary of total [¹⁴C]GS-9137 pharmacokinetic parameters in plasma by LSC (source: Study Report Table 7-2)

| PK Parameter	Total [¹⁴C]-Radioactivity in Plasma
C_{max} (ng eq/mL) Mean (%CV)	553.9 (28.0)
AUC_{last} (ng eq·h/mL) Mean (%CV)	7672.3 (31.7)
AUC_{inf} (ng eq·h/mL) Mean (%CV)	8878.6 (28.5)
%AUC_{exp} Mean (%CV)	14.3 (28.2)
T_{max} (h) Median (Q1, Q3)	2.00 (2.00, 3.27)
T_{last} (h) Median (Q1, Q3)	32.00 (30.02, 36.00)
$T_{½}$ (h) Median (Q1, Q3)	11.54 (10.29, 12.43)

%CV = percent coefficient of variation; Q1 = first quartile; Q3 = third quartile
Source: Section 11.1, Table 8.1

The blood to plasma ratio of [¹⁴C]-radioactivity based on mean AUC_{inf} was 0.73, indicating that [¹⁴C]GS-9137 remained primarily in the plasma.

Concentrations of GS-9137 in plasma

The mean (± SD) concentration-time profile of GS-9137 in plasma (measured by LC-MS/MS) after oral administration of a single dose of [^{14}C]GS-9137/r 50/100 mg is shown in Figure 3. Radioactivity was quantifiable up to 48 h post-dose; concentrations were BLQ at all timepoints thereafter.

Figure 3: Plasma GS-9137 concentration-time profile after a single oral dose of [^{14}C]GS-9137/r, measured by LC-MS/MS (mean ± SD; semilog scale; source: Study Report Figure 7-3)
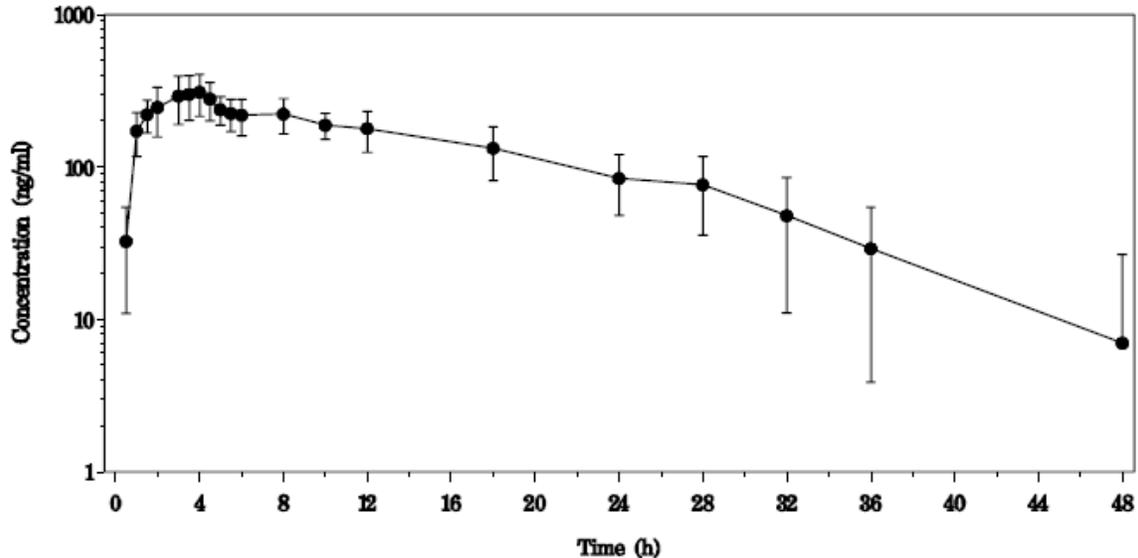
Pharmacokinetic parameters of GS-9137 in plasma measured by LC-MS/MS after a single dose of [^{14}C]GS-9137/r are listed in Table 4. The median T_{max} was 3.76 h and the median half-life was 9.18 h.

Table 4: Summary of GS-9137 pharmacokinetic parameters in plasma by LC-MS/MS (source: Study Report Table 7-3)

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PK Parameter	GS-9137 in Plasma
C_{max} (ng/mL) Mean (%CV)	320.8 (30.2)
AUC_{last} (ng•h/mL) Mean (%CV)	4915.4 (33.6)
AUC_{inf} (ng•h/mL) Mean (%CV)	5427.9 (34.5)
%AUC_{exp} Mean (%CV)	9.3 (30.6)
T_{max} (h) Median (Q1, Q3)	3.76 (3.50, 4.00)
T_{last} (h) Median (Q1, Q3)	36.00 (34.00, 36.00)
$T_{½}$ (h) Median (Q1, Q3)	9.18 (8.78, 9.81)

%CV = percent coefficient of variation; Q1 = first quartile; Q3 = third quartile
Source: Section 11.1, Table 8.2

Concentrations of GS-9200 (M4) in plasma

The mean (± SD) concentration-time profile of GS-9200 in plasma (measured by LC-MS/MS) after oral administration of a single dose of [^{14}C]GS-9137/r 50/100 mg is shown in Figure 4. Radioactivity was quantifiable only up to 8 h post-dose; concentrations were BLQ at all timepoints thereafter.

Figure 4: Plasma GS-9200 concentration-time profile after a single oral dose of [^{14}C]GS-9137/r, measured by LC-MS/MS (mean ± SD; semilog scale; source: Study Report Figure 7-4)
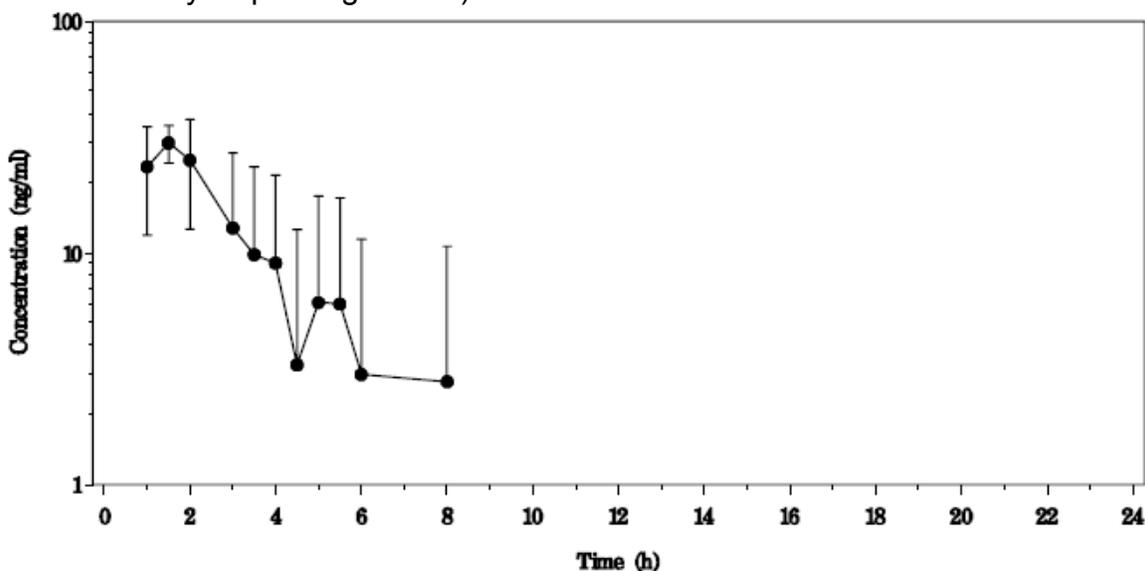
Pharmacokinetic parameters of GS-9200 in plasma measured by LC-MS/MS after a single dose of [^{14}C]GS-9137/r are listed in Table 5. Median GS-9200 peak levels were observed 1.5 h after a single dose of [^{14}C]GS-9137/r. Due to

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the short duration of measureable plasma concentrations, GS-9200 half-life could not be accurately estimated in 6 of 8 subjects.

Table 5: Summary of GS-9200 pharmacokinetic parameters in plasma by LC-MS/MS (source: Study Report Table 7-4)

| PK Parameter	GS-9200 in Plasma
C_{max} (ng/mL) Mean (%CV)	31.9 (23.4)
AUC_{last} (ng•h/mL) Mean (%CV)	73.1 (87.1)
AUC_{inf} (ng•h/mL)[a] Mean	223.8
T_{max} (h) Median (Q1, Q3)	1.50 (1.26, 1.50)
T_{last} (h) Median (Q1, Q3)	2.50 (2.00, 4.75)

CV% = percent coefficient of variation; Q1 = first quartile; Q3 = third quartile

a   n = 2

Source: Section 11.1, Table 8.4

Concentrations of GS-9202 (M1) in plasma

GS-9202 was observed in quantifiable plasma levels only between 1 and 2 h following a single dose of [14C]GS-9137/r. GS-9202 is formed via CYP3A4 metabolism of GS-9137; concentrations are therefore limited in the presence of RTV.

Urinary recovery

Approximately 6.7% of the administered [14C]GS-9137 dose was recovered in the urine, with the majority of recovery (5.6%) occurring within 24 h of administration, as displayed in Figure 5. The intersubject variability was low, ranging from 18.1 to 23.1% (CV).

Figure 5: Urinary recovery of total [14C]-radioactivity after a single oral dose of [14C]GS-9137/r (mean ± SD; source: Study Report Figure 7-5)
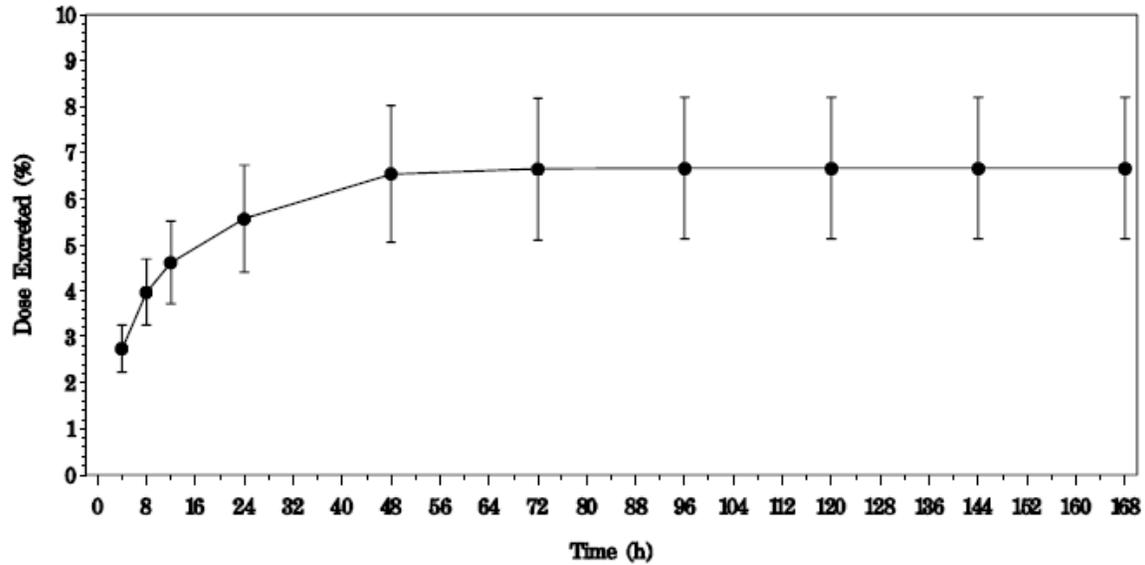
Fecal recovery

GS-9137 is predominantly eliminated in the feces. Approximately 94.8% of the administered [^{14}C]GS-9137 dose was recovered in the feces, with most of the recovery (87.5%) occurring within 120 h of administration, as displayed in Figure 6, although radioactivity continued to be recovered up to 312 h after dosing.

Figure 6: Fecal recovery of total [^{14}C]-radioactivity after a single oral dose of [^{14}C]GS-9137/r (mean ± SD; source: Study Report Figure 7-6)
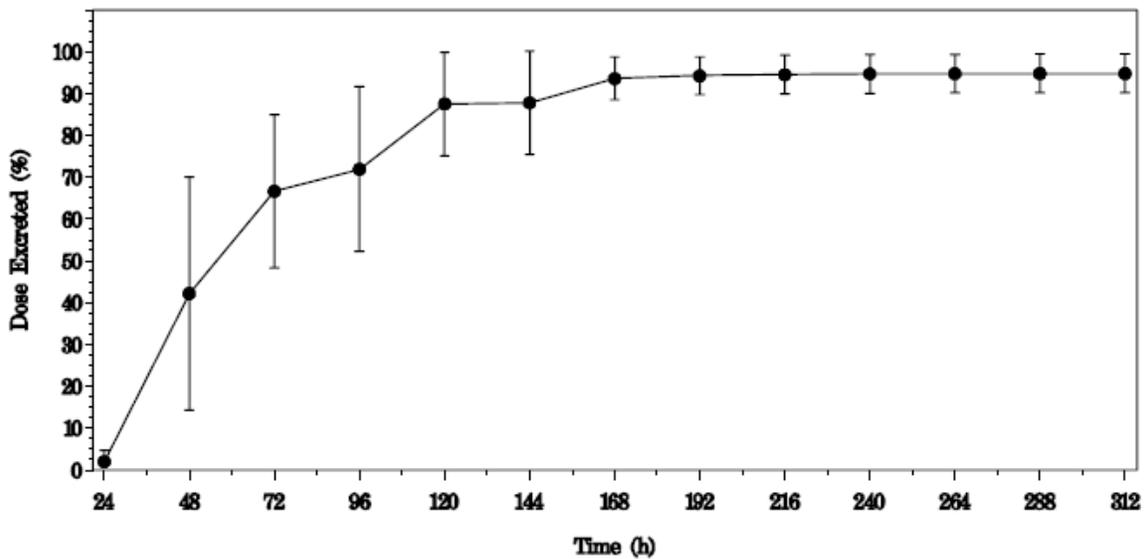
Combined urinary and fecal recovery

Urine and fecal samples were collected from individual subjects for a range of 192 to 312 h post-dose, at which time cumulative urinary and fecal radioactivity levels were >90% of the administered dose and radioactivity levels in samples from two consecutive collection intervals were ≤1% of the administered dose.

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Following a single dose of [^{14}C]GS-9137/r 50/100 mg, the sum of the cumulative urinary and fecal recoveries of [^{14}C]-radioactivity was 101.4% (SD = 4.2%) of the administered radioactivity, accounting for the entire dose. More than 94% of the cumulative dose recovery occurred during the first 120 h after [^{14}C]GS-9137/r administration (see Figure 7) and the majority of the dose was recovered from the feces.

Figure 7: Cumulative urinary and fecal recovery of total [^{14}C]-radioactivity after a single oral dose of [^{14}C]GS-9137/r (mean ± SD; source: Study Report Figure 7-7)
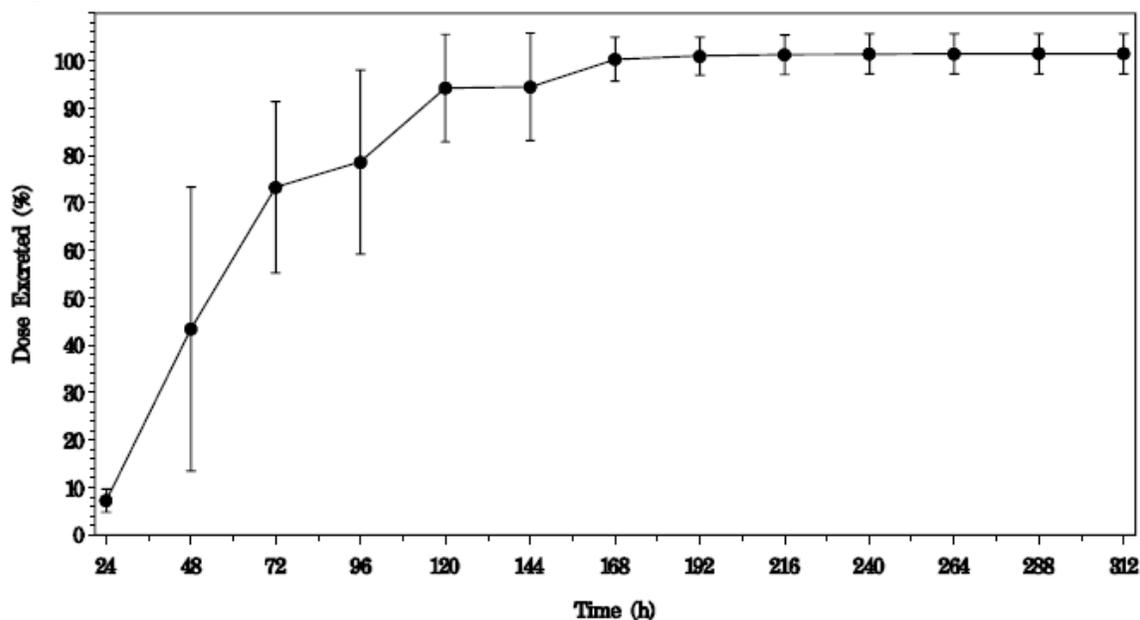
GS-9137 metabolite profiling in plasma, urine, and feces
In plasma samples pooled by collection timepoint up to 32 h after the [^{14}C]GS-9137/r 50/100 mg dose, HPLC-radiochemical detection demonstrated that GS-9137 was the predominant circulating species of [^{14}C]-radioactivity. Comparison of the AUC values of GS-9137 to total [^{14}C]-radioactivity indicates that GS-9137 accounted for approximately 94% of the total radioactivity (see Table 6). Low levels of the GS-9137 metabolites GS-9200 (M4), GS-9202 (M1), M7, and M19 were observed in samples collected at earlier timepoints (i.e. up to 10 h post-dose).

Table 6: Composite estimates of [^{14}C]-radioactivity and GS-9137 pharmacokinetic parameters in pooled plasma by HPLC-radiochemical detection (N=8; source: Study Report Table 7-5)

PK Parameter	[^{14}C]-Radioactivity	GS-9137
C_{max} (ng eq/mL)	472	388
AUC_{last} (ng eq•h/mL)	6602.1	6204.2
AUC_{inf} (ng eq•h/mL)	7346.1	6935.7
%AUC_{exp}	10.1	10.5
T_{max} (h)	2	4
T_{last} (h)	32	32
$T_{\frac{1}{2}}$ (h)	9.64	9.59

Source: Section 11.1, Table 8.5

Urine samples pooled by subject and analyzed by HPLC-radiochemical detection indicated that the [^{14}C]GS-9137/r dose eliminated in the urine primarily consisted of the GS-9137 metabolites GS-9200, M7, M19, and M20 in roughly equal proportions, as presented in Table 7. GS-9137 was not observed in urine samples from any of the subjects.

Table 7: Summary of total [^{14}C]-radioactivity as metabolites in pooled urine from all sampling collection intervals by HPLC-radiochemical detection (N=8; source: Study Report Table 7-6)

GS-9137 Metabolite	Mean Percent of Total [^{14}C]-Radioactivity (%CV)
GS-9200	1.26 (22.7)
GS-9202	0.20[a]
M7	1.44 (30.7)
M19	1.48 (33.3)
M20	1.41 (37.3)

%CV = percent coefficient of variation

a   n = 1

Source: Section 11.1, Table 6.1

Fecal samples pooled by subject and analyzed by HPLC-radiochemical detection indicated that the [^{14}C]GS-9137/r dose eliminated in the feces primarily consisted of GS-9137 (30.8%) and its metabolite GS-9202 (33.8%), although the metabolites M9 (1.49-3.26%), M13 (1.44-2.83%), and M15 (3.48-7.37%) were also observed in some samples (as documented in Study Report Appendix 10). The cumulative fecal recovery of GS-9137 and GS-9202 is shown as the percent of [^{14}C]-radioactive dose excreted in Figures 8 and 9, respectively.

Figure 8: Cumulative fecal recovery of GS-9137 (N=8; source: Study Report Table 7-8)
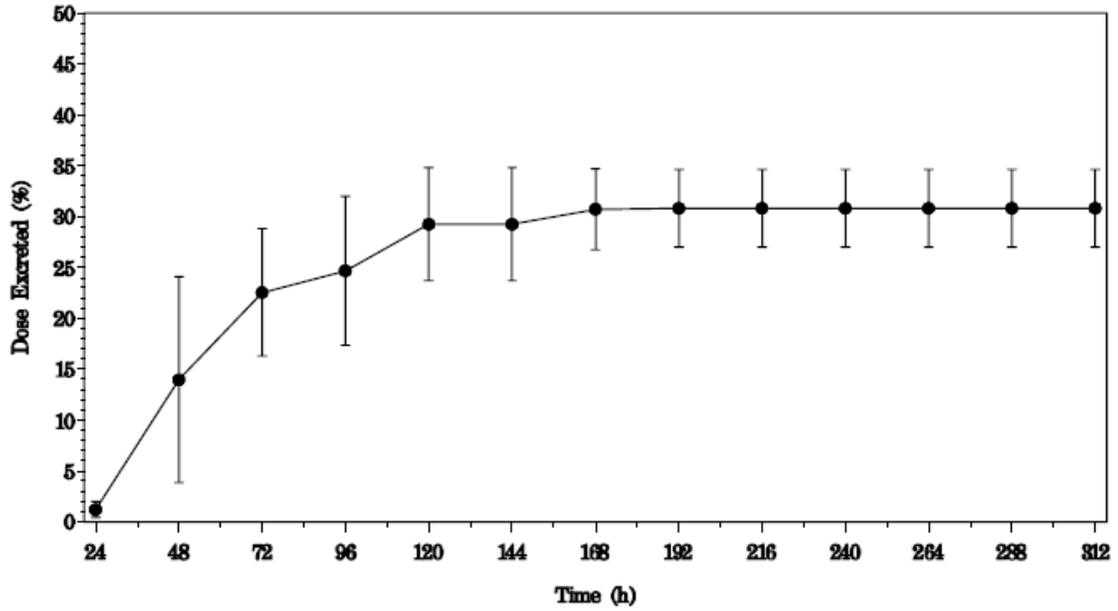
Values presented as mean ± standard deviation.
Source: Section 11.1, Figure 6.2

Figure 9: Cumulative fecal recovery of GS-9202 (N=8; source: Study Report Table 7-9)
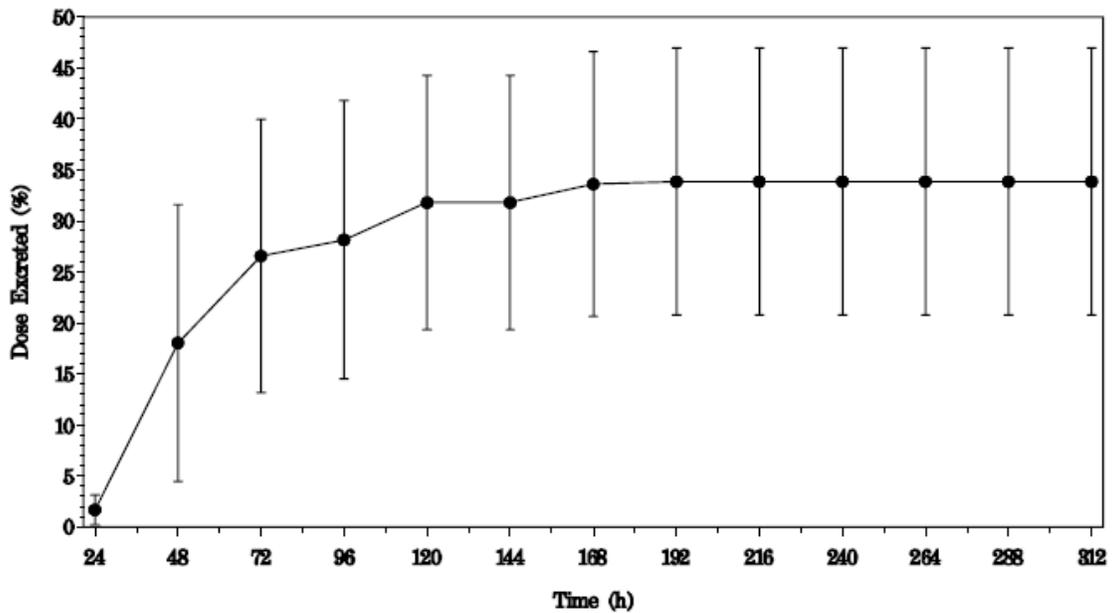
Values presented as mean ± standard deviation.
Source: Section 11.1, Figure 6.3

Identification and characterization of GS-9137 metabolites in plasma, urine, and feces

The characterization of GS-9137 metabolites in plasma, urine, and fecal samples was performed by HPLC-radiochemical analysis, and structural identities were assigned using mass spectrometry. In plasma, the predominant circulating species was GS-9137 (94%). Low levels of GS-9202, M7, M19, and GS-9200 accounted for the remaining radioactivity in the plasma. In fecal samples, the predominant circulating species were GS-9137 and GS-9202, with low levels of M9, M13, and M15. Approximately 7% of the administered radioactivity was excreted in the urine, with M19, M7, M20, and GS-9200 accounting for similar percentages of urine radioactivity. A proposed biotransformation pathway for radiolabeled GS-9137 is via aromatic and aliphatic hydroxylation and/or primary or secondary glucuronidation and is shown in Figure 10.

Figure 10: Proposed biotransformation pathway for [^{14}C]GS-9137 (source: Study Report Figure 7-10)
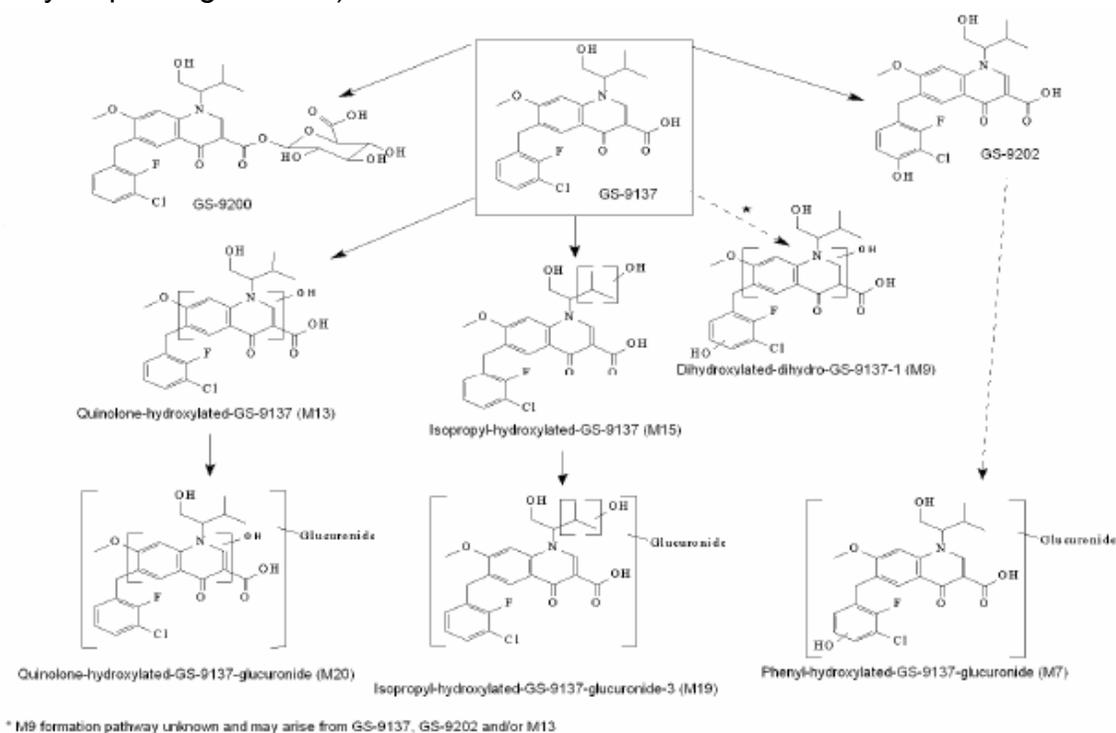
Source: Figure 14 from the metabolite profiling report, provided in report Appendix 10

Pharmacokinetics of RTV in plasma

Ritonavir plasma concentrations were quantified using LC-MS/MS. Peak plasma concentrations were observed approximately 4.50 h after a single dose of [^{14}C]GS-9137/r 50/100 mg. The median half-life was 4.87 h. Pharmacokinetic parameters are displayed in Table 8.

Table 8: Summary of plasma ritonavir pharmacokinetic parameters by LC-MS/MS detection (N=8; source: Study Report Table 7-7)

Reference ID: 3153809

PK Parameter	Ritonavir in Plasma
C_{max} (ng/mL) Mean (%CV)	388.9 (63.6)
AUC_{last} (ng•h/mL) Mean (%CV)	2943.7 (34.4)
AUC_{inf} (ng•h/mL) Mean (%CV)	3008.1 (34.4)
%AUC_{exp} Mean (%CV)	2.1 (34.6)
T_{max} (h) Median (Q1, Q3)	4.50 (4.01, 4.50)
T_{last} (h) Median (Q1, Q3)	30.01 (28.00, 36.00)
$T_{1/2}$ (h) Median (Q1, Q3)	4.87 (4.11, 5.76)

%CV = percent coefficient of variation; Q1 = first quartile; Q3 = third quartile
Source: Section 11.1, Table 8.3

Results of safety analysis

In total, 2 (16.7%) study subjects reported a treatment-emergent AE (one reported constipation, the other reported sensitivity of the teeth). All adverse events were judged to be of mild (Grade 1) severity and unrelated to treatment. There were no clinically significant changes in mean laboratory parameters, and there were no Grade 3 or Grade 4 treatment-emergent lab abnormalities in individual subjects. No serious adverse events occurred and no subjects discontinued the study due to adverse events.

Trial Summary

This mass-balance study characterized the pharmacokinetics and investigated the metabolism and excretion of GS-9137 following a single oral dose of [^{14}C]GS-9137/r 50/100 mg to healthy adult subjects. Radioactivity was primarily excreted in the feces (94.8% of the administered dose), while urinary recovery was low (6.7%). Sources of GS-9137 in the feces are likely unabsorbed drug from the gastrointestinal tract, biliary secretion of GS-9137, and potential biliary secretion of GS-9200 that was converted back to GS-9137 by the β-glucuronidases in the intestinal microflora.

The primary composition of plasma radioactivity was GS-9137 (approximately 94% of circulating species). Minor levels of the metabolites GS-9202 and GS-9200 (M1 and M4) were also detected in plasma; these metabolites have substantially less antiviral activity (5- to 38-fold lower) than GS-9137. Biotransformation is primarily via aromatic and aliphatic hydroxylation and/or primary or secondary glucuronidation.

Two mild treatment-emergent adverse events were reported by two different subjects: constipation and sensitivity of the teeth. No serious adverse events

occurred and no subjects discontinued the study due to adverse events. Overall, a single oral dose of [^{14}C]GS-9137/r 50/100 mg was safe and well-tolerated in healthy adult male subjects.

Trial GS-US-183-0140
A Phase 1 Study to Determine the Multiple Dose Relative Bioavailability of a Test Formulation of Elvitegravir Boosted with Ritonavir

Trial Period
9 May to 11 July 2007
Final report date: 24 March 2008 (submitted to IND ⬛(b) (4))

Trial Site
SeaView Research, Inc., Miami, Florida, USA

Trial Rationale
GS-9137 (elvitegravir, EVG) is an inhibitor of the human immunodeficiency virus (HIV) integrase, currently under development for the treatment of HIV infection. Results from *in vitro* studies have demonstrated potent anti-HIV activity, including activity against viruses that are resistant to nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PI). Ritonavir (RTV) is an HIV PI indicated for use in combination with other antiretroviral drugs for the treatment of HIV infection. Coadministration of antiretroviral drugs that are cytochrome P450 (CYP) isoform 3A substrates with the potent CYP3A inhibitor RTV increases the systemic exposure of the substrate drugs. The primary goal of this study was to evaluate exposure from multiple 150 mg doses of a formulation of EVG (Formulation 2) to be used in Phase 3 studies and commercially.

Trial Objectives
The primary objective of the trial was to:

- evaluate the relative bioavailability of the Phase 3 formulation (Formulation 2) of elvitegravir boosted with ritonavir
-

The secondary objective of the trial was to:

- evaluate the safety of two formulations of elvitegravir boosted with ritonavir

Trial Design
This was a randomized, open-label, multiple-dose, two-sequence, two-way crossover study stratified by gender and ethnicity (Hispanic/non-Hispanic). There were two treatments:

Treatment A: EVG 125 mg tablet (reference formulation) with RTV 100 mg capsule QD
Treatment B: EVG 150 mg tablet (test formulation; Formulation 2) with RTV 100 mg capsule QD

Eligible subjects were randomized to one of two treatment sequences (A-B or B-A), each of which lasted 20 days, followed by 7 days of follow-up. There was no washout period. Subjects were confined to the clinic for the 20 days during which study treatment was administered. The study schema is shown in Figure 1.

Figure 1: Study schema (source: Study Report Figure 5-1)
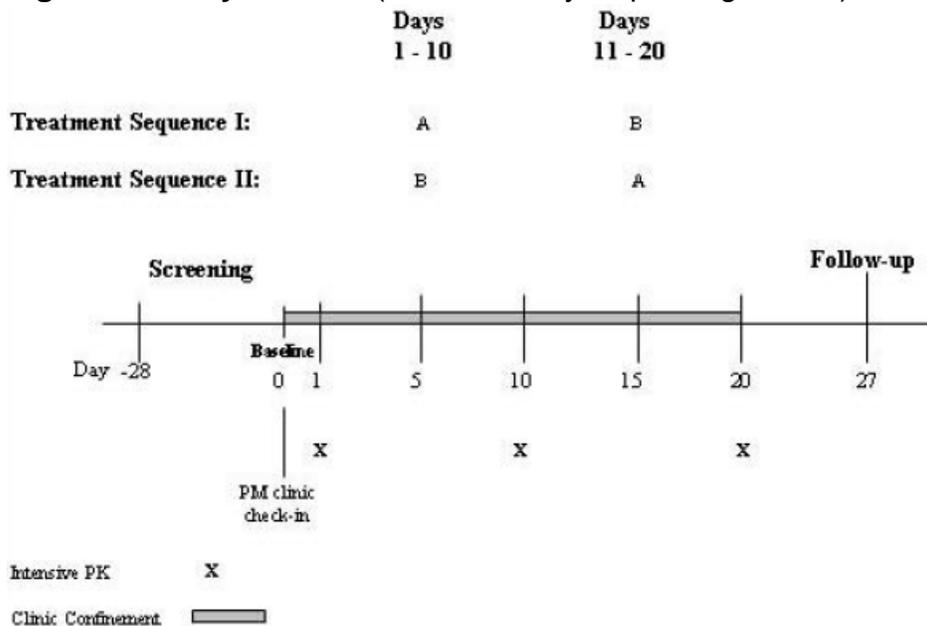
Rationale for Dose Selection

The EVG/r dose of 125/100 mg (reference formulation) QD was selected as Treatment A in the current study after reviewing results from GS-US-183-0105, a Phase 2 dose-finding study in treatment-experienced HIV-infected subjects examining the efficacy (time-weighted mean change from baseline in HIV-1 RNA at Week 24) of EVG/r 20/100, 50/100, and 125/100 mg QD. Results from the relative bioavailability study GS-US-183-0121 indicated that EVG exposures from a test formulation (Formulation 2) were 9.9% lower than exposures from the reference formulation used in GS-US-183-0105. Therefore, an EVG/r dose of 150/100 mg (Formulation 2) QD was selected as Treatment B in the current study.

Drug Administration

Subjects were confined to the study facility for the duration of treatment (i.e. Days 1-20). Study drugs were administered in an open-label fashion with 240 mL of water, within 5 min of completion of a standardized meal (containing approximately 400 calories and 13 g fat) at approximately the same time each day. On Days 1, 10, and 20 (on which intensive PK sampling occurred), study drugs were administered after an overnight fast of at least 8 h and no water was permitted (except for 240 mL at the time of dosing) from 1 h before until 2 h after

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dosing. After dosing, subjects fasted until the 4 h post-dose blood sample was drawn. Food and beverages containing caffeine and/or xanthine were restricted within 48 h of Days 1, 10, and 20. Alcohol was not permitted for the duration of study treatment.

Investigational Product
EVG 125 mg tablets (reference formulation, Lot AJ603A1) were administered in Treatment A, and EVG 150 mg tablets (Formulation 2, Log AJ0701D1) were administered in Treatment B. Ritonavir (NORVIR®, Abbott Laboratories, Lot 441552E21) 100 mg soft gelatin capsules were used in both treatments.

Key Inclusion and Exclusion Criteria
Subjects were healthy nonsmoking males and females between the ages of 18 and 45 years, inclusive. Potential subjects were excluded from enrollment if they had any serious or active medical or psychiatric illness or if they tested positive for HIV-1 antibody, hepatitis C antibody, or hepatitis B surface antigen. Potential subjects were excluded if they were pregnant or lactating. Exclusion criteria also included previous exposure to investigational drugs (within 30 days of study drug administration), the use of alcohol or illicit drugs, and the use of certain medications within specified timeframes (refer to "Concomitant Medications" section).

Concomitant Medications
The following medications were disallowed while subjects were participating in the study:

- all prescription and over-the-counter medications (including herbal products), with the exception of vitamins, acetaminophen, ibuprofen, and hormonal contraceptives including Depo-Provera; exceptions were subject to Sponsor approval
- certain citrus fruits and citrus fruit products (from 3 days prior to Day 1 until study completion)
- antacids that contain calcium, magnesium, or aluminum; sucralfate; or vitamin or mineral supplements that contain calcium, iron, or zinc (from at least 30 days prior to Day 1 until study completion)
- contraindicated drugs: $_1$-adrenoreceptor antagonist, antiarrhythmics, antihistamines, ergot derivatives, GI motility agent, neuroleptics, sedative/hypnotics, antineoplastics, herbal products, HMG-CoA reductase inhibitors, proton-pump inhibitors (from 30 days prior to study screening)
- hepatotoxic drugs: e.g. anabolic steroids, itraconazole, isoniazid, ketoconazole, lovastatin, HIV protease inhibitors, rifabutin, rifampin, simvastin (within 3 months prior to study screening)

- other drugs: systemic steroids, immunosuppressants, chemotherapeutic agents, immune- or cytokine-based therapies (within 3 months prior to study screening)

Sample Collection

Blood was collected for pharmacokinetic analysis of EVG and RTV in plasma at each of the following timepoints (in hours after dosing):

Days 1, 10, and 20 0:00 (predose), 2:00, 3:00, 3:30, 4:00, 4:30, 5:00, 6:00, 8:00, 10:00, 12:00, 18:00, and 24:00 [i.e. predose on Days 2 and 11]

Analytical Plan

Pharmacokinetic data

The pharmacokinetic parameters C_{max}, T_{max}, C_{last}, T_{last}, C_{tau}, λ_z, $t_{1/2}$, $AUC_{0\text{-}last}$, AUC_{inf}, $\%AUC_{exp}$, and AUC_{tau} were estimated for EVG and RTV in plasma. The primary PK parameters for EVG were AUC_{tau} and C_{max} and the secondary PK parameter was C_{tau}. The secondary PK parameters for RTV were AUC_{tau}, C_{max}, and C_{tau}. All pharmacokinetic parameters were estimated using a nonlinear model derived using standard noncompartmental methods (WinNonlin® Professional Edition, Pharsight Corporation, Mountain View, California, USA). Pharmacokinetic parameters that depend on an accurate estimation of the terminal elimination phase (λ_z and $t_{1/2)}$ were reported when the pharmacokineticist deemed the data evaluable after examination.

A mixed-effects model was fitted to log-transformed estimates of pharmacokinetic parameters (AUC_{tau}, C_{max}, and C_{tau}) and used in a parametric analysis of variance (ANOVA) of Treatments A and B at steady-state (Days 10 and 20) using SAS® PROC MIXED (SAS Institute, Cary, North Carolina, USA). Ratios of the geometric least-squares means of EVG and RTV PK parameters and their 90% confidence intervals (CI) were calculated.

Trial Results

Bioanalytical methods

Concentrations of EVG and RTV in plasma samples were measured by LC-MS/MS by Gilead Sciences   (b) (4) ((b) (4)
        Report S-183-24). Frozen plasma samples were received between 5 June and 10 July 2007 and stored at -80°C. Analysis was performed between 17 June and 19 July 2007. The first day of sample collection was 22 May 2007, so the maximum storage sample time was 58 days, which is within the validated long-term frozen stability duration of 268 days for EVG and RTV. The LC-MS/MS method M-GS-9137-16511V7 was used.

For EVG, the calibration standards ranged from 20-10,000 ng/mL and the quality control (QC) concentrations were 50.0, 750, and 7500 ng/mL. For RTV, the

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calibration standards ranged from 5 to 5000 ng/mL and the QC concentrations were 15.0, 750, and 4000 ng/mL. All inter-assay accuracy and precision estimates (% CV, displayed in Table 1) were within the acceptable range (≤20% deviation from nominal at the LLoQ concentration, and ≤15% from nominal at all other concentrations).

Table 1: Bioanalytical assay validation for EVG and RTV in human plasma (source: Study Report Table 5-6)

Validation Parameter	Elvitegravir	Ritonavir
Linear Range (ng/mL)	20 to 10,000	5 to 5,000
LLQ[a] (ng/mL)	20	5
Inter-Assay Precision Range[b]	2.1% to 6.3%	8.0% to 11.6%
Inter-Assay Accuracy Range[c]	−13.0% to −2.4%	−2.0% to 9.4%
Stability in Frozen Matrix (days)[d]	268	268

a   LLQ, lower limit of quantitation
b   Relative standard deviation
c   Difference from nominal concentrations
d   Samples stored at approximately −80°C
Source: Appendix 10

Trial population
A total of 26 healthy adults between the ages of 18 and 45 were randomized in the study: 12 in treatment sequence AB and 14 in treatment sequence BA. All 26 subjects received study drug and were included in the safety analysis set. Two subjects discontinued the study prematurely: Subject 1053 discontinued on Day 6 because of a positive pregnancy test, and Subject 1075 withdrew consent and discontinued on Day 13. The pharmacokinetic analysis set included the 24 subjects who completed study treatment.

In the safety analysis set, 54% of the subjects were black, 42% were white, and 4% were classified as "other." Half of the subjects were Hispanic/Latino. There were slightly more females (54%) than males. The subjects in the PK analysis set had similar demographic and baseline characteristics to those in the safety set.

Pharmacokinetics of single-dose elvitegravir
A single dose of the EVG/r test (150/100 mg) and reference (125/100 mg) formulations resulted in mean EVG plasma concentrations that were similar at each time point. EVG pharmacokinetic parameters of single-dose EVG/r were also similar between the test and reference formulations (Table 2).

Table 2: Summary of single-dose EVG pharmacokinetic parameters (PK analysis set; source: Study Report Table 7-1)

Elvitegravir PK Parameter	Elvitegravir/r 125/100 mg Reference Formulation (N = 12)	Elvitegravir/r 150/100 mg Formulation 2 (N = 12)
C_{max} (ng/mL), Mean (% CV)	1438.2 (52.9)	1414.1 (49.7)
T_{max} (h), Median (Min, Max)	4.00 (3.50, 8.00)	4.25 (4.00, 5.00)
AUC_{0-last} (ng•h/mL), Mean (% CV)	14914.5 (51.9)	14430.5 (51.8)
AUC_{inf} (ng•h/mL), Mean (% CV)	20796.3 (58.5)	19977.9 (55.8)
$T_{\frac{1}{2}}$ (h), Median (Min, Max)	11.00 (6.90, 13.58)	11.19 (7.34, 14.31)

Source: Section 11.1, Table 5.1

Pharmacokinetics of steady-state elvitegravir

A ten-day course of once-daily doses of the EVG/r test (150/100 mg) and reference (125/100 mg) formulations resulted in similar pharmacokinetic profiles (Table 3). Statistical comparisons of the EVG pharmacokinetic parameters C_{max}, AUC_{tau}, and C_{tau} indicated that the two formulations provide comparable EVG exposure (Table 4).

Table 3: Summary of steady-state EVG pharmacokinetic parameters (PK analysis set; source: Study Report Table 7-2)

Elvitegravir PK Parameter	Elvitegravir/r 125/100 mg Reference Formulation (N = 24)	Elvitegravir/r 150/100 mg Formulation 2 (N = 24)
C_{max} (ng/mL), Mean (% CV)	2033.3 (39.3)	2126.3 (37.6)
AUC_{tau} (ng•h/mL), Mean (% CV)	20584.8 (35.7)	22120.7 (32.3)
C_{tau} (ng/mL), Mean (% CV)	407.7 (51.1)	439.9 (47.8)
T_{max} (h), Median (Min, Max)	4.00 (3.00, 5.00)	4.00 (2.00, 6.00)
$T_{\frac{1}{2}}$ (h), Median (Min, Max)	8.89 (6.13, 11.74)	9.13 (6.03, 13.96)
T_{last} (h), Median (Min, Max)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)

Source: Section 11.1, Table 5.1

Table 4: Statistical comparisons of EVG steady-state PK parameters for test vs. reference formulations (PK analysis set; source: Study Report Table 7-3)

Elvitegravir PK Parameters	Geometric Least-Squares Means		Geometric Least-Squares Mean Ratio (%)	90% Confidence Interval
	Test[a] (N = 24)	Reference[a] (N = 24)		
C_{max} (ng/mL)	1997.7	1896.0	105.4	98.8, 112.3
AUC_{tau} (ng•h/mL)	21086.1	19396.5	108.7	102.6, 115.2
C_{tau} (ng/mL)	397.2	360.7	110.1	99.0, 122.6

a   Test Treatment: Elvitegravir/r 150/100 mg (Formulation 2); Reference Treatment: Elvitegravir/r 125/100 mg (Reference Formulation); each treatment was given for 10 consecutive days

Source: Section 11.1, Table 6

Pharmacokinetics of ritonavir

A single dose of RTV 100 mg dosed with EVG 125 mg (reference formulation) or 150 mg (test formulation) resulted in pharmacokinetic profiles that were similar at most timepoints, although RTV exposures were slightly higher when administered with the reference formulation (C_{max} and AUC_{0-last} for the reference formulation were 754.1 ng/mL and 4724.7 ng·h/mL, compared to 663.9 ng/mL and 4054.0 ng·h/mL for the test formulation). The pharmacokinetic profiles of RTV after multiple doses of EVG/r were more similar between the test and reference formulations and were determined to be statistically bioequivalent (Table 5).

Table 4: Statistical comparisons of RTV steady-state PK parameters for test vs. reference formulations (PK analysis set; source: Study Report Table 7-6)

Ritonavir PK Parameters	Geometric Least-Squares Means		Geometric Least-Squares Mean Ratio (%)	90% Confidence Interval
	Test[a] (N = 24)	Reference[a] (N = 24)		
C_{max} (ng/mL)	1024.0	1092.2	93.8	85.9, 102.3
AUC_{tau} (ng•h/mL)	6394.0	6684.0	95.7	90.2, 101.4
C_{tau} (ng/mL)	48.6	50.7	95.8	90.0, 102.0

a   Test Treatment: Elvitegravir/r 150/100 mg (Formulation 2); Reference Treatment: Elvitegravir/r 125/100 mg (Reference Formulation); each treatment was given for 10 consecutive days

Source: Section 11.1, Table 6

Results of safety analysis

The safety analysis set included all 40 subjects who received at least one dose of study drug. Two of the subjects in the safety analysis set discontinued the study prematurely due to a positive pregnancy test (n=1) and withdrawal of consent (n=1). Treatment-emergent adverse events were more common in the EVG/r 150/100 mg (test) formulation, especially nervous system events (8 subjects with 4 reports of headache and 5 reports of dizziness, compared to 2 subjects with headache in the reference formulation treatment group). Constipation, vomiting, diarrhea, nausea, headache, and dizziness were considered treatment-related in the test formulation treatment group, while constipation, vomiting, pain in

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extremity, and headache were considered treatment-related in the reference formulation treatment group. All treatment-emergent adverse events were Grade 1 or 2 and no subjects discontinued study drug due to adverse events. No serious adverse events or deaths occurred during the study.

Trial Summary

In this study, two formulations of elvitegravir were tested for bioequivalence: the reference formulation, which had been used in the Phase 2 dose-finding study GS-US-183-0105, and a test formulation (Formulation 2) which was to be used in future Phase 3 studies. Because of differences in exposure between the two formulations characterized in GS-US-183-0121, EVG/r was dosed at 150/100 mg (reference formulation, Treatment A) and 125/100 mg (test formulation, Treatment B). The results of this study demonstrate the bioequivalence of both EVG and RTV in the test and reference formulations as assessed by the ratios of the geometric least-squares means and their corresponding 90% confidence intervals for AUC_{tau}, C_{max}, and C_{tau}.

Both formulations of EVG/r were generally well-tolerated. All treatment-emergent adverse events were Grade 1 or 2 and no subjects discontinued study drug due to adverse events.

Trial GS-US-183-0146
A Phase 1, Multiple-Dose Pharmacokinetic Drug Interaction Study of Elvitegravir/r and Ketoconazole

Trial Period
8 Nov to 21 Dec 2007
Final report date: 11 Sep 2009 (submitted to IND   (b) (4)   )

Trial Site
Charles River Clinical Services Northwest, Inc., Tacoma, Washington, USA

Trial Rationale
Elvitegravir (EVG) is an inhibitor of the human immunodeficiency virus (HIV) integrase, currently under development for the treatment of HIV infection. Results from *in vitro* studies have demonstrated potent anti-HIV activity, including activity against viruses that are resistant to nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PI). Coadministration of EVG with the HIV PI ritonavir (RTV; EVG/r) results in a 20-fold increase in EVG exposures due to the inhibition of CYP3A activity by RTV. Similarly, ketoconazole (KTZ) is a potent CYP3A inhibitor, although it also inhibits other metabolic pathways such as UGT1A1 and UGT2B7 (K_i = 3.3 uM). This study investigated the ability of KTZ to provide additional pharmacoenhancement of EVG exposures beyond that of RTV. It also evaluated the additional effect of KTZ on CYP3A activity (beyond that of RTV) using the CYP3A probe substrate midazolam (MDZ).

Trial Objectives
The primary objectives of the trial were to:

- examine the effect of KTZ on the pharmacokinetics of EVG/r
- determine the additional effect of KTZ on CYP3A enzyme activity using midazolam (MDZ) as the CYP3A probe substrate when administered concomitantly with EVG/r

The secondary objective of the trial was to:

- evaluate the safety and tolerability of EVG/r and the combination of EVG, RTV, and KTZ

Trial Design
This was a nonrandomized, open-label, multiple-dose, drug interaction study. There was a single treatment sequence that lasted 15 days, followed by a 7-day follow-up phase.

Treatment A EVG/r 150/100 mg QD (Days 2-11)

Treatment B EVG/r 150/100 mg QD plus KTZ 200 mg BID (Days 12-15)

MDZ oral syrup (5 mg) was also administered on Days 1, 11, and 15.

Subjects were confined to the clinic between Days 0 and 2 and Days 10 and 16 and returned to the study site on Days 3 through 10 in the morning for observed dosing of study drugs. The study schema is shown in Figure 1.

Figure 1: Study schema (source: adapted from Study Report Figure 5-1)
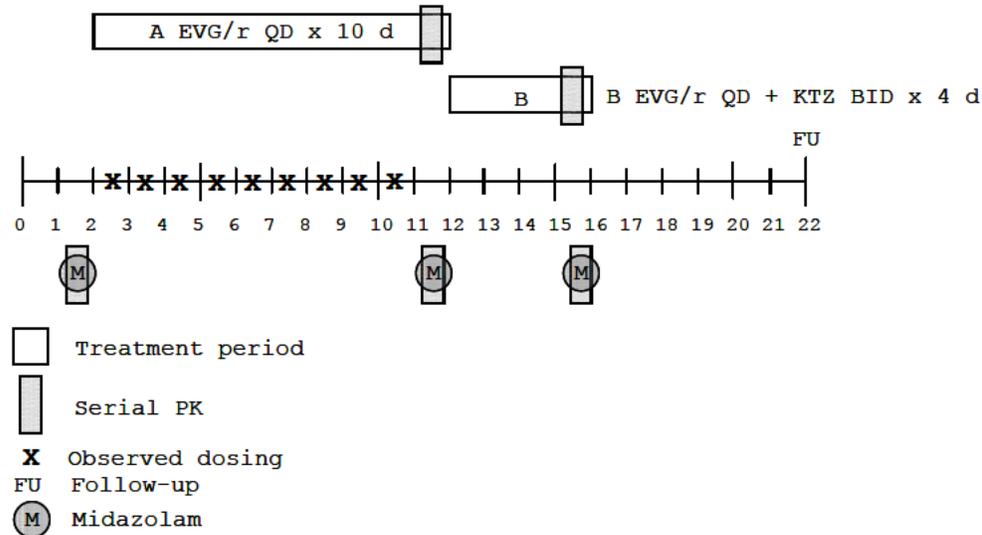
Drug Administration
EVG/r and MDZ were administered in the morning. When applicable, KTZ was coadministered with EVG/r and MDZ in the morning. On mornings of intensive PK sampling days (Days 1, 11, and 15), study drugs were administered with 240 mL of water within 5 minutes of completion of a standardized meal (containing approximately 400 calories and 13 g fat). At all other times, study drugs were administered with 240 mL of water and unspecified food items.

On intensive sampling days (Days 1, 11, and 15), subjects were required to fast for at least 8 hours prior to study drug administration. Subjects were also required to fast starting with administration of each EVG/r dose until after the collection of the blood sample 4 h later. Water was also restricted from 1 h before until 2 h after EVG/r dosing.

To maximize subject safety, subjects were required to maintain a seated position after MDZ administration and to stay at the study site for at least 24 h after dosing.

Rationale for Dose Selection

The EVG/r dose of 150/100 mg was selected for this study because it was the dose being evaluated in the concurrent Phase 3 study.

The MDZ dose of 5 mg was selected for this study because MDZ and RTV are contraindicated at their therapeutic doses (maximum recommended MDZ dose is 20 mg); the 5 mg dose is frequently used in PK studies in which MDZ is a probe drug and has minimal clinical effects in healthy subjects.

The KTZ dose of 200 mg BID was selected because literature reports indicated that this dosing regimen would allow for maintenance of average plasma concentrations above the K_i values for CYP3A and UGT throughout the entire dosing interval and therefore maximize the potential for a drug interaction. KTZ was dosed for 5 days because it was expected that this duration of dosing would be sufficient to reach steady-state (the half-life of KTZ is approximately 3-4 h).

Investigational Product

Tablets containing 150 mg of EVG were manufactured by ▓▓▓▓ (b) (4) (▓▓▓▓ (b) (4) ▓▓▓▓ Lot AJ0701D1). Soft gelatin capsules containing 100 mg RTV (NORVIR®) were manufactured by Abbott Laboratories (Abbott Park, Illinois, USA; Lot 526442E21). Midazolam hydrochloride syrup 2 mg/mL (VERSED®) was manufactured by Roxane Laboratories, Inc. (Columbus, Ohio, USA; Lot 658337A). Tablets containing 200 mg KTZ (NIZORAL®) were manufactured by Mutual Pharmaceutical Co. (Philadelphia, Pennsylvania, USA; Lot 60274).

Key Inclusion and Exclusion Criteria

Subjects were healthy nonsmoking males and females between the ages of 18 and 45 years, inclusive. Potential subjects were excluded if they were pregnant or lactating, or if they had taken any prescription or over-the-counter medication (including herbal products and antacids, with the exception of vitamins, acetaminophen, ibuprofen, and hormonal contraceptives) within 30 days prior to study drug dosing.

Potential subjects were excluded for the use of specified medications from the following drug classes within 30 days of the baseline visit: alpha$_1$-adrenoreceptor antagonist, antiarrhythmics, anticoagulants, antihistamines, ergot derivatives, GI motility agents, neuroleptics, sedative/hypnotics, antineoplastics, herbal products, proton-pump inhibitors, or H$_2$-receptor antagonists. Potential subjects were also excluded if they had received hepatotoxic drugs, systemic steroids, immunosuppressants, or chemotherapeutic agents within 3 months prior to study screening.

Concomitant Medications

In addition to the medications detailed in the "Key Inclusion and Exclusion Criteria" section above, food or beverages containing caffeine, xanthine, or soda were not permitted starting 48 h before study drug administration and during all

confinement periods. Alcohol was not permitted for the duration of the study. Consumption of certain citrus fruits, fruit and vegetable juices, antacids, and calcium channel blockers was restricted during the study.

Sample Collection

Blood was collected for the analysis of EVG (and metabolites GS-9202 and GS-9200), RTV, MDZ (and metabolite 1'-OH MDZ), and KTZ on the specified study days at the times (in hours post-dose) listed below:

EVG, RTV: Days 11, 15   0:00 (predose), 0:15, 0:30, 0:45, 1:00, 1:30, 2:00, 2:30, 3:00, 4:00, 5:00, 6:00, 8:00, 10:00, 12:00, 18:00, and 24:00

MDZ: Days 1, 11, 15   0:00 (predose), 0:15, 0:30, 0:45, 1:00, 1:30, 2:00, 2:30, 3:00, 4:00, 5:00, 6:00, 8:00, 10:00, 12:00, 18:00, and 24:00

KTZ: Day 15   0:00 (predose), 0:15, 0:30, 0:45 1:00, 1:30, 2:00, 2:30, 3:00, 4:00, 5:00, 6:00, 8:00, 10:00, and 12:00

Analytical Plan

Pharmacokinetic data

The primary pharmacokinetic parameters evaluated in this study were EVG AUC_{tau}, C_{max}, and C_{tau}. Secondary pharmacokinetic parameters included AUC_{tau}, AUC_{inf}, C_{max}, C_{tau}, $t_{1/2}$, T_{last}, λ_z, CL/F, and V/F for some or all of these analytes: EVG, RTV, GS-9202, GS-9200, KTZ, MDZ, and 1'-OH MDZ. All pharmacokinetic parameters were estimated using a nonlinear model derived using standard noncompartmental methods (WinNonlin® Professional Edition, Pharsight Corporation, Mountain View, California, USA). Pharmacokinetic parameters that depend on an accurate estimation of the terminal elimination phase (λ_z, and $t_{1/2}$) were reported when the pharmacokineticist deemed the data evaluable after examination.

Differences between treatments were estimated using a parametric analysis of variance (ANOVA) using a mixed effects model, with treatment as a fixed effect and subject as a random effect. The model was used to generate the point estimate and 90% CI between treatments. EVG PK parameters were considered unaltered if the 90% CI values were within the lack of interaction boundaries of 70-143%.

Trial Results

Bioanalytical methods

Concentrations of EVG, its metabolites GS-9200 (M4, produced by UGT1A1/3) and GS-9202 (M1, produced by CYP3A), and RTV in plasma samples were determined using LC-MS/MS (Method M-GS-9137-16511 Version 7) by Gilead

Sciences   (b) (4) Frozen
plasma samples were received between 5 Dec and 19 Dec 2007 and stored at -
80˚C. Analysis was performed between 20 Dec 2007 and 4 Feb 2008. The first
day of sample collection was 30 Nov 2007, so the maximum storage sample time
was 66 days, which is within the validated long-term frozen stability duration of
268 days for EVG, GS-9200, GS-9202, and RTV.

Concentrations of KTZ were determined using LC-MS/MS (Project 60-0719B) by
                                                              (b) (4) Frozen plasma
samples were received on 17 Dec 2007 and stored at -20˚C. Analysis was
performed between 17 Dec 2007 and 7 Jan 2008. Samples were collected on 14
Dec 2007, so the maximum storage sample time was 24 days, which is within the
validated long-term frozen (-20˚C) stability duration of 58 days.

Concentrations of MDZ and its metabolite 1'-OH MDZ were determined using
LC-MS/MS (Project 60-0719A) by   (b) (4)
                        Frozen plasma samples were received between 4 and 17 Dec
2007 and stored at -20˚C. Analysis was performed between 18 Dec 2007 and 16
Jan 2008. Samples were collected between 30 Nov and 14 Dec 2007, so the
maximum storage sample time was 46 days, which is within the validated long-
term frozen (-70˚C) stability duration of 135 days.

The EVG calibration standards ranged from 20-10,000 ng/mL and the quality
control (QC) concentrations were 50.0, 750, and 7500 ng. For GS-9200 and GS-
9202, the calibration standards ranged from 20-1000 ng/mL and the QC
concentrations were 50.0, 150, and 750 ng/mL. For RTV, the calibration
standards ranged from 5-5000 ng/mL and the QC concentrations were 15.0, 750,
and 4000 ng/mL. For KTZ, the calibration standards ranged from 20-10,000
ng/mL and the QC concentrations were 20, 60, 750, and 9000 ng/mL. For MDZ,
the calibration standards ranged from 0.1-100 ng/mL and the QC concentrations
were 0.1, 0.3, 5, 30, and 75 ng/mL. For 1'-OH MDZ, the calibration standards
ranged from 0.1-100 ng /mL and the QC concentrations were 0.3, 5, 30, and 75
ng/mL. All inter-assay accuracy and precision estimates (displayed in Table 1)
were within the acceptable range (≤20% deviation from nominal at the LLoQ
concentration, and ≤15% from nominal at all other concentrations).

**Table 1: Bioanalytical assay validation for EVG, GS-9200, GS-9202, RTV,
KTZ, and MDZ in human plasma** (source: Study Report Table 5-7)

Validation Parameter	EVG (GS-9137)	M4 (GS-9200)	M1 (GS-9202)	RTV	MDZ	1'-OH-MDZ	KTZ
Linear Range (ng/mL)	20 to 10,000	20 to 1000	20 to 1000	5 to 5000	0.1 to 100	0.1 to 100	20 to 10,000
LLQ (ng/mL)	20	20	20	5	0.1	0.1	20
Inter-Assay Precision Range[a]	2.1% to 6.3%	4.7% to 14.7%	3.5% to 10.9%	8.0% to 11.6%	2.4% to 7.1%	3.0% to 8.2%	5.9% to 9.9%
Inter-Assay Accuracy Range[b]	−13.0% to 2.4%	−4.5% to 1.5%	−5.1% to 3.3%	−2.0% to 9.4%	−2.7% to −1.0%	−3.0% to −1.3%	−8.9% to −0.8%
Stability in Frozen Matrix	268 days at −80°C	268 days at −80°C	268 days at −80°C	268 days at −80°C	ongoing	ongoing	58 days at 20°C

EVG, elvitegravir; KTZ, ketoconazole; LLQ, lower limit of quantitation; MDZ, midazolam; RTV, ritonavir

a   Relative standard deviation

b   Difference from nominal concentrations

Source: Appendix 10

Trial population

A total of 18 healthy adult subjects were enrolled in and completed the study. The majority of subjects were white (61%); the remaining 39% of subjects were black. Most of the subjects were male (67%). Subjects had a mean age of 27 years (range: 19-44 years). All 18 subjects were included in both the pharmacokinetic and safety analysis sets.

Results of pharmacokinetic analyses

In this study, the effects of coadministration of KTZ and RTV on EVG pharmacokinetics were evaluated. The PK profile of the CYP3A probe substrate MDZ was used to calibrate the extent of CYP3A inhibition by RTV and KTZ, thereby allowing the inhibitory effects of KTZ on CYP3A and UGT1A1/3 (and on EVG PK if alterations were observed) to be teased apart. Midazolam PK was evaluated after MDZ was administered alone, in combination with EVG/r, and after coadministration with EVG/r and KTZ.

Four days of KTZ 200 mg BID coadministration with EVG/r 150/100 mg QD resulted in increased EVG plasma concentrations at all time points (see Figure 2). The PK parameters for EVG/r alone and with KTZ, listed in Table 2, indicate significant alterations in EVG AUCtau and Ctau, with 90% CI values above the prespecified bounds of 70-143% (see Table 3). Increases in EVG AUC_{tau}, C_{tau}, and C_{max} were observed while median $t_{1/2}$ remained similar between treatments, indicating enhanced oral bioavailability but unchanged elimination.

Figure 2: Steady-state EVG plasma concentrations after administration of EVG/r alone or with KTZ (mean ± SD; PK analysis set; source: Study Report Figure 7-1)
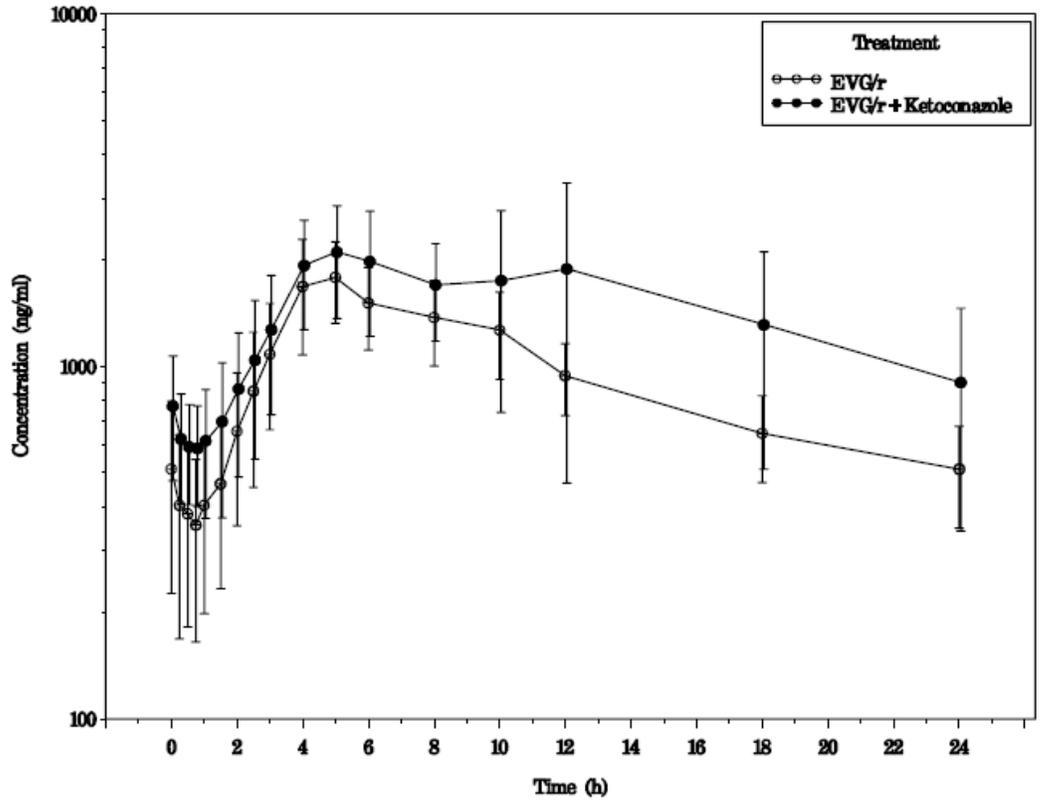
Note: Plasma concentrations below the lower limit of quantitation were treated as 0 for summary statistics and missing for log-normalized data.
EVG/r, elvitegravir and ritonavir; PK, pharmacokinetic; SD, standard deviation
Source: Section 11.1, Figure 1.1 (semi-logarithmic scale)

Table 2: Summary of steady-state EVG PK parameters after administration of EVG/r alone or with KTZ (PK analysis set; source: Study Report Table 7-1)

Elvitegravir Plasma PK Parameters	Elvitegravir/r (N = 18)	Elvitegravir/r + Ketoconazole (N = 18)
C_{max} (ng/mL) Mean (%CV)	1985.4 (24.6)	2450.7 (52.8)
AUC_{tau} (ng•h/mL) Mean (%CV)	22,389.9 (23.9)	34,817.6 (50.0)
C_{tau} (ng/mL) Mean (%CV)	511.4 (32.3)	900.1 (62.1)
$T_{1/2}$ (h) Median (Q1, Q3)	11.78 (10.22, 13.88)	12.64 (9.39, 16.56)
T_{max} (h) Median (Q1, Q3)	5.00 (4.00, 5.00)	5.00 (4.00, 5.00)
T_{last} (h) Median (Q1, Q3)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
CL/F (mL/h) Mean (%CV)	7114.6 (28.6)	4925.3 (34.8)
CL/F (mL/h•kg) Mean (%CV)	91.8 (21.7)	63.1 (28.5)

PK, pharmacokinetic
Source: Section 11.1, Table 5.1

Table 3: Statistical comparisons of EVG PK parameters after administration of EVG/r alone or with KTZ (PK analysis set; source: Study Report Table 7-2)

Test versus Reference Comparison PK Parameter	Geometric Least-squares Means		Geometric Least-squares Means Ratio (%)	90% Confidence Interval
	Test[a] (Mean)	Reference[b] (Mean)		
Elvitegravir/r + Ketoconazole versus Elvitegravir/r				
C_{max} (ng/mL)	2260.2	1926.3	117.3	103.8, 132.6
AUC_{tau} (ng•h/mL)	32286.0	21766.3	148.3	136.2, 161.6
C_{tau} (ng/mL)	804.8	482.9	166.7	148.2, 187.5

Note: Values are rounded. PK, pharmacokinetic

a   Test Treatment: Elvitegravir 1×150-mg tablet plus ritonavir 1×100-mg capsule once daily plus ketoconazole 1×200-mg tablet twice daily (dose of all three study drugs were taken together in the morning)

b   Reference Treatment: Elvitegravir 1×150-mg tablet plus ritonavir 1×100-mg capsule once daily (in the morning)
Source: Section 11.1, Table 6.1

Plasma concentrations of the EVG glucuronide metabolite M4 (GS-9200) were also higher after coadministration of EVG/r and KTZ, with increases in C_{tau} (35.0 ng/mL with KTZ compared to 20.0 ng/mL without) and AUC_{tau} (1621.5 ng·h/mL with KTZ compared to 1337.3 ng·h/mL without). The 90% CI for C_{tau} fell slightly above the predefined threshold for alterations in EVG PK. There was also a slight decrease in the mean AUC_{tau} M4:EVG ratio after KTZ treatment (6.1% compared to 5.0%), which may be caused by changes in the total amount of EVG metabolized in the presence of KTZ.

Plasma concentrations of the EVG metabolite M1 (GS-9202), formed by CYP3A, were below the limit of quantitation at all timepoints in the study.

Like EVG, ritonavir exposures were increased in the presence of KTZ. The results of a statistical comparison indicate that KTZ significant impacts RTV PK, with RTV 90% CIs falling outside of the prespecified 70-143% boundaries (Table 4).

Table 4: Statistical comparisons of RTV PK parameters after administration of EVG/r alone or with KTZ (PK analysis set; source: Study Report Table 7-6)

Test versus Reference Comparison Ritonavir Plasma Pharmacokinetic Parameters	Geometric Least-squares Means		Geometric Least-squares Means Ratio (%)	90% Confidence Interval
	Test[a] (Mean)	Reference[b] (Mean)		
Elvitegarvir/r + Ketoconazole versus Elvitegarvir/r				
C_{max} (ng/mL)	902.1	685.3	131.6	115.6, 149.9
AUC_{tau} (ng·h/mL)	7349.5	4539.1	161.9	142.7, 183.7
C_{tau} (ng/mL)	68.7	33.1	207.3	168.5, 255.0

Note: Values are rounded.

a   Test Treatment: Elvitegravir 1×150-mg tablet plus ritonavir 1×100-mg capsule once daily plus ketoconazole 1×200-mg tablet twice daily (dose of all three study drugs were taken together in the morning)

b   Reference Treatment: Elvitegravir 1×150-mg tablet plus ritonavir 1×100-mg capsule once daily (in the morning)

Source: Section 11.1, Table 6.1

The PK profile of KTZ was compared to historical data obtained following coadministration with RTV-boosted darunavir (DRV/r). The values for KTZ mean AUC_{tau} (97,879 ng·h/mL compared to 94,343 ng·h/mL after coadministration with DRV/r) and C_{max} (10,688 ng/mL compared to 10,501 ng/mL after coadministration with DRV/r) were similar to the historical data, while C_{tau} was higher (7495 ng/mL in this study compared with 5354 ng/mL in the DRV/r study).

The PK parameters of MDZ alone and after coadministration with EVG/r and EVG/r+KTZ are listed in Table 5; statistical comparisons are listed in Table 6. Plasma concentrations of MDZ were significantly higher after coadministration with EVG/r. Addition of KTZ resulted in a further increase in MDZ exposures, although this increase was slight.

Table 5: Summary of MDZ PK parameters after administration of MDZ alone, with EVG/r, or with EVG/r+KTZ (PK analysis set; source: Study Report Table 7-8)

Midazolam Plasma PK Parameters	Midazolam (N = 18)	Midazolam + Elvitegravir/r (N = 18)	Midazolam + Elvitegravir/r + Ketoconazole (N = 18)
C_{max} (ng/mL) Mean (%CV)	14.4 (30.2)	51.9 (16.6)	52.7 (26.2)
AUC_{inf} (ng•h/mL) Mean (%CV)	60.4 (62.9)	773.8 (29.7)	912.1 (29.4)
AUC_{0-last} (ng•h/mL) Mean (%CV)	58.1 (60.2)	555.7 (19.2)	593.5 (18.3)
T_{last} (h) Median (Min, Max)	23.75 (18.00, 23.75)	23.75 (23.72, 23.75)	24.00 (24.00, 24.00)
$T_{1/2}$ (h) Median (Q1, Q3)	4.77 (3.08, 5.42)	11.87 (9.27, 14.53)	14.94 (10.24, 19.59)
T_{max} (h) Median (Q1, Q3)	1.00 (0.75, 1.50)	2.00 (1.50, 2.50)	2.00 (1.50, 2.50)
C_{last} (ng/mL) Mean (%CV)	0.3 (110.0)	10.8 (35.5)	12.4 (35.7)
CL/F (mL/h) Mean (%CV)	104180.8 (44.2)	6955.7 (27.1)	5989.9 (31.7)
CL/F (mL/h•kg) Mean (%CV)	1351.2 (40.8)	90.3 (24.5)	77.4 (26.7)

PK, pharmacokinetic

Source: Section 11.1, Table 5.5

Table 6: Statistical comparisons of MDZ PK parameters after administration of MDZ alone, with EVG/r, or with EVG/r+KTZ (PK analysis set; source: Study Report Table 7-9)

Test versus Reference Comparison Midazolam Plasma PK Parameters	Geometric Least-squares Means		Geometric Least-squares Mean Ratio (%)	90% Confidence Interval
	Test (Mean)	Reference (Mean)		
Midazolam + Elvitegravir/r versus Midazolam[a]				
C_{max} (ng/mL)	51.1	13.8	371.1	334.4, 411.8
AUC_{inf} (ng•h/mL)	745.0	53.1	1402.7	1247.6, 1577.1
C_{last} (ng/mL)	10.2	0.18	5545.6	4437.9, 6929.9
Midazolam + Elvitegravir/r + Ketoconazole versus Midazolam + Elvitegravir/r[b]				
C_{max} (ng/mL)	51.2	51.1	100.2	90.3, 111.2
AUC_{inf} (ng•h/mL)	873.6	745.0	117.3	104.3, 131.8
C_{last} (ng/mL)	11.5	10.2	113.3	90.7, 141.6

PK, pharmacokinetic

a   Test = midazolam plus elvitegravir/r; Reference = midazolam

b   Test = midazolam plus elvitegravir/r plus ketoconazole; Reference = midazolam plus elvitegravir/r

Source: Section 11.1, Tables 5.5 and 6.1

Both EVG/r and KTZ coadministration influenced the PK of the 1'-OH MDZ metabolite, which is formed by CYP3A, as can be seen in Figure 3 and Tables 7 and 8. As expected, coadministration with the CYP3A inhibitor RTV significantly decreased formation of 1'-OH MDZ. However, the addition of the CYP3A and UGT inhibitor KTZ (i.e. MDZ+EVG/r+KTZ) resulted in a significant increase in 1'-OH MDZ AUC_{inf} compared to MDZ+EVG/r treatment. The Applicant proposes that this increase is due to a reduction in 1'-OH MDZ clearance and cites reports that demonstrate glucuronidation of 1'-OH MDZ by UGT2B7.

Figure 3: Plasma concentration-time profile of 1'-OH MDZ after administration of MDZ alone, with EVG/r, or with EVG/r+KTZ (mean ± SD; PK analysis set; source: Study Report Figure 7-6)
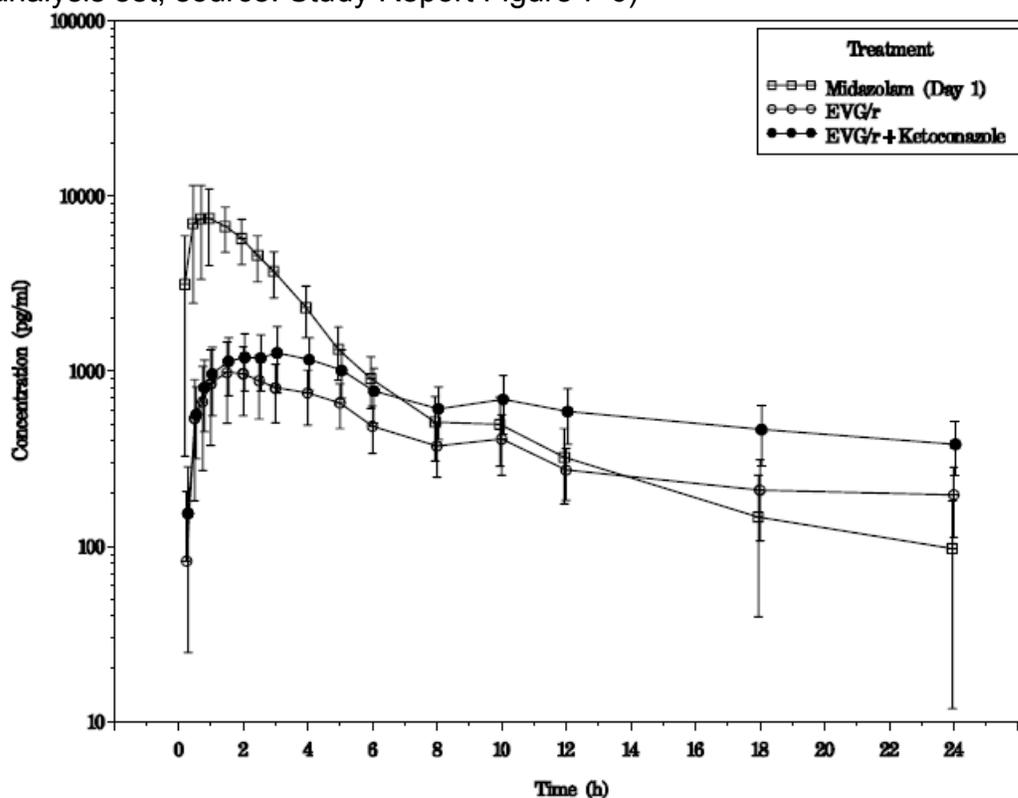
Note: Plasma concentrations below the lower limit of quantitation were treated as 0 for summary statistics and missing for log-normalized data.

EVG/r, elvitegravir and ritonavir; PK, pharmacokinetic

Source: Section 11.1, Figure 1.6

Table 7: Summary of 1'-OH MDZ PK parameters after administration of MDZ alone, with EVG/r, or with EVG/r+KTZ (PK analysis set; source: Study Report Table 7-10)

1'-OH-midazolam Plasma PK Parameters	Midazolam (N = 18)	Midazolam + Elvitegravir/r (N = 18)	Midazolam + Elvitegravir/r + Ketoconazole (N = 18)
C_{max} (ng/mL) Mean (%CV)	8.5 (49.1)	1.1 (43.7)	1.4 (37.4)
AUC_{inf} (ng·h/mL) Mean (%CV)	28.7 (31.7)	13.0 (34.1)	27.0 (33.0)
AUC_{0-last} (ng·h/mL) Mean (%CV)	27.4 (31.7)	9.2 (33.4)	15.5 (30.5)
C_{last} (ng/mL) Mean (%CV)	0.2 (27.2)	0.2 (134.3)	0.4 (33.7)

PK, pharmacokinetic

Source: Section 11.1, Table 5.6

Table 8: Statistical comparisons of 1'-OH MDZ PK parameters after administration of MDZ alone, with EVG/r, or with EVG/r+KTZ (PK analysis set; source: Study Report Table 7-11)

Test versus Reference Comparison 1'-OH-midazolam Plasma Pharmacokinetic Parameters	Geometric Least-squares Means		Geometric Least-squares Means Ratio (%)	90% Confidence Interval
	Test (Mean)	Reference (Mean)		
Midazolam + Elvitegravir/r versus Midazolam[a]				
C_{max} (ng/mL)	1.0	7.8	12.9	11.2, 14.8
C_{last} (ng/mL)	0.2	0.2	133.4	114.4, 155.6
AUC_{inf} (ng·h/mL)	12.3	27.3	45.0	40.7, 49.9
Midazolam + Elvitegravir/r + Ketoconazole versus Midazolam + Elvitegravir/r[b]				
C_{max} (ng/mL)	1.3	1.0	127.7	111.2, 146.8
C_{last} (ng/mL)	0.4	0.2	184.4	158.1, 215.1
AUC_{inf} (ng·h/mL)	25.5	12.3	207.5	187.4, 229.8

Note: Values are rounded.

a   Test = midazolam plus elvitegravir/r; Reference = midazolam

b   Test = midazolam plus elvitegravir/r plus ketoconazole; Reference= midazolam plus elvitegravir/r

Source: Section 11.1, Table 6.1

Results of safety analysis

Study drugs were generally safe and well-tolerated. Headache was the most common AE. All AEs but one (Grade 2 somnolence during EVG/r treatment) were mild in severity. There were no serious adverse events, discontinuations due to adverse events, or deaths during this trial.

Trial Summary

This study was designed to evaluate the effect of coadministration of EVG/r and the CYP3A/UGT inhibitor KTZ r on EVG pharmacokinetics, and to investigate the influence of KTZ and EVG/r administration on the pharmacokinetics of the CYP3A substrate MDZ.

Coadministration of MDZ and EVG/r resulted in increased MDZ exposures compared to MDZ alone. The addition of KTZ led to a further increase in MDZ exposures, albeit a statistically insignificant one. Coadministration of MDZ and EVG/r led to decreased plasma levels of 1'-OH MDZ, as was expected due to the inhibitory effects of RTV on CYP3A. Conversely, the addition of KTZ resulted in increased 1'-OH MDZ plasma concentrations compared to EVG/r alone. The Applicant hypothesizes that this is due to inhibition of 1'-OH MDZ metabolism by UGT2B7, of which KTZ is an inhibitor. Based on published reports, KTZ inhibition of UGT2B7 appears to be a reasonable explanation for increased MDZ metabolism via CYP3A.

Coadministration of KTZ and EVG/r resulted in modest increases in the exposures of EVG, its glucuronide metabolite GS-9200, and RTV compared to administration of EVG/r alone. The presence of KTZ increased EVG concentrations to a small degree compared to RTV, indicating that inhibition of CYP3A is maximal or near-maximal under the current EVG/r dosing regimen; therefore, no additional clinically relevant drug interactions are expected via inhibition of CYP3A. Based on the MDZ data, the increases in EVG AUC upon addition of KTZ could not be fully explained by CYP3A inhibition. EVG is also metabolized by UGT1A1; this route of metabolism was likely inhibited by KTZ, leading to the small increase in EVG AUC.

The extent of alterations in the PK of EVG after coadministration of EVG/r and KTZ is not expected to be clinically relevant, so no adjustment to EVG dose is recommended by the Applicant when KTZ is coadministered. However, the Applicant is recommending that a daily maximum KTZ dose of 200 mg be used when EVG/r is also being administered. Although the KTZ PK did not appear to be substantially influenced by EVG/r coadministration in this study, the Applicant notes that the 200 mg maximum KTZ dose is consistent with KTZ dosing recommendations for other RTV-boosted antiviral therapies.

In vivo trials of Cobicistat

Trial Number	Title	Page Number
GS-US-216-101	A Single- and Multiple-Dose Ranging Study Evaluating the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of GS-9350	191
GS-US-216-111	A Phase 1 Study to Evaluate the Pharmacokinetics, Metabolism, and Excretion of GS-9350	215
GS-US-216-112	A Phase 1 Study to Evaluate the Effect of GS-9350 on Selected P450 Enzymes or Drug Efflux Transporter P-glycoprotein (P-gp)	230
GS-US-216-113	A Phase 1 Study to Evaluate the Pharmacokinetics, Safety and Tolerability of GS-9350 in Healthy Volunteers	245
GS-US-216-116	A Phase 1 Multiple Dose Study to Evaluate Two Formulations of GS-9350 Tablets and the Pharmacokinetics of Elvitegravir Tablets administered with GS-9350 Tablets	258
GS-US-216-121	A Randomized, Blinded, Placebo-Controlled Phase 1 Study Evaluating the Effect of GS-9350 and Ritonavir on Renal Function as Assessed by Markers of Glomerular Filtration Rate	270

<u>GS-US-216-101</u>

1. Title

A Single- and Multiple-Dose Ranging Study Evaluating the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of GS-9350

2. Information Regarding the Clinical Trial Site and Duration of the Trial

The trial was conducted at Charles River Clinical Services, Tacoma WA from April 1, 2008 (first subject screened) to August 27, 2008 (last subject observation).

3. Objectives

The objective of the trial was to evaluate the following after oral dosing: a) the single and multiple dosing pharmacokinetics of GS-9350 (cobicistat), and b) the effect of cobicistat on CYP3A activity.

4. Trial Design

GS-US-216-101 was a Phase I, randomized, double blind, clinical trial that enrolled healthy male and female subjects between 18 and 45 years old. The overall trial design is displayed in Figure 1 and the trial designs for Part A single dosing, Part A multiple dosing, and Part B multiple dosing are displayed in Figures 2, 3, and 4, respectively. Three cohorts were planned and within each cohort subjects were randomized in a 4:1:1 ratio to receive cobicistat, 100 mg of ritonavir or placebo. Cobicistat doses of 50 mg, 100 mg, or 200 mg were evaluated in the three cohorts. A total of 54 subjects were to be enrolled. Within each cohort, 12 subjects were to receive cobicistat with ritonavir placebo, 3 subjects were to receive cobicistat placebo and ritonavir placebo and 2 subjects were to receive cobicistat placebo with 100 mg of ritonavir.

Figure 1-Overall GS-US-216-113 trial design

Part A – Cohorts 1 and 2

Cohort 1
GS-9350 50 mg

Period 1 (Single Dose) · · · · · Period 2 (Multiple Dose)

Cohort 2
GS-9350 100 mg

Period 1 (Single Dose) · · · · · Period 2 (Multiple Dose)

Part B – Cohort 3

Cohort 3
GS-9350 200 mg

Cohort 3 Dosing Period (Multiple Dose)

▬▬ Screening (must occur within 28 days of first dose)
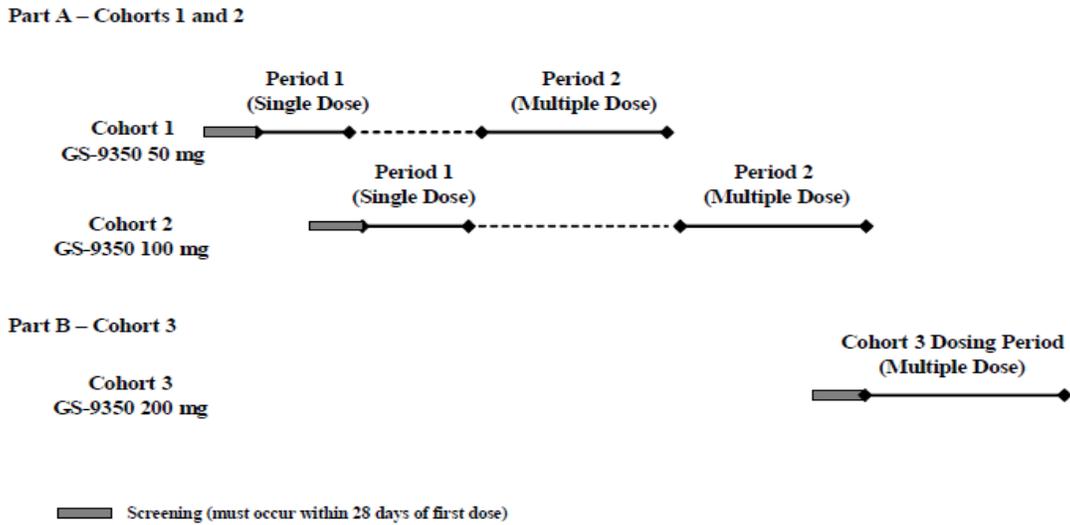
Figure 2-Part A single dosing trial design

Single Dose

Day -27   -2 -1 0   1   2   3   7

Screening

PM Clinic Check-In

Follow-up

Clinic Confinement

Intensive PK   X   X

Midazolam Dose   X

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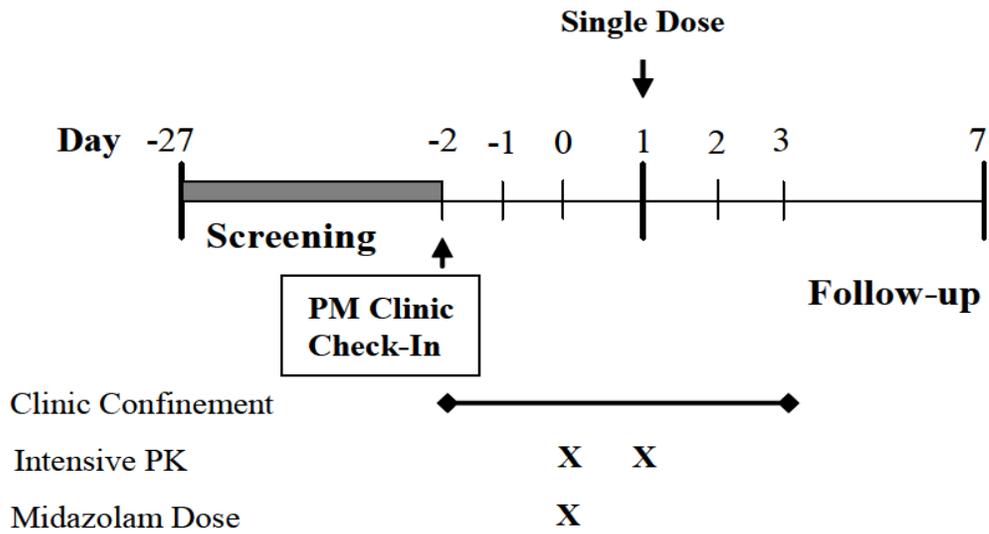
Figure 3-Part A multiple dosing trial design

Begin Multiple Dose (qd)

Last Dose

Day 0 1 4 7 13 14 15 21 28

Clinic Check-In

Follow-up

Clinic Confinement

Intensive PK X

Midazolam Dose X
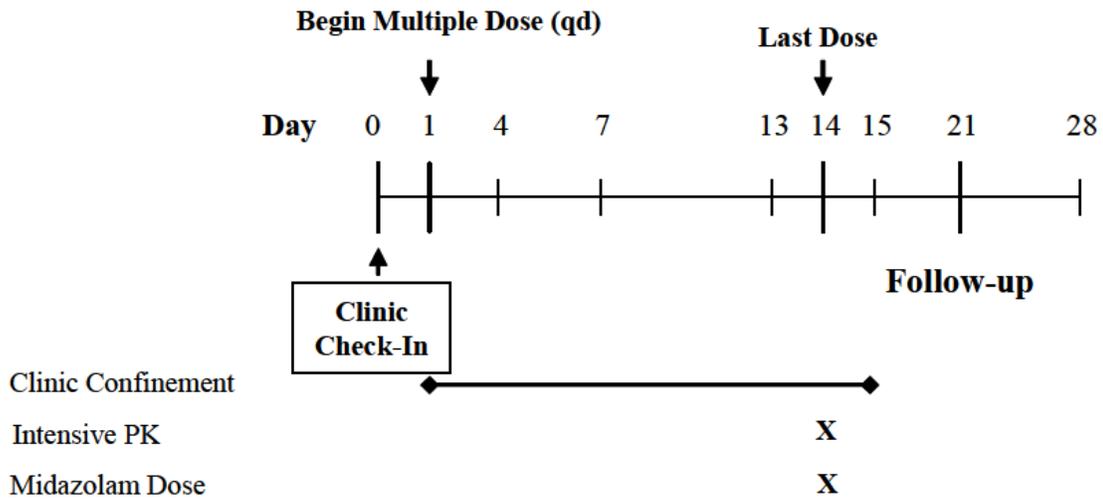
Figure 4-Part B multiple dosing trial design
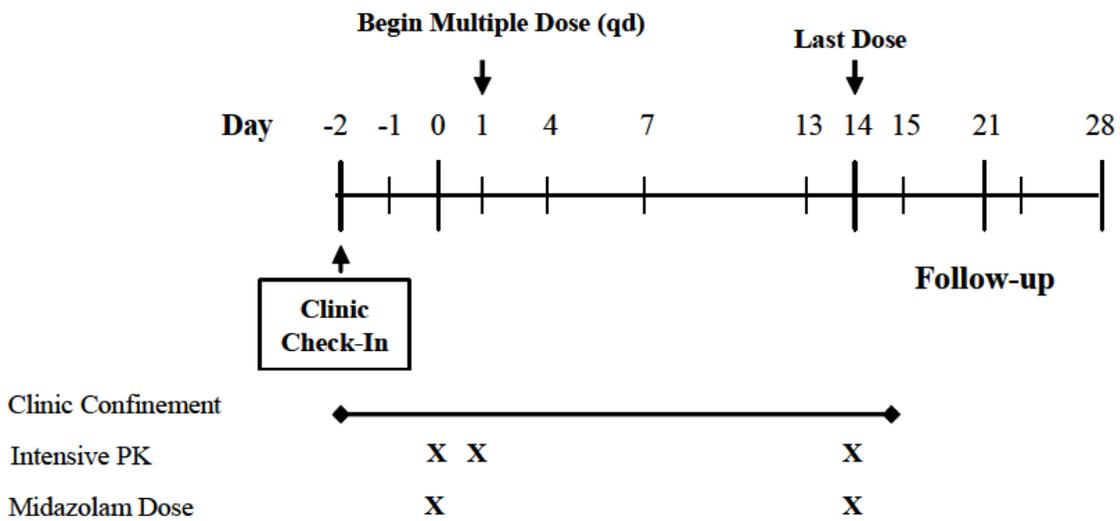
Begin Multiple Dose (qd)

Last Dose

Day -2 -1 0 1 4 7 13 14 15 21 28

Clinic Check-In

Follow-up

Clinic Confinement

Intensive PK .... X X X

Midazolam Dose ...... X X

5. Medication Restrictions and Exceptions

Use of vitamins, acetaminophen, ibuprofen, oral contraceptives, and topical hydrocortisone cream or A&D ointment to treat skin irritation from ECG leads was permitted. All other prescription and nonprescription medications, including herbal medications, were not permitted 28 days before initiation of dosing for the trial and during the trial.

6. Dosage and Administration

In Part A, Period 1, in each cohort, subjects were to receive a single dose of one of the following: 50 mg of cobicistat (cohort 1), 100 mg of cobicistat (cohort 2), 100 mg of ritonavir, cobicistat placebo, or ritonavir placebo. The trial report does not specify the number of days between the end of Period 1 and the start of Period 2 in each cohort. In Part A, Period 2, in each cohort, subjects were to receive once daily doses of one of the following for 14 days: 50 mg of cobicistat (cohort 1), 100 mg of cobicistat (cohort 2), 100 mg of ritonavir, cobicistat placebo, or ritonavir placebo. In Part B, subjects received cobicistat 200 mg once daily, ritonavir 100 mg once daily or placebo for 14 days.

In Part A, a single 5 mg dose of midazolam was administered on day 0 (Period 1) and day 14 (Period 2). In part B, a single 5 mg dose of midazolam was administered on day 0 and day 14.

On intensive pharmacokinetic sampling days or on days when midazolam was administered, subjects fasted for approximately 8 hours overnight and trial medications were administered within 5 minutes of completion of a standardized meal. Water was not permitted from one hour before dosing to two hours after dosing, except with the 240 mL of water that was provided with the trial medication.

7. Rationale for Doses Used in the Trial

The 50 mg and 100 mg cobicistat doses were anticipated to achieve sufficient CYP 3A inhibition. A 200 mg cobicistat dose was also evacuated to more fully characterize cobicistat's effects on CYP 3A activity.

The trial report states that a 5 mg oral midazolam dose was chosen by the applicant because the dose is commonly used in trials where midazolam is used as a CYP3A substrate.

A specific rationale for selecting a ritonavir dose of 100 mg was not provided in the trial report, however when used with other protease inhibitors for the treatment of HIV-1 infection, a ritonavir dose of 100 mg is commonly used to increase the systemic exposure of the concurrently administered HIV-1 protease inhibitor.

8. Drugs Used in the Trial

A summary of the cobicistat, ritonavir, midazolam, and placebo formulations that were administered in the GS-US-216-101 trial is displayed in Table 1 below.

Table 1-Cobcistat, ritonavir, midazolam, and placebo formulations administered in the GS-US-216-101 trial

	GS-9350	GS-9350 Placebo	Ritonavir	Ritonavir Placebo	Midazolam
Strength (mg)	25 and 100 mg	NA	100 mg	NA	5 mg[a]
Lot No.	25 mg: BB0702A1 100 mg: BB0703A1	BB0704A1	AK0801B1	AK0802C1	757363A
Expiration Date	January 2010	January 2013	November 2009	February 2010	August 2009
Manufacturer or Supplier	GSI 333 Lakeside Dr. Foster City, CA 94404	GSI 333 Lakeside Dr. Foster City, CA 94404	Abbott Labs 100 Abbott Park Rd, Abbott Park, IL 60064	(b) (4)	Clinical study site[b]
Site of Release in Europe	NA	NA	NA	NA	NA

a   2.5 mL of a 2 mg/mL oral syrup

b   Commercial drug product

Note: GS-9350, ritonavir, and the two matching placebos were overencapsulated by   (b) (4)

GSI, Gilead Sciences, Inc.; NA, not applicable

9. Sample Collection, Bioanalysis, Pharmacokinetic Assessments, and StatisticalAnalysis

Sample Collection

Blood samples for analysis of cobicistat, ritonavir, and midazolam and 1-hydroxymidazolam plasma concentrations were according to the schedule displayed in Table 2.   If a subject discontinued from the trial early, a cobicistat blood sample was also collected.

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Table 2-Cobcistat, ritonavir, and midazolam blood sampling schedule for the GS-US-216-101 trial

| | Part A, Period 1 | | Part A, Period 2 | Part B | | |
	Day 0	Day 1	Day 14	Day 0	Day 1	Day 14
GS-9350		48 hours postdose	24 hours postdose		24 hours postdose	24 hours postdose
Ritonavir		48 hours postdose	24 hours postdose		24 hours postdose	24 hours postdose
Midazolam (PD Probe)	16 hours postdose		24 hours postdose	16 hours postdose		24 hours postdose

PD, pharmacodynamic

Bioanalysis

The method and bioanalysis of cobicistat, ritonavir and midazolam are acceptable, with the exception of long term stability for cobicistat, ritonavir and midazolam.

Cobicistat plasma samples were analyzed using a validated LC/MS/MS method with K_2EDTA anticoagulated plasma by ⬛⬛⬛ (b) (4) The lower limit of quantification for cobicistat was 5 ng/mL and the upper limit of quantification was 1000 ng/mL. There were no precision or accuracy issues identified for cobicistat based on the bioanalytical report. However, out of the 29 analytical runs where cobicistat samples were analyzed, 2 analytical runs failed. The bioanalytical report does not discussion whether an investigation was conducted to determine the cause(s) of the analytical run failures or whether the cause(s) of the run failures were identified and corrective actions taken.

For the GS-US-216-101 trial, cobicistat precision and accuracy were evaluated using the low (15 ng/mL), medium (75 ng/mL), and high (750 ng/mL) QC samples. A dilution QC (5000 ng/mL) was also analyzed in six of the analytical runs. The cobicistat inter-run accuracy values were 6% for the low QCs, 1.5% for the medium QCs, -0.1% for the high QCs, and -2% for the dilution QCs. The cobicistat inter-run precision values were 5% for the low QCs, 4.7% for the medium QCs, 4.8% for the high QCs and 4.7% for the dilution QCs.

Of the 26 cobicistat samples selected for incurred sample reanalysis, 1 sample was not within 20% using the mean value of the repeat and original concentrations. However, it is not clear whether the total number of samples that were reanalyzed represents 5% to 10% of the total number of samples that were initially analyzed.

The submitted cobicistat long term sample stability data demonstrated that cobicistat was stable for 121 days at -10°C to -30°C and 365 days at -60°C to -80°C in K_2EDTA anticoagulated plasma. At the ⬛⬛⬛ (b) (4) bioanalytical

laboratory, subject samples from the GS-US-216-101 trial were stored at -60°C to -80°C but it is not clear whether samples were also stored at -80°C at the trial site or any intermediate storage facility, if applicable. Therefore, the long term stability of the cobicistat subject samples has not been established for the GS-US-216-101 trial.

Midazolam and 1-hydroxymidazolam plasma concentration were analyzed using a validated LC/MS/MS method with sodium heparin anticoagulated plasma by [(b) (4)] and cross validated to K_3EDTA anticoagulated plasma. Additionally, a cross validation using K_2EDTA anticoagulated was also conducted because subject samples from the GS-US-216-101 trial were collected in tubes containing K_2EDTA. Both the initial method validation and the bioanalysis of midazolam samples for the GS-US-216-101 were conducted at the [(b) (4)] laboratory. There were subsequent additional modifications made to the midazolam method by the [(b) (4)] site but there was no partial method validation data from the [(b) (4)] laboratory using the modified midazolam method that was submitted. The lower limit of quantification for midazolam and 1-hydroxymidazolam was 0.1 ng/mL and the upper limit of quantification was 100 ng/mL. There were no precision or accuracy issues identified for midazolam or 1-hydroxymidazolam based on the bioanalytical report. Out of the 35 analytical runs where midazolam samples were analyzed, 1 analytical run failed for the midazolam analysis and no runs failed for the 1-hydroxymidazolam analysis. The bioanalytical report does not discussion whether an investigation was conducted to determine the cause(s) of the analytical run failure for midazolam or whether the cause(s) of the run failure were identified and corrective actions taken.

For the GS-US-216-101 trial, midazolam and 1-hydroxymidazolam precision and accuracy were evaluated using the low (0.3 ng/mL), medium (15 ng/mL), and high (70 ng/mL) QC samples. There were no dilution QCs analyzed in the analytical runs. The midazolam inter-run accuracy values were -1.7% for the low QCs, 2% for the medium QCs, and -0.3% for the high QCs. The midazolam inter-run precision values were 6.2% for the low QCs, 6.5% for the medium QCs, and 4.3% for the high QCs. The corresponding 1-hydroxymidazolam inter-run accuracy values were -3.7% for the low QCs, 4% for the medium QCs, and 0.1% for the high QCs. The inter-run precision values for 1-hydroxymidazolam were 7.6% for the low QCs, 19.6% for the medium QCs (two runs each had one medium QC with a difference of more than 15% when compared to 15 ng/mL for accuracy), and 4.4% for the high QCs.

Of the 22 samples selected for incurred sample reanalysis for midazolam and 1-hydroxymidazolam, all samples for midazolam were within 20% and 1 sample was not within 20% for 1-hydroxymidazolam using the mean value of the repeat and original concentrations. However, it is not clear whether the total number of samples that were reanalyzed represents 5% to 10% of the total number of samples that were initially analyzed.

The submitted midazolam and 1-hydroxymidazolam long term sample stability data of 439 days at -60°C to -80°C (presumably in sodium heparin anticoagulated plasma) had multiple medium and high QCs that exceeded 15% and additional stability data is necessary to characterize midazolam and 1-hydroxymidazolam long term sample stability at -60°C to -80°C in K_2EDTA anticoagulated plasma. The bioanalytical report states that additional midazolam and 1-hydroxymidazolam long term sample stability for 622 days at -60°C to -80°C in K_3EDTA anticoagulated plasma was generated but this data was not submitted by the applicant. In the bioanalytical report, data was provided demonstrating long term midazolam and 1-hydroxymidazolam sample stability in K_2EDTA anticoagulated plasma for 51 days at -10°C to -30°C. At the (b) (4) bioanalytical laboratory, subject samples from the GS-US-216-101 trial were stored at -60°C to -80°C but it is not clear whether samples were also stored at -80°C at the trial site or any intermediate storage facility, if applicable. Therefore, the long term stability of the midazolam and 1-hydroxymidazolam subject samples has not been established for the GS-US-216-101 trial.

Ritonavir plasma concentrations were analyzed using a validated LC/MS/MS method with K_3EDTA anticoagulated plasma by (b) (4). Additionally, a cross validation using K_2EDTA anticoagulated plasma was also conducted because subject samples from the GS-US-216-101 trial were collected in tubes containing K_2EDTA. The lower limit of quantification for ritonavir was 10 ng/mL and the upper limit of quantification was 2000 ng/mL. There were no precision or accuracy issues identified for ritonavir based on the bioanalytical report. Out of the 7 analytical runs where ritonavir samples were analyzed, no runs failed for the ritonavir analysis.

For the GS-US-216-101 trial, ritonavir precision and accuracy were evaluated using the low (30 ng/mL), medium (700 ng/mL), and high (1400 ng/mL) QC samples. Dilution QCs (7000 ng/mL) were analyzed in one of the analytical runs. The ritonavir inter-run accuracy values were -1.3% for the low QCs, -1.4% for the medium QCs, 0.1% for the high QCs, and -0.1% for the dilution QCs. The midazolam inter-run precision values were 5.4% for the low QCs, 3.9% for the medium QCs, and 4.1% for the high QCs.

Of the 22 ritonavir samples selected for incurred sample reanalysis, all ritonavir samples were within 20% using the mean value of the repeat and original concentrations. However, it is not clear whether the total number of samples that were reanalyzed represents 5% to 10% of the total number of samples that were initially analyzed.

The bioanalytical report states that ritonavir long term sample stability for 840 days at -60°C to -80°C in K_3EDTA anticoagulated plasma was generated but this data was not submitted by the applicant. At the (b) (4) bioanalytical laboratory, subject samples from the GS-US-216-101 trial were stored at -60°C to -80°C but it is not clear whether samples were also stored at -80°C at the trial

site or any intermediate storage facility, if applicable. Therefore, the long term stability of the ritonavir subject samples has not been established for the GS-US-216-101 trial.

Pharmacokinetic Assessments

Noncompartmental analysis was performed to calculate cobicistat, midazolam and 1-hydroxymidazolam, and ritonavir plasma pharmacokinetic parameters, including C_{max} and $AUC_{(0-inf)}$ for single dosing, and C_T, C_{max}, and $AUC_{(0-T)}$ for multiple dosing.

Statistical Analysis

Descriptive statistics were calculated for cobicistat, midazolam and 1-hydroxymidazolam, and ritonavir plasma concentrations and pharmacokinetic parameters, including the number of subjects (n), mean, standard deviation, the coefficient of variation (CV%), median, and the minimum and maximum values. The geometric mean was also calculated for the individual pharmacokinetic parameters.

For cobicistat, dose proportionality was evaluated using a power model, using a mixed effects power model initially and if the mixed effects power model did not converge, a fixed effect power model was used. There were no specific criteria that were used for determining whether cobicistat pharmacokinetics was dose proportional.

ANOVA was used to compare cobicistat, midazolam, and ritonavir pharmacokinetic parameters including AUC and C_{max}. Nonparametric statistical methods (Kruskal-Wallis for all groups, Wilcoxon rank sum test for pairwise comparisons) were used to compare cobicistat, midazolam, and ritonavir clearance and half life.

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10. Results

10.1 Subject Demographics and Disposition

Table 3-GS-US-216-101 subject demographics

Characteristic	Treatment Group					Total (N = 60)
	GS-9350 50 mg (N = 12)	GS-9350 100 mg (N = 15)	GS-9350 200 mg (N = 15)	Ritonavir 100 mg (N = 9)	Placebo (N = 9)	
Sex (n, %)						
Male	4 (33.3%)	7 (46.7%)	7 (46.7%)	4 (44.4%)	7 (77.8%)	29 (48.3%)
Female	8 (66.7%)	8 (53.3%)	8 (53.3%)	5 (55.6%)	2 (22.2%)	31 (51.7%)
Age (Years)						
Mean (SD)	28 (9.9)	26 (7.8)	26 (6.7)	27 (8.0)	25 (6.0)	26 (7.6)
Min, Max	19, 44	18, 45	20, 41	19, 45	20, 39	18, 45
Ethnicity (n, %)						
White	7 (58.3%)	10 (66.7%)	9 (60.0%)	5 (55.6%)	7 (77.8%)	38 (63.3%)
Black	3 (25.0%)	3 (20.0%)	5 (33.3%)	4 (44.4%)	2 (22.2%)	17 (28.3%)
American Indian or Alaska Native	1 (8.3%)	1(6.7%)	0	0	0	2 (3.3%)
Native Hawaiian or Pacific Islander	1 (8.3%)	0	1 (6.7%)	0	0	2 (3.3%)
Asian	0	1 (6.7%)	0	0	0	1 (1.7%)
Screening Weight (kg)						
Mean (SD)	71.6 (9.3)	70.6 (11.7)	74.9 (11.0)	72.2 (12.1)	77.1 (17.7)	73.1 (12.0)
Min, Max	57.1, 89.0	54.0, 98.8	63.8, 102.2	54.7, 91.1	56.0, 104.0	54.0, 104.0
Screening BMI (kg/m²)[a]						
Mean (SD)	25.3 (2.54)	24.8 (3.21)	25.6 (3.43)	25.3 (2.96)	24.9 (3.93)	25.2 (3.13)
Min, Max	21.5, 29.3	20.3, 29.3	19.8, 29.9	21.4, 29.3	19.8, 30.0	19.8, 30.0
Screening Creatinine Clearance (mL/min)[b]						
Mean (SD)	122 (19.5)	123 (26.9)	134 (28.4)	125 (28.3)	138 (29.4)	128 (26.5)
Median	124	120	128	116	132	125
Min, Max	88, 146	81, 175	92, 186	96, 193	108, 196	81, 196

a   Body Mass Index (BMI) = (weight [kg]/height [cm]^2) × 10,000

b   Creatinine clearance estimated by Cockcroft-Gault equation:
    For male subjects: [(140 − Age) × body weight (kg)] / (72 × serum creatinine concentration)
    For female subjects: [(140 − Age) × body weight (kg) × 0.85] / (72 × serum creatinine concentration)

Figure 5-GS-US-216-101 subject disposition
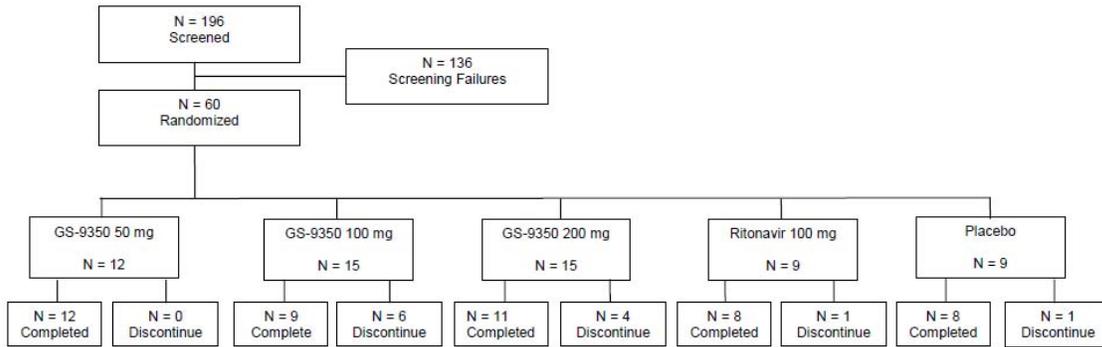
10.2 Concomitant Medications

The concomitant medications that subjects received during the trial included various oral contraceptive medications, acetaminophen and ibuprofen. None of the administered concomitant medications are anticipated to significantly impact the results of the trial.

10.3 Pharmacokinetic and Statistical Analysis

Subject 1009 that received 50 mg of cobicistat on Day 14 had a quantifiable predose midazolam concentration (0.104 ng/mL) that was ≤ 5% of the C_{max} (35.4 ng/mL). Therefore, no adjustments to the pharmacokinetic analysis for these two subjects were required. All other subjects did not have quantifiable predose cobicistat concentrations, ritonavir or midazolam on Day 0 or Day 1 or Day 14 for midazolam in either cohort 1 or cohort 2 (Part A) or Part B.

<u>Comparison of the pharmacokinetics for single doses of 50 mg and 100 mg of cobicistat and 200 mg of cobicistat (day 1)</u>

The mean plasma-concentration time profiles for single doses of 50 mg and 100 mg of cobicistat and 200 mg of cobicistat (day 1) are displayed in Figure 6. The pharmacokinetic parameters for single doses of 50 mg and 100 mg of cobicistat and 200 mg of cobicistat (day 1) are displayed in Table 4 and the statistical analyses comparing: a) a single 100 mg dose of cobicistat to a single 50 mg dose of cobicistat, b) 200 mg of cobicistat (day 1) to a single 50 mg dose of cobicistat, and c) 200 mg of cobicistat (day 1) to a single 100 mg dose of cobicistat, are displayed in Table 5.

Based on the comparison of the pharmacokinetic data, greater than dose proportional changes in cobicistat exposure were observed for both C_{max} and AUC (both overall and when comparing different doses). There was also a decrease in cobicistat clearance and an increase in the elimination half life as the dose was increased from 50 mg to 100 mg. Of the three treatment groups, the lowest clearance and the highest elimination half life observed occurred with 200 mg cobicistat (day 1). However, the differences in the total duration of sampling (24 hours for 200 mg cobicistat [day 1] dosing versus 48 hours for a single 50 mg and 100 mg doses of cobicistat) also contributes to the observed difference in AUC values and a direct comparison of the cobicistat elimination half life and clearance can not be made for single dosing versus Day 1 pharmacokinetic data.

Figure 6-Mean plasma-concentration time profiles for single doses of 50 mg and 100 mg of cobicistat and 200 mg of cobicistat (day 1)

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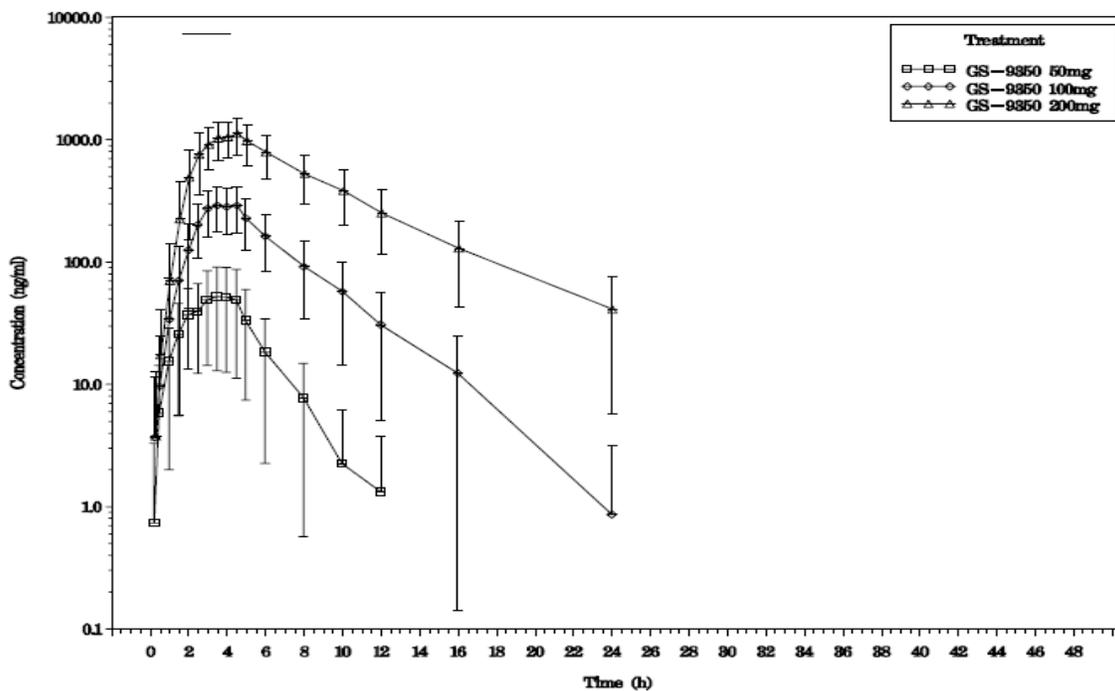
Table 4-Pharmacokinetic parameters for single doses of 50 mg and 100 mg of cobicistat and 200 mg of cobicistat (day 1)

GS-9350 Plasma PK Parameters	GS-9350 50 mg (N = 12)	GS-9350 100 mg (N = 15)	GS-9350 200 mg (N = 15)
C_{max} (ng/mL) Mean (%CV)	61.6 (57.2)	342.6 (34.6)	1200.1 (30.1)
AUC_{last} (ng·h/mL) Mean (%CV)	228.9 (71.8)	1610.8 (49.0)	8111.1 (40.0)
AUC_{inf} (ng·h/mL) Mean (%CV)	242.9 (69.5)	1650.7 (48.3)	8422.0 (41.4)
C_{last} (ng/mL) Mean (%CV)	6.8 (27.4)	10.7 (51.2)	45.7 (70.1)
T_{max} (h) Median (Q1, Q3)	3.26 (2.53, 4.25)	4.00 (3.50, 4.50)	4.00 (3.50, 4.50)
$T_{\frac{1}{2}}$ (h) Median (Q1, Q3)	1.41 (0.98, 1.78)	2.68 (2.31, 2.95)	4.17 (3.32, 5.07)
T_{last} (h) Median (Q1, Q3)	8.00 (4.50, 10.02)	16.00 (16.00, 16.00)	24.00 (24.00, 24.00)
CL/F (mL/h) Mean (%CV)	435292.1 (92.0)	77125.2 (58.9)	29097.6 (53.3)

Table 5-Statistical analyses for single doses of 50 mg and 100 mg of cobicistat and 200 mg of cobicistat (day 1)

Test/Reference Comparison of GS-9350 Plasma PK Parameters	Geometric Least-Squares Mean Ratio (%) (90% CI)	
Reference: GS-9350 50 mg (N = 12)	Test: GS-9350 100 mg (N = 15)	Test: GS-9350 200 mg (N = 15)
AUC_{last} (ng·h/mL)	909.39 (585.16, 1413.28)	4710.5 (3031.02, 7320.58)
AUC_{inf} (ng·h/mL)	849.22 (554.87, 1299.71)	4419.6 (2887.72, 6764.18)
C_{max} (ng/mL)	659.42 (474.45, 916.50)	2322.6 (1671.14, 3228.14)
CL/F (mL/h)	23.55 (15.39, 36.05)	9.05 (5.91, 13.85)
Reference: GS-9350 100 mg (N = 15)		
AUC_{last} (ng·h/mL)	—	517.98 (341.81, 784.95)
AUC_{inf} (ng·h/mL)	—	520.44 (348.42, 777.37)
C_{max} (ng/mL)	—	352.22 (258.24, 480.41)
CL/F (mL/h)	—	38.43 (25.73, 57.40)

Note: Ratios estimated as the GLSM (geometric least-squares mean) ratio of Test CL/F vs. Reference CL/F

Comparison of the multiple dosing pharmacokinetics for 50 mg, 100 mg, and 200 mg of cobicistat administered once daily

The mean multiple dosing plasma-concentration time profiles for 50 mg, 100 mg, and 200 mg of cobicistat administered once daily are displayed in Figure 7. The pharmacokinetic parameters for 50 mg, 100 mg, and 200 mg of cobicistat administered once daily on Day 14 are displayed in Table 6 and the statistical analyses comparing the statistical analyses comparing the following multiple dosing cobicistat comparisons: a) 100 mg to 50 mg cobicistat, b) 200 mg to 50 mg, and c) 200 mg to 100 mg, are displayed in Table 7.

Based on the comparison of the multiple dosing pharmacokinetic data, greater than dose proportional changes in cobicistat exposure were observed for both C_{max} and $AUC_{(0-\tau)}$ [both overall and when comparing different doses]. A reliable comparison of C_{24h} using the 50 mg once daily arm could not made due to the fact that the cobicistat concentration at 24 hours was below the limit of quantification in all but one subject. A greater than dose proportional increase in the C_{24h} was observed when comparing 200 mg to 100 mg. In addition, the cobicistat clearance with multiple dosing decreased as the dose was increased from 50 mg to 200 mg.

Figure 7-Mean multiple dosing (Day 14) plasma-concentration time profiles for 50 mg, 100 mg, and 200 mg of cobicistat administered once daily
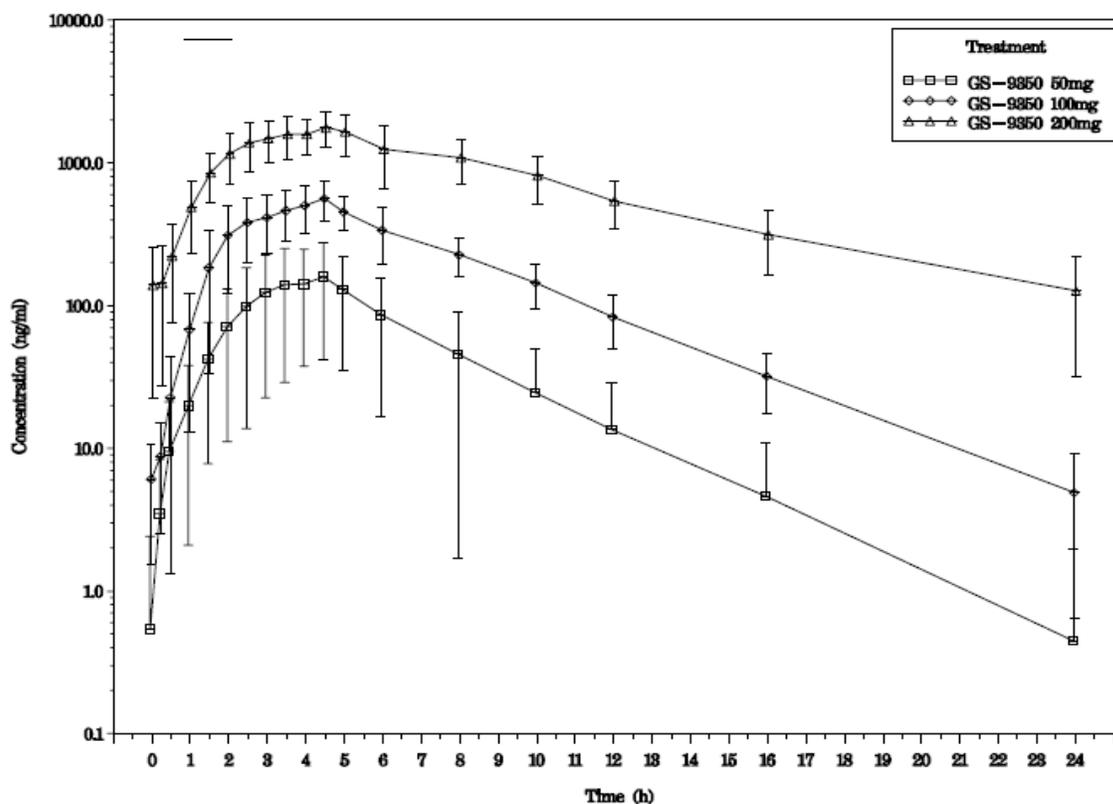
Table 6-Pharmacokinetic parameters for 50 mg, 100 mg of cobicistat and 200 mg of cobicistat once daily (day 14)

GS-9350 Plasma PK Parameters	GS-9350 50 mg (N = 12)	GS-9350 100 mg (N = 11)	GS-9350 200 mg (N = 12)
C_{max} (ng/mL) Mean (%CV)	170.0 (70.1)	563.3 (30.7)	1854.8 (28.0)
AUC_{tau} (ng·h/mL) Mean (%CV)	827.0 (81.6)	3435.8 (34.3)	16108.3 (34.3)
C_{tau} (ng/mL) Mean (%CV)	0.4 (346.4)	4.9 (87.0)	126.6 (74.9)
T_{max} (h) Median (Q1, Q3)	4.50 (3.50, 4.50)	4.50 (4.50, 4.53)	4.50 (4.50, 4.50)
$T_{1/2}$ (h) Median (Q1, Q3)	2.19 (1.34, 2.48)	3.12 (2.55, 3.36)	5.20 (4.12, 6.10)
T_{last} (h) Median (Q1, Q3)	12.00 (8.03, 16.00)	24.00 (16.00, 24.00)	24.00 (24.00, 24.00)
CL_{ss}/F (mL/h) Mean (%CV)	154288.3 (106.9)	33190.3 (43.6)	13952.5 (38.4)

Table 7-Statistical analyses for 50 mg, 100 mg of cobicistat and 200 mg of cobicistat once daily (day 14)

Test/Reference Comparison of GS-9350 Plasma PK Parameters	Geometric Least-Squares Mean Ratio (%) (90% CI)	
Reference: GS-9350 50 mg (N = 12)	Test: GS-9350 100 mg (N = 11)	Test: GS-9350 200 mg (N = 12)
AUC_{tau} (ng·h/mL)	597.17 (366.66, 972.60)	2811.5 (1744.85, 4530.23)
C_{max} (ng/mL)	436.84 (284.54, 670.65)	1455.9 (957.29, 2214.17)
C_{tau} (ng/mL)	138.83 (38.89, 495.60)[a,b]	1793.3 (519.53, 6190.36)[a]
CL_{ss}/F (mL/h)	33.49 (20.56, 54.55)	14.23 (8.83, 22.92)
Reference: GS-9350 100 mg (N = 11)		
AUC_{tau} (ng·h/mL)	—	470.81 (289.07, 766.80)
C_{max} (ng/mL)	—	333.28 (217.09, 511.66)
C_{tau} (ng/mL)	—	1291.7 (733.35, 2275.22)[b]
CL_{ss}/F (mL/h)	—	42.48 (26.08, 69.19)

Note: Ratios estimated as the GLSM (geometric least-squares mean) ratio of Test CL/F versus Reference CL/F

a   n = 1 for GS-9350 50 mg group

b   n = 7 for GS-9350 100 mg group

The applicant also provided information that was described as the cobicistat accumulation indices; however, the appropriate reference for the accumulation index would be the $AUC_{(0-T)}$ after Day 1, not $AUC_{(0-inf)}$. Nonetheless, in comparing single dosing to multiple dosing, the information in Table 8 does support the fact that cobicistat pharmacokinetics is nonlinear with respect to time.

Table 8-Comparison of cobicistat single and multiple dosing pharmacokinetic parameters

Test (AUC_{tau})	Reference (AUC_{inf})	Geometric Least-Squares Means Ratio (%) (90% CI)
GS-9350 50 mg Multiple Dose (N = 12)	GS-9350 50 mg Single dose (N = 12)	312.04 (236.93, 410.96)
GS-9350 100 mg Multiple Dose (N = 11)	GS-9350 100 mg Single dose (N = 15)	209.57 (174.47, 251.72)
GS-9350 200 mg Multiple Dose (N = 12)	GS-9350 200 mg Single dose (N = 15)	197.46 (175.18, 222.57)

Note: All available PK data were used for these determinations.

Comparison of the pharmacokinetics of midazolam when coadministered with ritonavir or cobicistat

Figure 8-Mean plasma-concentration time profiles for midazolam on day 0 and on day 14 when coadministered with either cobicistat or ritonavir
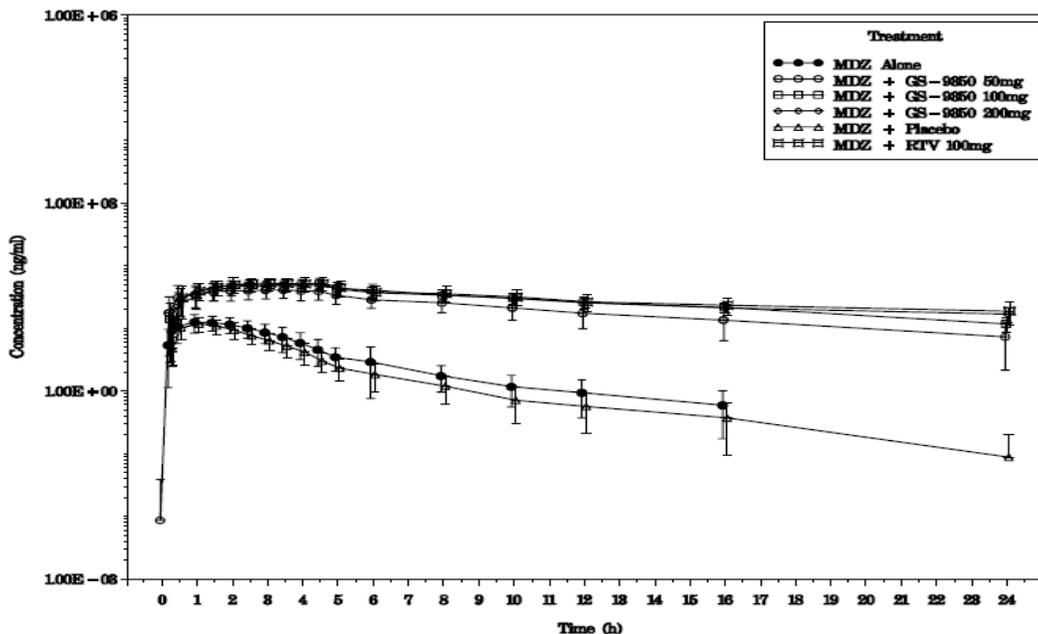
As shown in Table 10 below, the concentrations of midazolam were significantly increased when 50 mg of cobicistat once daily was coadministered with a single 5 mg dose of midazolam compared to midazolam administered by itself. When 100 mg and 200 mg of cobicistat once daily was coadministered with a single 5 mg dose of midazolam compared to midazolam administered by itself, a further increase in midazolam concentrations was observed. However, with midazolam and cobicistat coadministration, minimal differences were observed with the increase in midazolam $AUC_{(0-inf)}$ when the cobicistat dose was increased from 100 mg to 200 mg once daily compared to the increase in midazolam $AUC_{(0-inf)}$ when the cobicistat dose was increased from 50 mg to 100 mg once daily.

Concentrations of midazolam were also significantly increased when 100 mg of ritonavir once daily was coadministered with a single 5 mg dose of midazolam compared to midazolam administered by itself. With midazolam coadministration with either cobicistat or ritonavir, the increase in midazolam $AUC_{(0-last)}$ with 100 mg of ritonavir once daily was similar to the increase in midazolam $AUC_{(0-last)}$ with 200 mg of cobicistat once daily. The midazolam $AUC_{(0-inf)}$ was lower with 200 mg once daily of cobicistat coadministered with a single 5 mg dose of midazolam when compared to ritonavir 100 mg once daily coadministered with a single 5 mg dose of midazolam (see Table 11).

Table 9-Pharmacokinetic parameters for midazolam when coadministered with 50 mg, 100 mg of cobicistat and 200 mg of cobicistat once daily or ritonavir 100 mg once daily (day 14)

MDZ Plasma PK Parameters[a]	MDZ Alone (N = 60)	MDZ + GS-9350 50 mg (N = 12)	MDZ + GS-9350 100 mg (N = 11)	MDZ + GS-9350 200 mg (N = 12)	MDZ + RTV 100 mg (N = 9)
C_{max} (ng/mL) Mean (%CV)	14.5 (28.5)	45.3 (26.6)	55.0 (17.5)	56.0 (20.7)	58.5 (16.4)
AUC_{last} (ng•h/mL) Mean (%CV)	58.0 (29.2)	4•93.2 (34.1)	665.3 (17.8)	690.1 (16.2)	729.4 (22.9)
AUC_{inf} (ng•h/mL) Mean (%CV)	64.1 (33.3)	626.0 (49.9)	855.7 (18.2)	1219.6 (26.6)[a]	1526.3 (42.8)
C_{last} (ng/mL) Mean (%CV)	0.6 (69.0)	7.4 (70.6)	11.8 (26.9)	16.9 (25.4)	18.9 (40.2)
T_{max} (h) Median (Q1, Q3)	1.00 (1.00, 2.00)	1.75 (1.27, 3.75)	4.00 (2.50, 4.52)	2.25 (1.75, 4.25)	3.00 (2.00, 4.00)
$T_{½}$ (h) Median (Q1, Q3)	5.23 (3.85, 6.89)	8.89 (6.35, 10.89)	11.23 (8.24, 12.92)	19.35 (11.34, 28.22)[a]	25.79 (16.37, 35.10)
T_{last} (h) Median (Q1, Q3)	16.00 (16.00, 16.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
CL/F (mL/h) Mean (%CV)	87336.1 (35.4)	9660.6 (41.8)	6019.8 (17.9)	4351.4 (24.3)[a]	3935.5 (45.4)

a   n = 11

Table 10-Statistical analysis comparing midazolam pharmacokinetics when coadministered with either cobicistat or ritonavir compared to midazolam administered without concurrent use of either cobicistat or ritonavir and comparison of the three cobicistat once daily dosage regimens

Test	Geometric Least-Squares Means Ratio (%) (90% CI)			
	MDZ + GS-9350 50 mg (N = 12)	MDZ + GS-9350 100 mg (N = 11)	MDZ + GS-9350 200 mg (N = 12)	MDZ + Ritonavir 100 mg (N = 9)
Reference: MDZ Alone (N = 60)				
AUC_{last} (ng•h/mL)	802.32 (703.77, 914.68)	1175.8 (1025.77, 1347.67)	1218.6 (1068.91, 1389.26)	1269.7 (1050.91, 1534.06)
AUC_{inf} (ng•h/mL)	863.86 (755.92, 987.21)	1375.3 (1196.63, 1580.59)	1921.3 (1671.77, 2208.17)[a]	2247.8 (1851.20, 2729.50)
C_{max} (ng/mL)	308.26 (275.02, 345.52)	402.95 (357.77, 453.82)	386.80 (345.09, 433.56)	413.35 (344.12, 496.51)
CL/F (mL/h)	11.58 (10.13, 13.23)	7.27 (6.33, 8.36)	5.20 (4.53, 5.98)[a]	4.45 (3.66, 5.40)
Reference: MDZ + GS-9350 50 mg (N = 12)				
AUC_{last} (ng·h/mL)	—	139.95 (118.14, 165.78)	145.60 (123.37, 171.84)	—
AUC_{inf} (ng·h/mL)	—	148.95 (118.25, 187.63)	208.99 (165.91, 263.26)[a]	—
C_{max} (ng/mL)	—	123.26 (105.69, 143.75)	125.19 (107.71, 145.51)	—
CL/F (mL/h)	—	67.14 (53.30, 84.57)	47.85 (37.99, 60.27)[a]	—
Reference: MDZ + GS-9350 100 mg (N = 11)				
AUC_{last} (ng·h/mL)	—	—	104.04 (87.83, 123.25)	—
AUC_{inf} (ng·h/mL)	—	—	140.31 (110.84, 177.62)[a]	—
C_{max} (ng/mL)	—	—	101.57 (87.09, 118.45)	—
CL/F (mL/h)	—	—	71.27 (56.30, 90.22)[a]	—

a   n = 11 for MDZ + GS-9350 200 mg

Table 11-Statistical analysis comparing midazolam pharmacokinetics when coadministered with the three cobicistat once daily dosage regimens compared to coadministration with ritonavir 100 mg once daily

Test	Geometric Least-Squares Means Ratio (%) (90% CI)		
	MDZ + GS-9350 50 mg (N = 12)	MDZ + GS-9350 100 mg (N = 11)	MDZ + GS-9350 200 mg (N = 12)
Reference: MDZ + Ritonavir 100 mg (N = 9)			
AUC_{last} (ng•h/mL)	65.83 (55.09, 78.66)	92.12 (76.83, 110.46)	95.85 (80.21, 114.53)
AUC_{inf} (ng•h/mL)	40.56 (31.10, 52.91)	60.42 (46.08, 79.22)	84.78 (64.66, 111.16)[a]
C_{max} (ng/mL)	75.98 (65.05, 88.75)	93.65 (79.94, 109.72)	95.12 (81.43, 111.10)
CL/F (mL/h)	246.51 (188.98, 321.56)	165.50 (126.23, 217.00)	117.96 (89.96, 154.66)[a]

a   n = 11 for MDZ + GS-9350 200 mg

The data in Figure 9 displays the differences in midazolam clearance for ritonavir 100 mg once daily and cobicistat 50 mg, 100 mg, and 200 mg once daily. The following conclusion can be made based on the percentage change in midazolam clearance compared to midazolam administered by itself: a) minimal differences were observed in the inhibitory effects of cobicistat 50 mg, 100 mg and 200 mg on midazolam clearance, and b) the inhibitory effects on midazolam clearance with 100 mg of ritonavir once daily and 200 mg once daily of cobicistat were similar.

Figure 9- Comparison of the changes in midazolam clearance when coadministered with the three cobicistat once daily dosage regimens and ritonavir 100 mg once daily
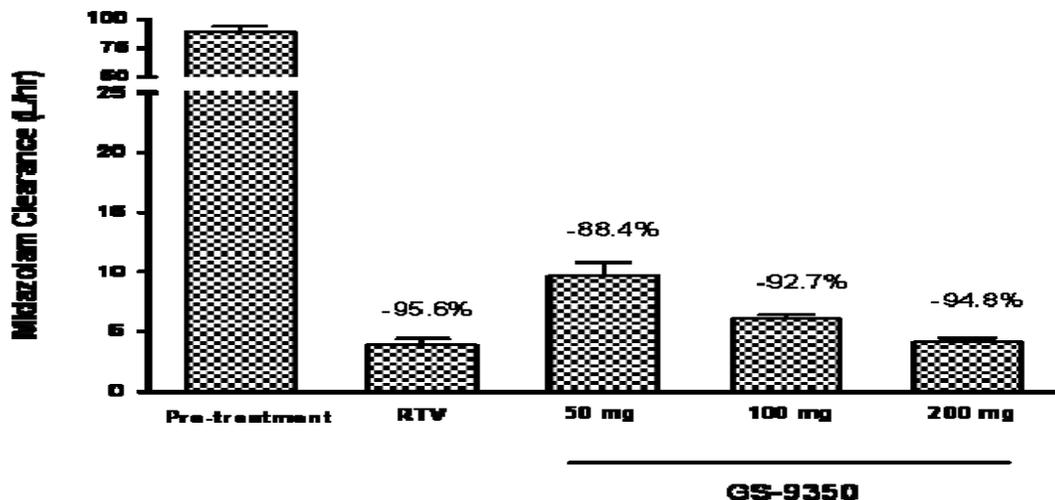
The observed trend in the mean 1-hydroxymidazolam plasma concentration time profiles are consistent with the reported midazolam pharmacokinetic results: with increasing cobicistat doses and the subsequent increased midazolam concentrations, a corresponding decrease in the concentrations of 1-hydroxymidazolam is observed (see Figure 10).

Figure 10-Mean plasma-concentration time profiles for 1-hydroxymidazolam on day 0 and on day 14 when coadministered with either cobicistat or ritonavir

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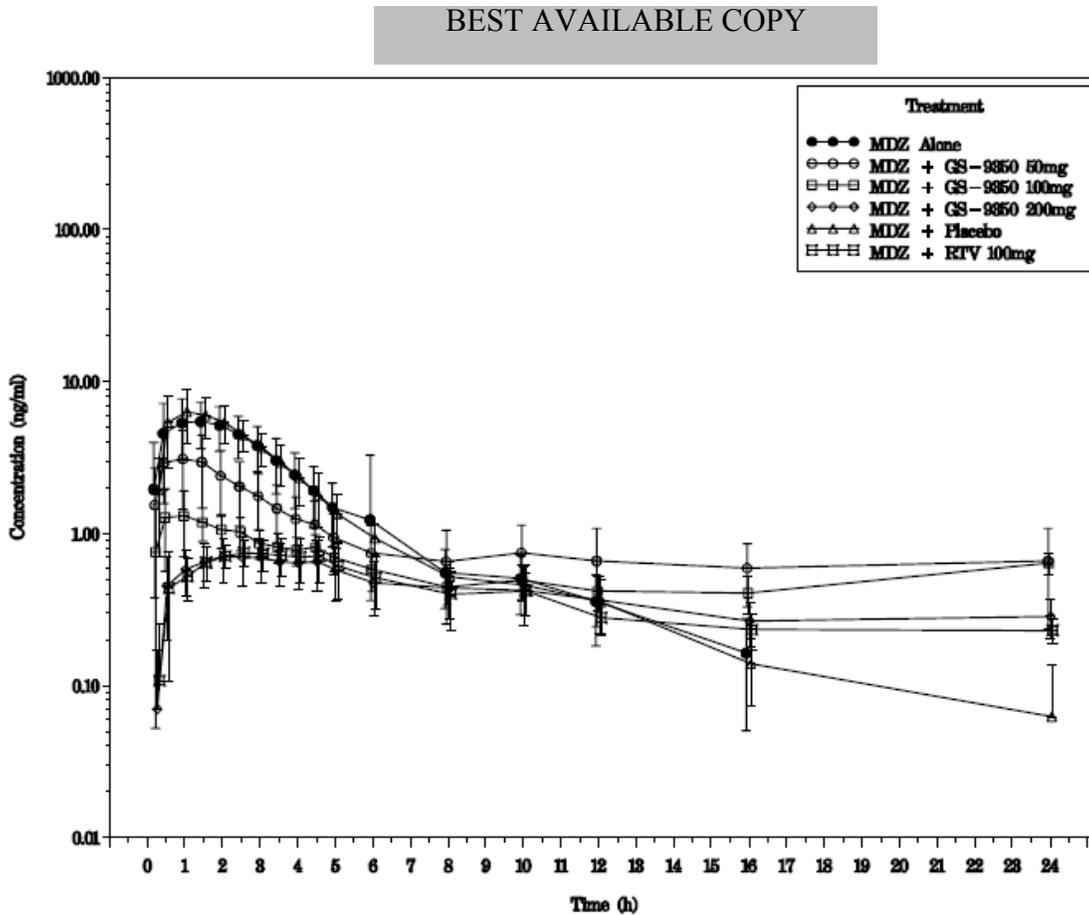
The duration of cobicistat's inhibitory effects was also evaluated based on comparing the changes in the midazolam metabolite: parent ratio at 12, 16, and 24 hours with the three cobicistat once daily dosage regimens when coadministered with midazolam. Based on the data in Table 12 below, an increase in the midazolam metabolite: parent ratio was observed after 16 hours for cobicistat with 50 mg once daily or 100 mg once daily dosing. The increase in the midazolam metabolite: parent ratio after 16 hours was less pronounced with 200 mg once daily dosing, indicating that cobicistat's inhibitory effects were maintained.

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Table 12-Analysis of midazolam metabolite: parent ratios with the three cobicistat once daily dosage regimens and ritonavir 100 mg once daily

	Plasma Concentrations (ng/mL) Mean (% CV)		
	Time Point After Dosing (h)		
	12 hours	**16 hours**	**24 hours**
MDZ Alone			
Metabolite	0.4 (49.3)	0.2 (69.3)	NA
Parent MDZ	0.9 (60.1)	0.6 (71.0)	NA
Metabolite:Parent Ratio	0.444	0.333	NA
MDZ + GS-9350 50 mg			
Metabolite	0.7 (63.1)	0.6 (44.2)	0.7 (64.2)
Parent MDZ	17.3 (43.3)	13.7 (53.4)	7.4 (70.6)
Metabolite:Parent Ratio	0.040	0.043	0.095
MDZ + GS-9350 100 mg			
Metabolite	0.4 (26.8)	0.4 (27.0)	0.6 (16.2)
Parent MDZ	26.1 (21.2)	21.5 (17.0)	11.8 (26.9)
Metabolite:Parent Ratio	0.015	0.019	0.051
MDZ + GS-9350 200 mg			
Metabolite	0.4 (40.8)	0.3 (32.7)	0.3 (28.6)
Parent MDZ	25.8 (18.6)	21.2 (14.6)	16.9 (25.4)
Metabolite:Parent Ratio	0.016	0.014	0.018
MDZ + RTV 100 mg			
Metabolite	0.3 (22.2)	0.2 (26.9)	0.2 (18.3)
Parent MDZ	26.3 (29.7)	23.4 (30.1)	18.9 (40.2)
Metabolite:Parent Ratio	0.011	0.009	0.011

Ritonavir single and multiple dosing pharmacokinetic data

The ritonavir single and multiple dosing pharmacokinetic data pharmacokinetic data are displayed in Table 13 below. There was no specific statistical analysis that was conducted for ritonavir in the current trial.

Table 13-Ritonavir single and multiple dosing pharmacokinetic data

Ritonavir Plasma PK Parameters	Single-Dose Ritonavir (N = 9)	Multiple-Dose Ritonavir (14 days of dosing) (N = 9)
C_{max} (ng/mL) Mean (%CV)	742.0 (42.8)	1386.1 (50.7)
AUC_{last} (ng•h/mL) Mean (%CV)	5379.2 (48.7)	—
AUC_{inf} (ng•h/mL) Mean (%CV)	5699.1 (47.8)	—
AUC_{tau} (ng•h/mL) Mean (%CV)	—	8743.3 (38.0)
C_{last} (ng/mL) Mean (%CV)	31.9 (70.6)	—
C_{tau} (ng/mL) Mean (%CV)	—	86.9 (55.7)
T_{max} (h) Median (Q1, Q3)	4.50 (4.50, 5.00)	4.50 (4.50, 5.00)
$T_{½}$ (h) Median (Q1, Q3)	6.15 (5.73, 6.65)	6.15 (5.83, 6.53)
T_{last} (h) Median (Q1, Q3)	24.00 (24.00, 36.00)	24.00 (24.00, 24.00)
CL/F (mL/h) (Single-Dose) CL_{ss}/F (mL/h) (Multiple-Dose) Mean (%CV)	21954.8 (52.2)	13018.7 (39.1)

11. Discussion and Conclusions

Based on the results from the GS-US-216-101 trial, the following conclusions can be made:

- At doses of 50 mg, 100 mg, and 200 mg, greater than dose proportional increases in cobicistat exposure were observed with single and multiple dosing, supporting the conclusion that the pharmacokinetics of cobicistat are nonlinear. Based on a comparison of the single and multiple dosing data, the cobicistat pharmacokinetics of cobicistat are also nonlinear with respect to time.

- The concentrations of midazolam were significantly increased when 50 mg of cobicistat once daily was coadministered with a single 5 mg dose of midazolam compared to midazolam administered by itself, with the midazolam $AUC_{(0-inf)}$ increasing by 764%. However, there was minimal additional increase in midazolam exposure when comparing the change in midazolam exposure with an increase from cobicistat 50 mg once daily to

100 mg once daily (49 % increase) to the change in midazolam exposure with an increase from cobicistat 100 mg once daily to 200 mg once daily once daily (40% increase).

- The cobicistat clearance with multiple dosing decreased as the dose was increased from 50 mg to 200 mg.

- With a single 5 mg dose of midazolam, the increase in midazolam $AUC_{(0-last)}$ with 100 mg of ritonavir once daily was similar to the increase in midazolam $AUC_{(0-inf)}$ with 200 mg of cobicistat once daily. The midazolam $AUC_{(0-inf)}$ was lower by 15% for 200 mg of cobicistat once daily coadministered with a single 5 mg dose of midazolam when compared to ritonavir 100 mg once daily coadministered with a single 5 mg dose of midazolam.

- Minimal differences were observed in the inhibitory effects of cobicistat 50 mg, 100 mg and 200 mg on midazolam clearance, and the inhibitory effects on midazolam clearance with 100 mg of ritonavir once daily and 200 mg once daily of cobicistat were similar.

- In evaluating the duration of cobicistat's inhibitory effects based on comparing the changes in the midazolam metabolite: parent ratio at 12, 16, and 24 hours with the three cobicistat once daily dosage regimens when coadministered with midazolam, an increase in the midazolam metabolite: parent ratio was observed for cobicistat with 50 mg once daily or 100 mg once daily dosing after 16 hours. The increase in the midazolam metabolite: parent ratio was less pronounced with 200 mg once daily dosing, indicating that cobicistat's inhibitory effects were maintained.

GS-US-216-111

1. Title

A Phase 1 Study to Evaluate the Pharmacokinetics, Metabolism, and Excretion of GS-9350

2. Objectives

The objectives of the trial were to obtain information on the excretion and metabolic profile subsequent to administering a single dose of ^{14}C-labeled GS-9350 (cobicistat) in humans.

3. Trial Design

GS-US-216-111 was a Phase I, open label trial that enrolled eight male subjects between 18 and 45 years old. The trial design is displayed in Figure 1.

Figure 1-GS-US-216-111 trial design
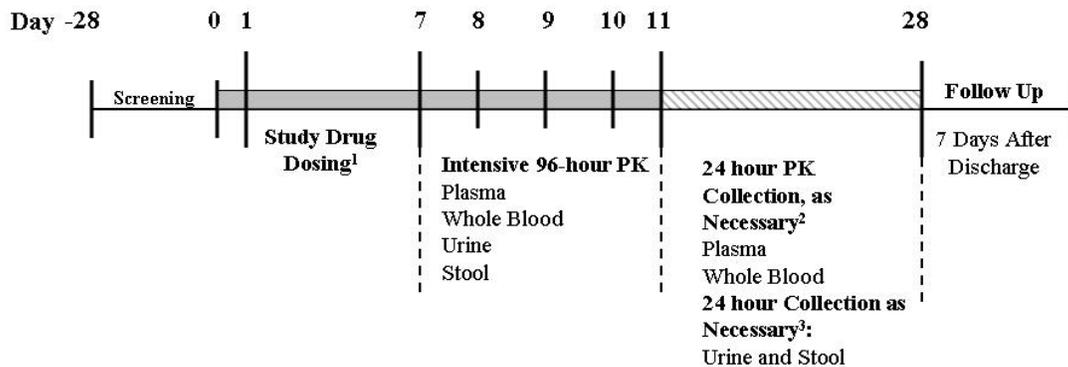
[1] Subjects will dose with GS-9350 on Days 1-6 and [^{14}C] GS-9350 on Day 7.
[2] Additional samples to be collected until assays indicate that the radioactivity levels in two consecutive samples have decreased to less than or equal to twice the level of background radioactivity, or both the urine and stool collections are discontinued, whichever comes first.
[3] Additional samples to be collected until assays indicate that the radioactivity levels in samples from two consecutive collection intervals are equal/less than 1% of the administered dose and the cumulative ^{14}C radioactivity recovered in urine and stools over the sampling period is greater than 90% of the administered dose.

Clinic confinement
Clinic confinement as needed for radiosafety monitoring

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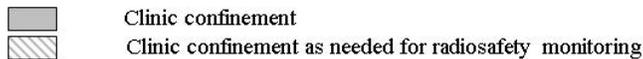
4. Rationale for Doses Used in the Trial

Multiple doses of nonradiolabeled cobicistat 150 mg once daily were administered on Day 1 through 6. On Day 7, a single 150 mg dose of cobicistat containing 148.5 mg of nonradiolabeled cobicistat and 100 µCi (1.5 mg) of ^{14}C-labeled cobicistat was administered.

The cobicistat doses administered in the trial reflect the proposed dosage regimen for cobicistat. Cobicistat 150 mg once daily is the proposed dosage regimen for cobicistat when combined with elvitegravir (cobicistat is utilized to achieve therapeutic concentrations of elvitegravir).

5. Drugs Used in the Trial

Two cobcicistat formulations were administered in the trial. The first formulation was a nonradiolabeled 150 mg cobicistat oral tablet. The second formulation contained 100 µCi of ^{14}C-labeled cobicistat (equal to 1.5 mg) and 148.5 mg of nonradiolabeled cobicistat in ethanol administered as capsules.

6. Sample Collection, Bioanalysis, Pharmacokinetic Assessments, and Statistical Analysis

Sample Collection

Blood samples were collected starting on Day 7 at predose and up to 96 hours postdose.

Urine samples were collected starting on Day 7 at predose (single void only) and during the following postdose collection intervals: 0-4, 4-8, 8-12, 12-24, 24-48, 48-72, and 72-96 hours.

Feces were collected at predose (within 24 hours prior to Day 7, if available) and starting on Day 7, at 0-24, 24-48, 48-72, and 72-96 hours postdose.

After collection of samples for the 96 hour timepoint was completed, additional blood samples were collected at 24 hour intervals until one the following timepoints: a) Day 28 (504 hours postdose), b) the radioactivity levels in two consecutive blood samples decreased to less than or equal to two times background radioactivity, or c) urine and feces collection were discontinued. The decision to discontinue blood sampling was made on an individual basis. After the 96 hour timepoint, urine and feces were collected at 24 hour intervals until one the following timepoints: a) Day 28 (504 hours postdose), or b) the radioactivity levels in two consecutive samples were less than or equal to 1% of the administered dose and the cumulative ^{14}C radioactivity was greater than or equal to 90% of the administered dose.

Bioanalysis

Liquid scintillation counting was utilized to determine the radioactivity (^{14}C) in whole blood, plasma, urine and fecal samples. The radioactivity in each sample was reported as the actual amount, the concentration, and the percentage of the administered ^{14}C cobicistat dose that was administered. The cumulative ^{14}C dose that was recovered in the urine and feces was also reported. Identification of ^{14}C cobicistat metabolites was conducted using high performance liquid chromatography with a radiochemical detector.

Pharmacokinetic Assessments

Noncompartmental analysis was performed using WinNonlin 5.0.1 to calculate pharmacokinetic parameters for the ^{14}C cobicistat dose, including C_{max} and $AUC_{(0-T)}$ for the plasma data, the percentage of the dose excreted for the urine data, and the percentage of the dose excreted for the fecal data.

Statistical Analysis

The descriptive statistics that were calculated for cobicistat plasma concentrations and pharmacokinetic parameters included the number of subjects (n), mean, standard deviation, the coefficient of variation (CV%), median, and the minimum and maximum values. For pharmacokinetic parameters additional descriptive statistics were derived that included the geometric mean and the 95% confidence intervals.

7. Results

7.1 Subject Disposition and Demographics

Table 1-GS-US-216-111 subject disposition
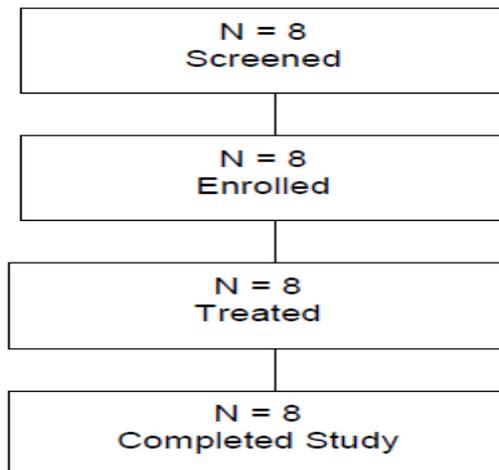
Table 2-GS-US-216-111 subject demographics

Characteristic	GS-9350 (N = 8)
Sex (n, %)	
Male	8 (100%)
Female	0
Age at baseline (years)	
Mean (SD)	32 (6.6)
Median (Q1, Q3)	32 (26, 38)
Min, Max	23, 40
Ethnicity (n, %)	
Hispanic/Latino	1 (12.5%)
Non-Hispanic/Latino	7 (87.5%)
Race (n, %)	
White	8 (100%)
Other	0
Weight (kg)	
Mean (SD)	82.6 (5.99)
Median (Q1, Q3)	82.0 (79.0, 86.1)
Min, Max	73.9, 92.9
Height (cm)	
Mean (SD)	181.8 (4.34)
Median (Q1, Q3)	181.5 (180.7, 184.9)
Min, Max	173.1, 187.1
BMI (kg/m^2)[a]	
Mean (SD)	25.0 (1.85)
Median (Q1, Q3)	25.1 (23.9, 26.0)
Min, Max	22.0, 28.2
Serum creatinine clearance (mL/min)[b]	
Mean (SD)	137.79 (23.2)
Median (Q1, Q3)	136.58 (122.49, 151.30)
Min, Max	103.67, 177.89

a   BMI = (weight [kg]/height [cm]^2) × 10,000

b   Estimated creatinine clearance = (140 − age [y]) × lean body weight (kg)/(72 × serum creatinine [mg/dL]) for male subjects

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7.2 Pharmacokinetic and Statistical Analysis

For all eight subjects, using liquid scintillation counting, the total ^{14}C radioactivity was below the lower limit of quantification (BLQ) at 32 hours postdose in whole blood, and 96 hours postdose in plasma. The cumulative combined radioactivity in urine and feces was greater than 90% of the administered dose with the exception of one subject (1008).

Whole blood and plasma analysis

Figure 1 below displays the whole blood mean profile of total ^{14}C radioactivity after a 150 mg ^{14}C dose of cobicistat containing ^{14}C-labeled and nonradiolabeled cobicistat using liquid scintillation counting. Based on the mean whole blood total ^{14}C radioactivity profile, radioactivity was detected until 28 hours postdose.

Figure 1-Mean whole blood total ^{14}C radioactivity profile of a 150 mg ^{14}C dose of cobicistat containing ^{14}C-labeled and nonradiolabeled cobicistat using liquid scintillation counting
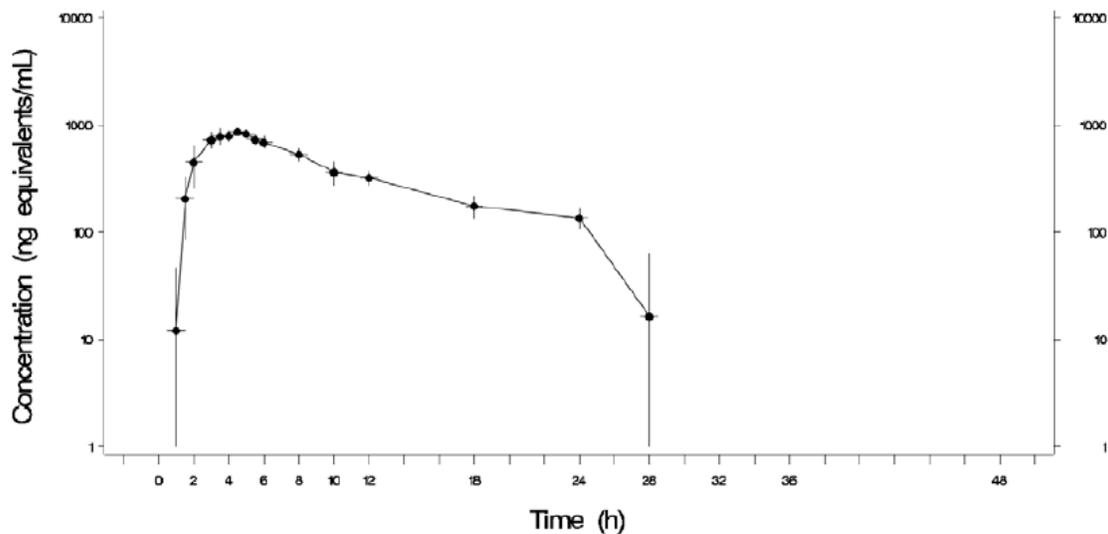
a   Values below the lower limit of quantification (BLQ) were treated as 0 for summary statistics and as missing for log-normalized data.

b   Values where no sample (NS) was available were treated as missing for summary statistics.

The total ^{14}C mean whole blood to plasma concentration ratios over 24 hours ranged from 0.49 to 0.75, indicating that cobicistat preferentially distributes to plasma. Based on this observation, the subsequent pharmacokinetic data was analyzed for plasma concentrations only.

Figure 2 below displays the mean plasma profile of total ^{14}C radioactivity after a 150 mg ^{14}C dose of cobicistat containing ^{14}C-labeled and nonradiolabeled

cobicistat using liquid scintillation counting. Based on the mean plasma total ^{14}C cobicistat radioactivity profile, radioactivity was detected until 84 hours postdose.

Figure 2-Mean plasma total ^{14}C radioactivity profile of a 150 mg ^{14}C dose of cobicistat containing ^{14}C-labeled and nonradiolabeled cobicistat using liquid scintillation counting
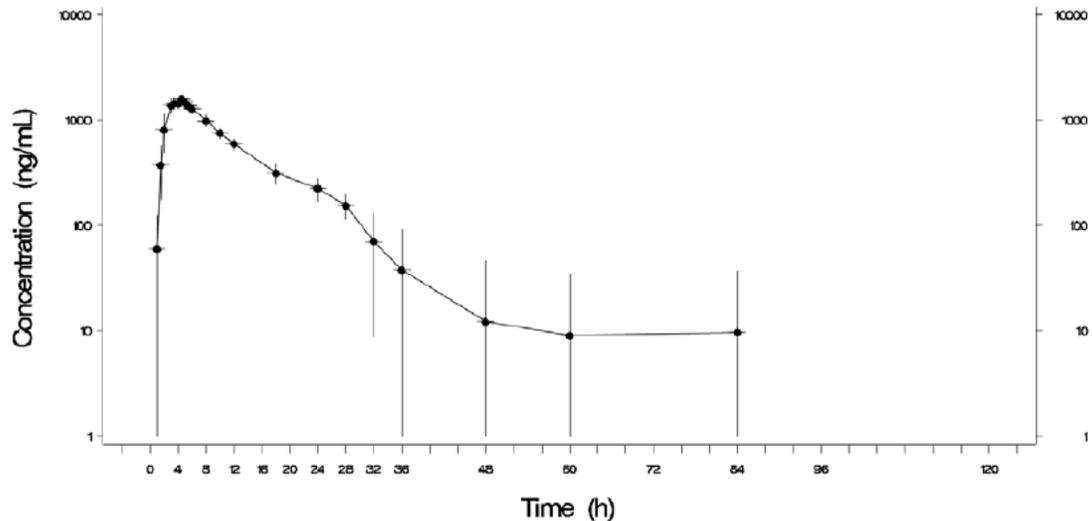
a   Values below the lower limit of quantification (BLQ) were treated as 0 for summary statistics and as missing for log-normalized data.

b   Values where no sample (NS) was available were treated as missing for summary statistics.

Using high performance liquid chromatography with a radiochemical detector, plasma was pooled for timepoints up to 24 hours to identify cobicistat metabolites. As a comparison of the results using a different assay, the pooled plasma profile of total ^{14}C radioactivity up to 24 hours using high performance liquid chromatography is displayed in Figure 3.

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Figure 3-Pooled plasma total ^{14}C radioactivity profile of a 150 mg ^{14}C dose of cobicistat containing ^{14}C-labeled and nonradiolabeled cobicistat using high performance liquid chromatography
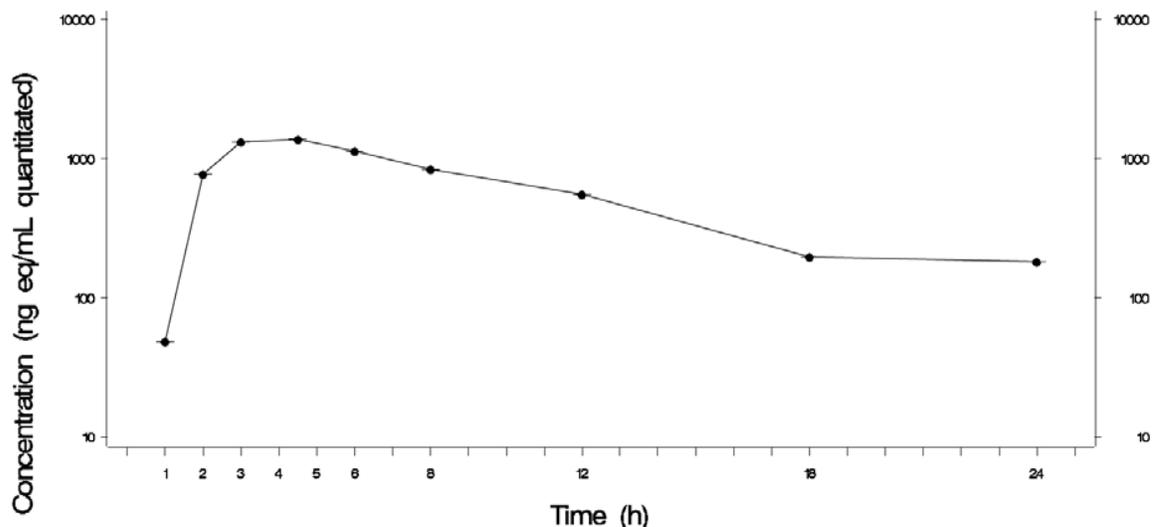

a   Values below the lower limit of quantification (BLQ) were treated as 0 for summary statistics and as missing for log-normalized data.

b   Values where no sample (NS) was available were treated as missing for summary statistics.

A comparison of the pharmacokinetic parameters after a 150 mg ^{14}C dose of cobicistat that were derived for total ^{14}C radioactivity using liquid scintillation counting and high performance liquid chromatography is displayed in Table 3. In general, major differences in the derived pharmacokinetic parameters were not observed for the two analytical methods.

Table 3-Pharmacokinetic parameters for total ^{14}C radioactivity using liquid scintillation counting compared to high performance liquid chromatography

PK Parameter	Total ^{14}C Radioactivity in Plasma	
	LSC[a]	HPLC[a]
C_{max} (ng eq/mL)	1,628.8	1,380.0
C_{tau} (ng eq/mL)	224.5	181.0
AUC_{tau} (h•[ng eq /mL])	15,390.0	13,283.3
T_{max} (h)	4.19	4.50
T_{last} (h)	40.50	24.00
$T_{½}$ (h)	11.84	7.42

a   HPLC parameter estimates are based on pooled data; to allow for direct comparison across radiochemical detection methods, mean values are presented for all parameters.

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The pooled ^{14}C cobicistat profile and derived pharmacokinetic parameters using high performance liquid chromatography are displayed in Figure 4 and Table 4, respectively

Figure 4-Pooled plasma ^{14}C cobicistat profile of a 150 mg ^{14}C dose of cobicistat containing ^{14}C-labeled and nonradiolabeled cobicistat using high performance liquid chromatography
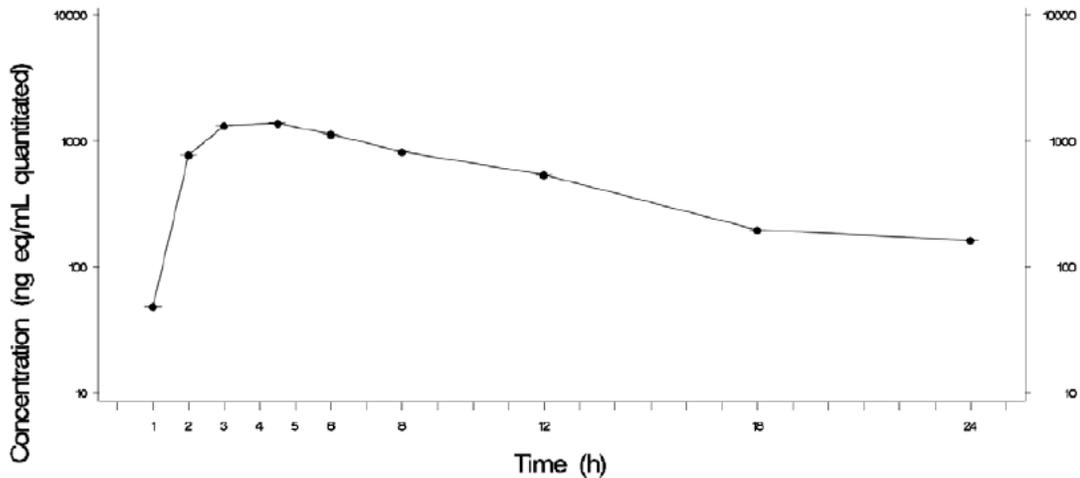
a   Values below the lower limit of quantification (BLQ) were treated as 0 for summary statistics and as missing for log-normalized data.

b   Values where no sample (NS) was available were treated as missing for summary statistics.

Table 4-Pharmacokinetic parameters for ^{14}C cobicistat using high performance liquid chromatography

PK Parameter	Pooled [^{14}C]GS-9350 Plasma Concentrations [a]
C_{max} (ng eq/mL)	1,380.0
C_{tau} (ng eq/mL)	163.0
AUC_{tau} (h•[ng eq /mL])	13,091.5
T_{max} (h)	4.50
T_{last} (h)	24.00
$T_{½}$ (h)	6.98

a   Parameter estimates are based on pooled data; mean values are presented.

Table 5 below compares the pharmacokinetic parameters for total ^{14}C radioactivity to ^{14}C cobicistat using high performance liquid chromatography. Of the total ^{14}C radioactivity, ^{14}C cobicistat accounted for 98.6% of the $AUC_{(0-T)}$ and 100% of the C_{max}.

Table 5-Pharmacokinetic parameters for total ^{14}C radioactivity compared to ^{14}C cobicistat using high performance liquid chromatography

PK Parameter	Total ^{14}C Radioactivity[a]	$[^{14}C]$GS-9350[a]
C_{max} (ng eq/mL)	1380.0	1380.0
C_{tau} (ng eq/mL)	181.0	163.0
AUC_{tau} (h•[ng eq /mL])	13283.3	13091.5
T_{max} (h)	4.50	4.50
T_{last} (h)	24.00	24.00
$T_{½}$ (h)	7.42	6.98

a   Parameter estimates are based on pooled data; mean values are presented.

Urine analysis

Figure 5 displays the mean cumulative urinary excretion (expressed at the percentage of the radioactive dose excreted) profile of total ^{14}C radioactivity after a 150 mg ^{14}C dose of cobicistat containing ^{14}C-labeled and nonradiolabeled cobicistat using liquid scintillation counting. The mean cumulative percentage of the radioactive dose that was excreted in the urine was 8.23% with urine collection up to 504 hours.

Figure 5- Mean cumulative urinary excretion (expressed at the percentage of the radioactive dose excreted) profile of total ^{14}C radioactivity using a 150 mg ^{14}C dose of cobicistat containing ^{14}C-labeled and nonradiolabeled cobicistat using liquid scintillation counting
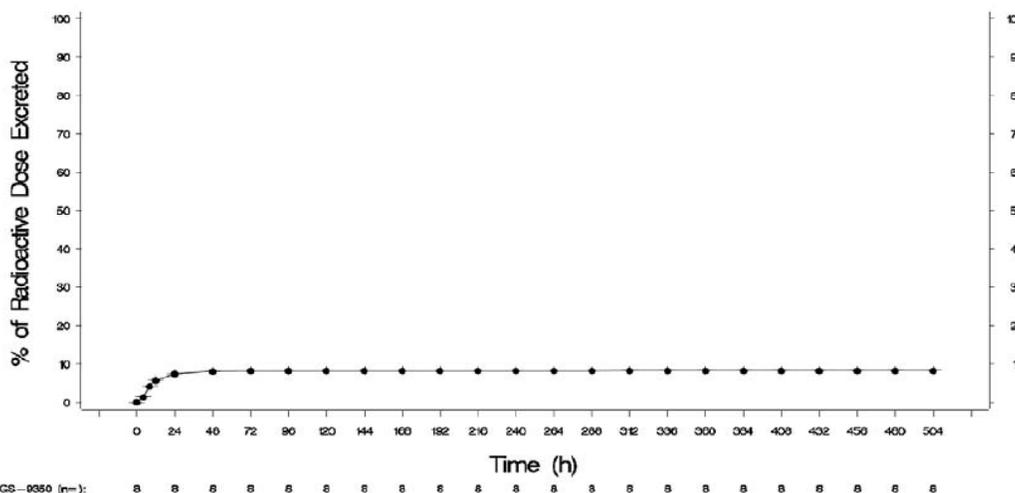
a   Values below the lower limit of quantification (BLQ) were treated as 0 for summary statistics and as missing for log-normalized data.
b   Values where no sample (NS) was available were treated as missing for summary statistics.

Fecal analysis

Figure 6 displays the mean cumulative fecal excretion (expressed at the percentage of the radioactive dose excreted) profile of total ^{14}C radioactivity after a 150 mg ^{14}C dose of cobicistat containing ^{14}C-labeled and nonradiolabeled cobicistat using liquid scintillation counting. The mean cumulative percentage of the radioactive dose that was excreted in the feces was 86.19% with fecal collection up to 504 hours.

Figure 6-Mean cumulative fecal excretion (expressed at the percentage of the radioactive dose excreted) profile of total ^{14}C radioactivity using a 150 mg ^{14}C dose of cobicistat containing ^{14}C-labeled and nonradiolabeled cobicistat using liquid scintillation counting
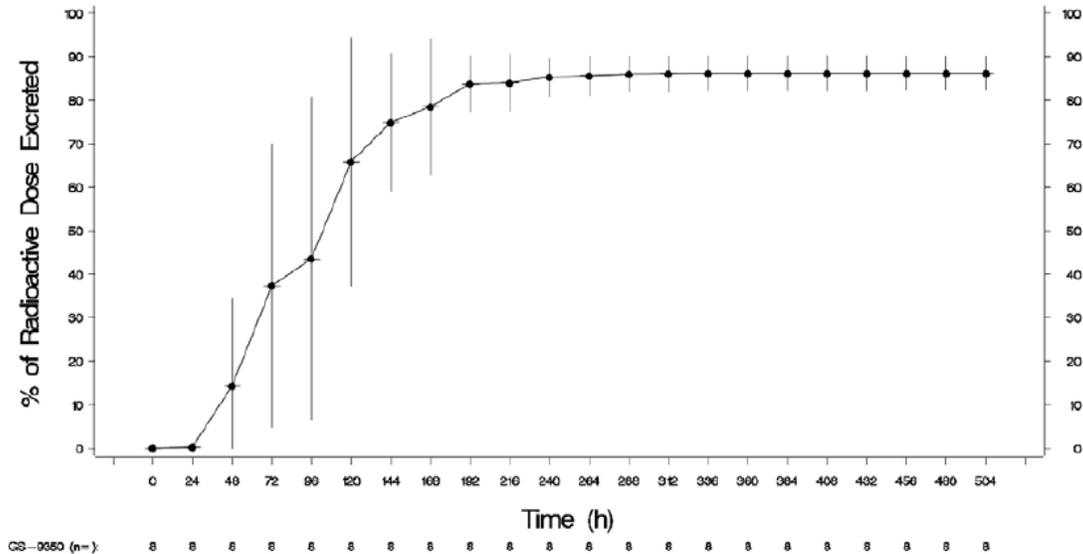
a   Values below the lower limit of quantification (BLQ) were treated as 0 for summary statistics and as missing for log-normalized data.
b   Values where no sample (NS) was available were treated as missing for summary statistics.

Combined urine and fecal analysis

Figure 7 displays the mean cumulative combined urine and fecal excretion (expressed at the percentage of the radioactive dose excreted) profile of total ^{14}C radioactivity after a 150 mg ^{14}C dose of cobicistat containing ^{14}C-labeled and nonradiolabeled cobicistat using liquid scintillation counting. The mean cumulative percentage of the radioactive dose that was excreted in the urine and feces was 94.42% with urine and fecal collection up to 504 hours.

Figure 7-Mean cumulative combined urine and fecal excretion (expressed at the percentage of the radioactive dose excreted) profile of total ^{14}C radioactivity using a 150 mg ^{14}C dose of cobicistat containing ^{14}C-labeled and nonradiolabeled cobicistat using liquid scintillation counting
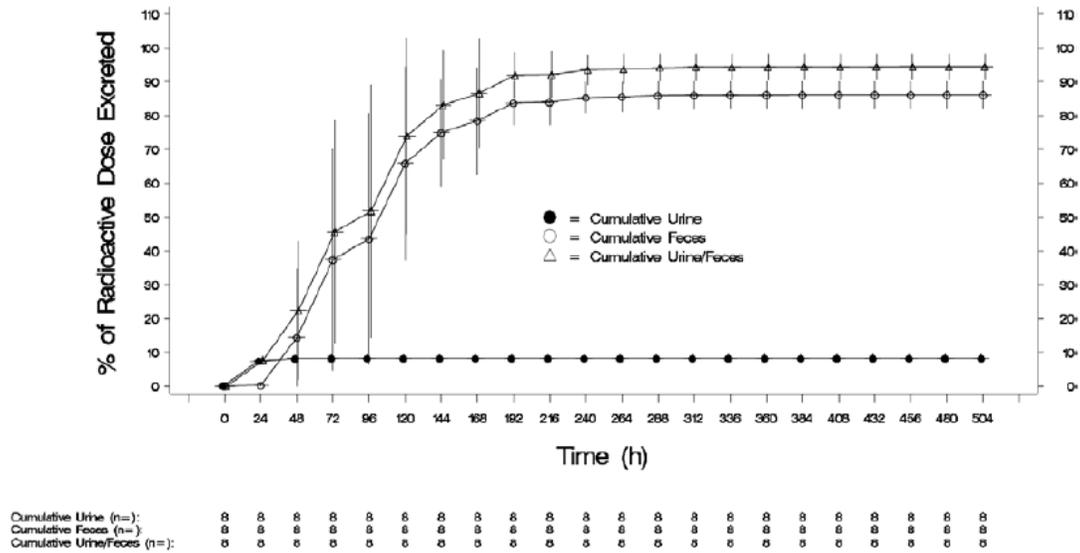
a   Values below the lower limit of quantification (BLQ) were treated as 0 for summary statistics and as missing for log-normalized data.

b   Values where no sample (NS) was available were treated as missing for summary statistics.

7.3 Metabolite Identification

As previously stated, using high performance liquid chromatography, the total ^{14}C radioactivity, ^{14}C cobicistat accounted for 98.6% of the $AUC_{(0-T)}$ and 100% of the C_{max} in plasma. The three metabolites quantified in plasma (M56, M31, and M77) did not have peaks detected in the majority of the time points ranging from 1 to 24 hours postdose.

Using high performance liquid chromatography, in pooled urine, the total percentage of the dose that was measured was 6.31%. The majority of the total radioactivity was attributed to cobicistat (5.45%) with the each of the identified cobicistat metabolites accounting for less than 1% of the total radioactivity in pooled urine (see Table 6 below).

Table 6-Perentage of total ^{14}C radioactivity attributed to cobicistat and cobicistat metabolites in pooled urine using high performance liquid chromatography

[^{14}C]GS-9350 Metabolite [a]	Mean (SD) Percent of Total ^{14}C Radioactivity
M55	0.03 (0.05)
M56	0.01 (0.03)
M10	0.01 (0.02)
M14	0.01 (0.02)
M21	0.09 (0.04)
M31	0.70 (0.20)
GS-9350	5.45 (0.99)
M77	0.02 (0.04)

Note: M55 and M56 are unknown; M10 is N-des isopropyl-4-methylthiazole-GS-342006; M14 is Oxy GS-342006; M21 is E1 (GS-9454, GS-342006); M31 is E3 (GS-9612, GS-364751); and M77 is Oxy GS-9350-4.

Using high performance liquid chromatography, in pooled feces, the total percentage of the dose that was measured was 62.3%. The majority of the total radioactivity was attributed to cobicistat (26.94%) and with the exception of two cobicistat metabolites, (M31 [14.04%] and M21 [5.47%]), each of the other identified cobicistat metabolites accounted for less than 3% of the total radioactivity in pooled feces (see Table 7 below).

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Table 7-Perentage of total ^{14}C radioactivity attributed to cobicistat and cobicistat metabolites in pooled feces using high performance liquid chromatography

[^{14}C]GS-9350 Metabolite [a]	Mean (SD) Percent of Total ^{14}C Radioactivity
M10	1.20 (0.53)
M57	0.21 (0.40)
M14	2.40 (0.42)
M59	0.10 (0.28)
M60	0.11 (0.30)
M21	5.47 (0.88)
M48	1.09 (0.26)
M26	2.37 (0.78)
M49	1.90 (1.34)
M72	0.34 (0.46)
M73	1.15 (0.10)
M31	14.04 (3.20)
M64	0.80 (0.55)
M74	1.16 (0.75)
M75	0.16 (0.46)
M76	0.41 (0.45)
M39	2.41 (0.42)
GS-9350	26.94 (2.82)

Note: M57, M59, M60, M72, M73, M64, and M75 are unknown; M10 is N-des isopropyl-4-methylthiazole-GS-342006; M14 is Oxy GS-342006; M21 is E1 (GS-9454, GS-342006); M31 is E3 (GS-9612, GS-364751); M48 is Dioxy GS-9350-1; M26 is E5 (GS-341842); M49 is Dioxy GS-9350-2; M74 is Oxy GS-9350-3; M76 is Desmethyl GS-9350; and M39 is Desethyl GS-9350.

Based on the proposed cobicistat metabolic pathways in human and various animal species (mice, rats, and dogs) in Figure 8, cobicistat appears to be mainly metabolized through oxidation in humans.

Figure 8-Proposed cobicistat metabolic pathways in human and various animal species (mice, rats, and dogs)
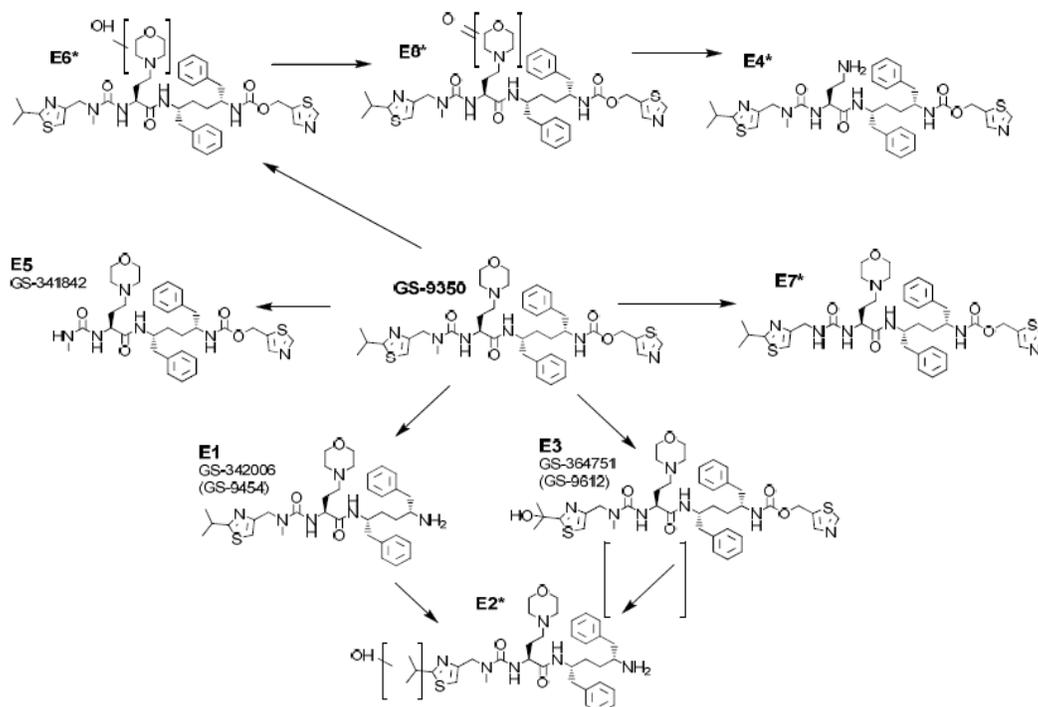
Note: E1, E3, and E5 were generated by humans, mice, rats, and dogs; E2, E4, E6, E7, and E8 were generated exclusively by dogs.

8. Discussion and Conclusions

The following conclusions can be made based on the results from GS-US-216-111:

a) In plasma, of the total ^{14}C radioactivity, ^{14}C cobicistat accounted for the majority of the observed exposure, and metabolite peaks were not detected in the majority of the time points ranging from 1 to 24 hours postdose. Of the total ^{14}C radioactivity, ^{14}C cobicistat accounted for 98.6% of the AUC$_{(0-\tau)}$ and 100% of the C$_{max}$.

b) Based on the urine and fecal excretion data, cobicistat is metabolized to multiple metabolites; however most of metabolites identified in the feces and urine are only present in small amounts relative to the total dose that is measured in the matrix:
-Cobicistat and cobicistat metabolites are minimally excreted in the urine. The total percentage of the dose that was measured was 6.31%. The majority of the total radioactivity was attributed to cobicistat (5.45%) with each of the identified cobicistat metabolites accounting for less than 1% of the total radioactivity in pooled urine.

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- In pooled feces, the total percentage of the dose that was measured was 62.3%. The majority of the total radioactivity was attributed to cobicistat (26.94%) and with the exception of two cobicistat metabolites (M31 and M21), each of the other identified cobicistat metabolites accounted for less than 3% of the total radioactivity in pooled feces.

c) Cobicistat appears to be mainly metabolized through oxidation in humans.

<u>GS-US-216-112</u>

1. Title

A Phase 1 Study to Evaluate the Effect of GS-9350 on Selected P450 Enzymes or Drug Efflux Transporter P-glycoprotein (P-gp)

2. Information Regarding the Clinical Trial Site and Duration of the Trial

The trial was conducted at SeaView Research, Miami, FL from February 22, 2010 (first subject screened) to April 29, 2010 (last subject observation).

3. Objectives

The objective of the trial was to evaluate the effects of cobicistat on CYP 2D6, CYP 2B6, and P-gp. Sensitive substrates of CYP 2D6 (desipramine), CYP 2B6 (efavirenz) were evaluated as well as digoxin as a P-gp substrate.

4. Trial Design

GS-US-216-112 was a Phase I, open label, clinical trial that enrolled healthy male and female subjects between 18 and 45 years old. The following cohorts were enrolled: a) cohort 1: cobicistat and desipramine, b) cohort 2: cobicistat and digoxin, and c) cohort 3: cobicistat and efavirenz.

Genotyping was performed as part of the screening process. Cohort 1 only enrolled subjects that were intermediate, extensive, or ultra-rapid CYP 2D6 metabolizers. Cohort 3 only enrolled subjects that were intermediate or extensive CYP 2B6 metabolizers.

A total of 52 subjects were to be enrolled. The number of subjects that were to be enrolled is as follows: a) 10 subjects (cohort1), b) 24 subjects (cohort 2), and c) 18 subjects (cohort 3).

5. Medication Restrictions and Exceptions

Use of vitamins, acetaminophen, ibuprofen, oral contraceptives, and topical hydrocortisone cream or A&D ointment to treat skin irritation from ECG leads was permitted during the trial. All other prescription and nonprescription medications, including herbal medications, were not permitted 28 days before initiation of dosing for the trial.

6. Dosage and Administration

Information regarding the treatments that will be administered in the trial for cohorts 1 and 2 and cohort 3 is displayed in Table 1 and Table 2, respectively.

In cohort 1, subjects received a single 50 mg desipramine dose (treatment A), and cobicistat 150 mg once daily for 10 days with concurrent administration with a single 50 mg desipramine dose on the tenth day (treatment B). In cohort 2, subjects received a single 0.5 mg digoxin dose (treatment C), and cobicistat 150 mg once daily for 10 days with concurrent administration with a single 0.5 mg digoxin dose on the tenth day (treatment D). In cohort 3, subjects received a single 600 mg efavirenz dose (treatment E), and cobicistat 150 mg once daily for 10 days with concurrent administration with a single 600 mg efavirenz dose on the tenth day (treatment F).

Table 1-Treatments administered in cohorts 1 and 2

Treatment Sequence	Day 1	Days 2–15	Days 16–24	Day 25
AB or CD	Probe	Washout	COBI	COBI + Probe
BA or DC	Days 1–9	Day 10	Days 11–24	Day 25
	COBI	COBI + Probe	Washout	Probe

Note: Shaded cells denote intensive pharmacokinetic sampling days.

Table 2-Treatments administered in cohort 3

Treatment Sequence	Day 1	Days 2–21	Days 22–30	Day 31
EF	Probe	Washout	COBI	COBI + Probe
FE	Days 1–9	Day 10	Days 11–30	Day 31
	COBI	COBI + Probe	Washout	Probe

Note: Shaded cells denote intensive pharmacokinetic sampling days.

Trial medications were administered within 5 minutes of completion of a meal. On intensive pharmacokinetic sampling days, subjects fasted for a minimum of 8 hours overnight prior to receiving a standardized meal and water was not

permitted from one hour before dosing to two hours after dosing, except for the 240 mL of water that was provided with the trial medication.

7. Rationale for Doses Used in the Trial

The selected cobicistat dosage regimen for the GS-US-216-112 trial, 150 mg once daily, is the proposed dosage regimen for cobicistat when combined with elvitegravir (cobicistat is utilized to achieve therapeutic concentrations of elvitegravir).

The recommended adult dosage regimen for desipramine in the U.S prescribing information is 100 mg to 200 mg daily, with an increase to 300 mg daily in severely ill patients. A lower dose of 50 mg was chosen for the GS-US-216-112 trial because of possible adverse events with potential inhibition of CYP 2D6 metabolism of despiramine.

The efavirenz dose that was selected for the GS-US-216-112 trial was 600 mg. This dose is consistent with the recommended adult dosage regimen for efavirenz in the U.S prescribing information (600 mg daily).

The digoxin dose that was selected for the GS-US-216-112 trial was 0.5 mg. This dose is consistent with the recommended range of maintenance adult dosage regimens (0.125 mg to 0.5 mg once daily) for heart failure patients in the U.S prescribing information for digoxin.

8. Drugs Used in the Trial

A summary of the cobicistat, desipramine, digoxin, and efavirenz formulations that were administered in the GS-US-216-112 trial is displayed in Table 3 below.

Table 3-Cobcistat, desipramine, digoxin, and efavirenz formulations administered in the GS-US-216-112 trial

	Cobicistat	Desipramine	Digoxin	Efavirenz (Sustiva®)
Strength (mg)	150 mg	50 mg	0.25 mg	600 mg
Lot No.	BB1001B1	182256	66943A	9B54552A
Expiration Date	01/31/2012	12/2011	03/2012	03/2012
Manufacturer/ Supplier	Gilead Sciences, Inc. Foster City, CA, USA	(b) (4)		Bristol-Myers Squibb Princeton, NJ, USA
Site of Release in Europe	Not applicable	Not applicable	Not applicable	Not applicable

9. Sample Collection, Bioanalysis, Pharmacokinetic Assessments, and Statistical Analysis

Sample Collection

Blood samples for analysis of cobicistat, desipramine, digoxin, and efavirenz plasma concentrations were collected. In cohort 1 and cohort 2, cobicistat, desipramine, and digoxin plasma samples were collected up to 72 hours postdose. For cohorts 1 and 2, subjects in treatment sequences AB or CD had blood samples collected on days 1 and 25 and subjects in treatment sequences BA or DC had blood samples collected on days 10 and 25. In cohort 3, cobicistat and efavirenz plasma samples were collected up to 336 hours (14 days) postdose. For cohort 3, subjects in treatment sequences EF had blood samples collected on days 1 and 31 and subjects in treatment sequences FE had blood samples collected on days 10 and 31.

Bioanalysis

The method and bioanalysis of cobicistat, ritonavir, midazolam, desipramine, digoxin, and efavirenz are acceptable, with the exception of long term stability for the desipramine, digoxin, and efavirenz analytes.

Blood samples for the GS-US-216-112 trial were collected in tubes containing K_2EDTA as the anticoagulant according to the bioanalytical reports for the cobicistat, desipramine, digoxin, and efavirenz analytes.

Cobicistat plasma samples were analyzed using a validated LC/MS/MS method with K_2EDTA anticoagulated plasma by ⬛ (b) (4) The lower limit of quantification for cobicistat was 5 ng/mL and the upper limit of quantification was 2500 ng/mL. There were no precision or accuracy issues identified for cobicistat based on the bioanalytical report. However, out of the 25 analytical runs where cobicistat samples were analyzed, 3 analytical runs failed. An investigation by ⬛ (b) (4) concluded that the cause was ⬛⬛⬛⬛ (b) (4)

Additionally, the cobicistat reference standard that was used for bioanalysis expired prior to the start of cobicistat sample analysis and the bioanalytical report does not provide information on why use of the expired cobicistat reference standard was acceptable.

For the GS-US-216-112 trial, cobicistat precision and accuracy were evaluated using the low (15 ng/mL), medium (200 ng/mL), and high (2000 ng/mL) QC samples. There were no dilution QCs analyzed in the analytical runs. The cobicistat inter-run accuracy values were 4.7% for the low QCs, 0.2% for the medium QCs, and -2.9% for the high QCs. The cobicistat inter-run precision values were 9.5% for the low QCs, 4.5% for the medium QCs, and 5.1% for the high QCs.

Of the 137 samples selected for incurred sample reanalysis, 1 sample was not within 20% using the mean value of the repeat and original concentrations. However, it is not clear whether the total number of samples that were reanalyzed represents 5% to 10% of the total number of samples that were initially analyzed.

(b) (4) did not conduct long term stability experiments for cobicistat. Instead, (b) (4) references the long term stability data for cobicistat that was generated by (b) (4). The cobicistat analytical method that was used by (b) (4) is not identical to the cobicistat analytical method used by (b) (4). The submitted cobicistat long term sample stability data demonstrated that cobicistat was stable for 121 days at -10°C to -30°C and 365 days at -60°C to -80°C in K_2EDTA anticoagulated plasma. At the (b) (4) bioanalytical laboratory, subject samples from the GS-US-216-112 trial were stored at -70°C and the bioanalytical report states that subject samples were also stored at -70°C at the trial site until they were shipped to (b) (4). Overall, while the submitted cobicistat long term stability data of 365 days at-70°C covers the duration of long term cobicistat stability data necessary for the GS-US-216-112 trial, the impact of the differences in the cobicistat analytical methods used by (b) (4) compared to (b) (4) is unknown.

One additional issue that was identified based on the method validation results for cobicistat was the multiple QCs at 200 ng/mL that exceeded 15% for the post preparative reinjection stability experiment at 4°C.

Desipramine plasma samples were analyzed using a validated LC/MS/MS method with K_2EDTA anticoagulated plasma by (b) (4). The lower limit of quantification for desipramine was 1 ng/mL and the upper limit of quantification was 2000 ng/mL. There were no precision or accuracy issues identified for desipramine based on the bioanalytical report. Out of the six analytical runs where desipramine samples were analyzed, no analytical run failed for the desipramine analysis.

For the GS-US-216-112 trial, desipramine precision and accuracy were evaluated at the following concentrations: 3 ng/mL, 20 ng/mL, 200 ng/mL, 900 ng/mL, and 1800 ng/mL. There were no dilution QCs analyzed in the analytical runs. The desipramine inter-run accuracy values were -0.9% for 3 ng/mL, -1% for 20 ng/mL, 0.2% for 200 ng/mL, 0.5% for 900 ng/mL, and -1.8% for 1800 ng/mL. The desipramine inter-run precision values were 4.4% for 3 ng/mL, 2.3% for 20 ng/mL, 2.4% for 200 ng/mL, 2.1% for 900 ng/mL, and 2.8% for 1800 ng/mL.

Of the 41 samples selected for incurred sample reanalysis, all samples were within 20% using the mean value of the repeat and original concentrations. However, it is not clear whether the total number of samples that were reanalyzed represents 5% to 10% of the total number of samples that were initially analyzed.

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The submitted desipramine long term sample stability data demonstrated that desipramine was stable for of 91 days at -20°C in K$_2$EDTA anticoagulated plasma. At the (b) (4) bioanalytical laboratory, subject samples from the GS-US-216-112 trial were stored at -20°and at -70°C at the trial site until they were shipped to (b) (4) according to the bioanalytical report. There was no -70°C long term sample stability data submitted for desipramine. Therefore, the long term stability of the desipramine subject samples has not been established for the GS-US-216-112 trial. The applicant will be requested to provide additional long term stability information.

Digoxin plasma concentrations were analyzed using a LC/MS/MS method with K$_2$EDTA anticoagulated plasma by (b) (4). Only the partial method validation report evaluating a change in the liquid chromatography gradient was submitted, therefore a determination by the clinical pharmacology reviewer regarding whether there are any issues with the method validation could not be made. The lower limit of quantification for digoxin was 100 pg/mL and the upper limit of quantification was 3000 pg/mL. There were no precision or accuracy issues identified for digoxin based on the bioanalytical report. Out of the ten analytical runs where digoxin samples were analyzed, no runs failed for the digoxin analysis.

For the GS-US-216-112 trial, digoxin precision and accuracy were evaluated using the low (300 pg/mL), medium (2000 pg/mL), and high (25000 pg/mL) QC samples. An additional QC (1000 pg/mL) that was not evaluated as part of the digoxin partial method validation was also included in two of the analytical runs. The bioanalytical report does not specify why the additional 1000 pg/mL QC was added. There were no dilution QCs analyzed in the analytical runs. The digoxin inter-run accuracy values were 0.4% for 300 pg/mL, 2.1% for 1000 pg/mL, 1.8% for 2000 pg/mL, and -7.1% for 25000 pg/mL. The digoxin inter-run precision values were 5.3% for 300 pg/mL, 4.2% for 1000 pg/mL, 4.1% for 2000 pg/mL, and 5.4% for 25000 pg/mL.

Of the 97 samples selected for incurred sample reanalysis, 1 sample was not within 20% using the mean value of the repeat and original concentrations. However, it is not clear whether the total number of samples that were reanalyzed represents 5% to 10% of the total number of samples that were initially analyzed.

There was no digoxin long term sample stability data at -20°C or -70°C that was submitted. Therefore, the long term stability of the digoxin subject samples has not been established for the GS-US-216-112 trial. The applicant will be requested to provide additional long term stability information.

Efavirenz plasma concentrations were analyzed using a LC/MS/MS method with K$_2$EDTA anticoagulated plasma by (b) (4) The lower limit of quantification for efavirenz was 5 ng/mL and the upper limit of quantification was 5000 ng/mL.

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There were no precision or accuracy issues identified for efavirenz based on the bioanalytical report. Out of the 11 analytical runs where efavirenz samples were analyzed, no runs failed for the efavirenz analysis.

For the GS-US-216-112 trial, efavirenz precision and accuracy were evaluated at the following concentrations: 15 ng/mL, 200 ng/mL, 750 ng/mL, and 4000 ng/mL. There were no dilution QCs analyzed in the analytical runs. The efavirenz inter-run accuracy values were -1.9% for 15 ng/mL, -0.6% for 200 ng/mL, 1.3% for 750 ng/mL, and 3.7% for 4000 ng/mL. The efavirenz inter-run precision values were 4% for 15 ng/mL, 3.6% for 200 ng/mL, 4% for 750 ng/mL, and 5.2% for 4000 ng/mL.

Of the 106 samples selected for incurred sample reanalysis, 3 samples were not within 20% using the mean value of the repeat and original concentrations. However, it is not clear whether the total number of samples that were reanalyzed represents 5% to 10% of the total number of samples that were initially analyzed.

The bioanalytical report states that long term sample stability for efavirenz was evaluated for 127 days at -20°C or -70°C. However, this information was not submitted. Therefore, the long term stability of the efavirenz subject samples has not been established for the GS-US-216-112 trial.

Pharmacokinetic Assessments

Noncompartmental analysis was performed to calculate cobicistat, desipramine, digoxin, and efavirenz plasma pharmacokinetic parameters, including C_{max} and $AUC_{(0-inf)}$ for desipramine, digoxin, and efavirenz, and C_τ, C_{max}, and $AUC_{(0-\tau)}$ for cobicistat.

Statistical Analysis

Descriptive statistics were calculated for cobicistat, desipramine, digoxin, and efavirenz plasma concentrations and pharmacokinetic parameters, including the number of subjects (n), mean, standard deviation, the coefficient of variation (CV%), median, and the minimum and maximum values. The geometric mean was also calculated for the individual pharmacokinetic parameters.

Statistical analysis involved comparison of desipramine, digoxin, and efavirenz natural log transformed pharmacokinetic parameters for the relevant test arms (treatments B, D, and F) compared to the reference arms (treatments A, C, and E) and 90% confidence intervals were derived. If the 90% confidence intervals were within 80%-125%, it was concluded that a clinically relevant drug-drug interaction did not exist.

The Wilcoxon signed-rank test was used to compare desipramine, digoxin, and efavirenz clearance and half life when administered alone and in combination with cobicistat.

10. Results

10.1 Subject Demographics and Disposition

Table 4-GS-US-216-112 subject demographics

	Cohort 1 (N=10)	Cohort 2 (N=25)	Cohort 3 (N=18)	Total (N=53)
Age at Day 0 (Years)				
N	10	25	18	53
Mean (SD)	35 (8.3)	33 (7.1)	33 (7.4)	33 (7.3)
Median	39	34	34	35
Q1, Q3	28, 42	27, 38	28, 38	28, 39
Min, Max	21, 43	20, 45	20, 43	20, 45
Sex				
Male	6 (60.0%)	13 (52.0%)	10 (55.6%)	29 (54.7%)
Female	4 (40.0%)	12 (48.0%)	8 (44.4%)	24 (45.3%)
Race				
American Indian or Alaska Native	0	0	0	0
Asian	0	0	0	0
Black or African Heritage	4 (40.0%)	8 (32.0%)	5 (27.8%)	17 (32.1%)
Native Hawaiian or Pacific Islander	0	0	0	0
White	6 (60.0%)	17 (68.0%)	11 (61.1%)	34 (64.2%)
Other	0	0	2 (11.1%)	2 (3.8%)
Ethnicity				
Hispanic/Latino	5 (50.0%)	18 (72.0%)	9 (50.0%)	32 (60.4%)
Non-Hispanic/Latino	5 (50.0%)	7 (28.0%)	9 (50.0%)	21 (39.6%)
Weight (kg)				
N	10	25	18	53
Mean (SD)	76.0 (12.39)	71.0 (10.42)	73.6 (11.36)	72.8 (11.07)
Median	77.5	70.8	74.6	72.0
Q1, Q3	64.5, 88.0	65.0, 78.0	65.8, 84.0	65.0, 79.1
Min, Max	58.2, 91.0	51.0, 100.0	52.7, 91.0	51.0, 100.0
Height (cm)				
N	10	25	18	53
Mean (SD)	170.1 (10.16)	167.9 (7.29)	170.1 (6.36)	169.1 (7.54)
Median	168.5	166.0	170.5	168.0
Q1, Q3	163.0, 178.0	164.5, 173.0	166.0, 176.0	165.0, 175.0
Min, Max	153.0, 187.0	153.5, 185.0	157.5, 179.0	153.0, 187.0
Body Mass Index (kg/m^2)				
N	10	25	18	53
Mean (SD)	26.2 (3.25)	25.1 (2.46)	25.3 (2.98)	25.4 (2.78)
Median	27.2	25.0	25.9	25.8
Q1, Q3	25.6, 28.3	23.2, 27.4	22.7, 27.9	23.2, 27.8
Min, Max	20.4, 29.9	20.1, 29.2	19.7, 29.4	19.7, 29.9

Figure 3-GS-US-216-112 subject disposition


```
                        N = 122
                        Screened
                            |
                            |------------------ N = 62
                            |                   Screening Failures
                            |
                            |------------------ N = 7
                            |                   Alternates
                            |
                        N = 53
                        Enrolled
         _____|_____
        |                      |                      |
    N = 10                 N = 25*                  N = 18
Enrolled into Cohort 1    Enrolled into Cohort 2   Enrolled into Cohort 3
and Randomized into       and Randomized into      and Randomized into
AB/BA                     CD/DC                    EF/FE
        |                      |                      |
    N = 1                  N = 3                    N = 1
Early Terminated          Early Terminated         Early Terminated
• Pregnancy in AB         • Withdrew Consent in CD  • Lost to Follow-up in EF
                          • Withdrew Consent in DC
                          • Adverse Event in CD
        |                      |                      |
    N = 9                  N = 22                   N = 17
Completed Study Drug      Completed Study Drug     Completed Study Drug
    ____|____              ____|____                ____|____
   |         |            |         |              |         |
 N = 4     N = 5        N = 10    N = 12          N = 8     N = 9
Sequence  Sequence     Sequence  Sequence        Sequence  Sequence
AB        BA           CD        DC              EF        FE
```

* Subject 3648-2003 was discontinued from the study on Day 1; Subject 3648-2103 was enrolled as a replacement. As a result, a total of 25 subjects were enrolled in Cohort 2.

10.2 Concomitant Medications

The concomitant medications that subjects received during the trial included various oral contraceptive medications, acetaminophen, and loratadine. None of the administered concomitant medications are anticipated to significantly impact the results of the trial.

10.3 Pharmacokinetic and Statistical Analysis

Nine subjects had a quantifiable predose efavirenz concentration with treatment E, Day 31 and six subjects had a quantifiable predose efavirenz concentration with treatment F, Day 31, that were ≤ 5% of the individual's C_{max}. The quantifiable predose efavirenz concentrations appear to be caused by carryover from the previous efavirenz single dose due to efavirenz's long half life (single dose half life ranging from 52-76 hours). No adjustments to the pharmacokinetic analysis for these subjects were required. There were no subjects with quantifiable predose desipramine or digoxin concentrations when administered alone or in combination with cobicistat.

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Comparison of desipramine single dose pharmacokinetics when administered alone and in combination with cobicsistat

The pharmacokinetic parameters for single 50 mg doses of desipramine when administered alone and in combination with cobicistat and the statistical analyses comparing the treatment arms are displayed in Table 5 and Table 6, respectively.

Based on the comparison of the pharmacokinetic data in Table 6, cobicistat increased the C_{max}, $AUC_{(0-last)}$, and $AUC_{(0-inf)}$ of desipramine, a sensitive CYP 2D6 substrate. In addition, the desipramine clearance also appears to be lower with minimal differences in desipramine half life when desipramine is coadministered with cobicistat compared to desipramine administered by itself (see Table 5). Based on the magnitude of increase in desipramine exposure, cobicistat is a weak inhibitor of CYP 2D6, with a weak inhibitor defined as an increase in AUC ranging from 25% to 100%.

Table 5-Pharmacokinetic parameters for single 50 mg doses of desipramine when administered alone and in combination with cobicistat

Desipramine PK Parameter	Desipramine 50 mg (N = 8)	Desipramine/COBI 50/150 mg (N = 8)
AUC_{last} (ng•h/mL), Mean (%CV)	650.9 (66.3)	970.4 (51.2)
AUC_{inf} (ng•h/mL), Mean (%CV)	752.0 (83.3)[a]	1205.0 (64.7)
C_{max} (ng/mL), Mean (%CV)	27.9 (43.6)	33.2 (28.2)
C_{last} (ng/mL), Mean (%CV)	3.4 (80.5)	5.0 (90.3)
T_{max} (h), Median (Q1, Q3)	5.25 (5.00, 6.00)	8.00 (6.00, 9.00)
T_{last} (h), Median (Q1, Q3)	60.00 (48.00, 72.50)	72.00 (72.00, 72.00)
$T_{½}$ (h), Median (Q1, Q3)	14.49 (13.45, 24.54)[a]	23.45 (15.23, 28.13)
CL/F (mL/h), Mean (%CV)	99,076.6 (49.9)[a]	57,848.0 (54.3)
Weight-adjusted CL/F (mL/h/kg), Mean (%CV)	1248.0 (48.8)[a]	732.2 (54.6)

%CV, percentage coefficient of variation

Note: Subjects 3648-1006 and 3648-1007 did not have PK data for a treatment pair of interest and were excluded from the summaries.

a   n=7; Desipramine $T_{1/2}$, AUC_{inf}, and CL/F could not be estimated for Subject 3648-1001 following the desipramine 50-mg treatment.

Table 6-Statistical analyses for single 50 mg doses of desipramine when administered alone and in combination with cobicistat

Desipramine PK Parameter	Geometric Least Squares Means		Geometric Least Squares Mean Ratio (%)	90% Confidence Interval
	Test Treatment Desipramine + COBI (N=8)	Reference Treatment Desipramine (N=8)		
AUC_{inf} (ng•h/mL)	1010.54	610.92[a]	165.41	135.76, 201.54
AUC_{last} (ng•h/mL)	866.23	549.93	157.52	134.88, 183.95
C_{max} (ng/mL)	32.18	25.89	124.31	107.54, 143.68

a   n=7; Desipramine AUC_{inf} could not be estimated for Subject 3648-1001 following the desipramine 50-mg treatment.

<u>Comparison of digoxin single dose pharmacokinetics when administered alone and in combination with cobicsistat</u>

The pharmacokinetic parameters for single 0.5 mg doses of digoxin when administered alone and in combination with cobicistat and the statistical analyses comparing the treatment arms are displayed in Table 7 and Table 8, respectively.

The issue of whether there was adequate sampling of digoxin concentrations was reviewed because of digoxin's long half life (1.5 to 2 days). When digoxin was administered alone, the mean percentage of the AUC that was extrapolated was 27.4% with minimum and maximum percentages of 18.4% and 38.6%. When digoxin was coadministered with cobicistat, the mean percent of the AUC that was extrapolated was 22.3% with minimum and maximum percentages of 14.9% and 35.4%. Therefore, the digoxin sampling up to 72 hours appeared to be adequate for the trial.

Based on the comparison of the pharmacokinetic data in Table 8, cobicistat increased digoxin C_{max} and $AUC_{(0-last)}$, but the 90% confidence interval for the increase in $AUC_{(0-inf)}$ of digoxin was within 80%-125%. The digoxin clearance was similar for the two treatment arms, with minimal differences in digoxin half life when digoxin is administered alone compared to coadministration with cobicistat (see Table 7).

Table 7-Pharmacokinetic parameters for single 0.5 mg doses of digoxin when administered alone and in combination with cobicistat

Digoxin PK Parameter	Digoxin 0.5 mg (N = 22)	Digoxin/COBI 0.5/150 mg (N = 22)
AUC_{last} (ng·h/mL), Mean (%CV)	22.0 (28.2)	26.5 (28.1)
AUC_{inf} (ng·h/mL), Mean (%CV)	31.9 (24.1)[a]	34.7 (26.6)[b]
C_{max} (ng/mL), Mean (%CV)	1.7 (24.9)	2.5 (32.3)
C_{last} (ng/mL), Mean (%CV)	0.2 (31.6)	0.2 (30.1)
T_{max} (h), Median (Q1, Q3)	2.00 (2.00, 2.00)	2.00 (1.00, 2.00)
T_{last} (h), Median (Q1, Q3)	72.00 (72.00, 73.00)	72.00 (72.00, 72.00)
$T_{½}$ (h), Median (Q1, Q3)	38.56 (33.03, 44.27)[a]	29.74 (28.16, 34.12)[b]
CL/F (mL/h), Mean (%CV)	16,693.0 (27.4)[a]	15,553.5 (30.4)[b]
Weight-adjusted CL/F (mL/h/kg), Mean (%CV)	237.2 (29.5)[a]	217.3 (29.8)[b]

%CV, percentage coefficient of variation

Note: Subjects 3648-2003, 3648-2006, and 3648-2023 did not have PK data for a treatment pair of interest and were excluded from the summaries.

a n=19; Digoxin $T_{1/2}$, AUC_{inf}, and CL/F could not be estimated for Subjects 3648-2002, 3648-2004, and 3648-2010 following the digoxin 0.5-mg treatment.

b n=21; Digoxin $T_{1/2}$, AUC_{inf}, and CL/F could not be estimated for Subject 3648-2014 following the digoxin/COBI 0.5/150-mg treatment.

Table 8-Statistical analyses for single 0.5 mg doses of digoxin when administered alone and in combination with cobicistat

Digoxin PK Parameter	Geometric Least Squares Means		Geometric Least Squares Mean Ratio (%)	90% Confidence Interval
	Test Treatment Digoxin + COBI (N=22)	Reference Treatment Digoxin (N=22)		
AUC_{inf} (ng·h/mL)	32.95[a]	30.58[b]	107.73	99.58, 116.55
AUC_{last} (ng·h/mL)	25.12	21.01	119.60	109.98, 130.06
C_{max} (ng/mL)	2.38	1.69	140.95	128.52, 154.58

Note: Subjects 3648-2003, 3648-2006, and 3648-2023 did not have PK data for a treatment pair of interest and were excluded from the summaries.

a n=21; Digoxin AUC_{inf} could not be estimated for Subject 3648-2014 following the digoxin/COBI 0.5/150-mg treatment.

b n=19; Digoxin AUC_{inf} could not be estimated for Subjects 3648-2002, 3648-2004, and 3648-2010 following the digoxin 0.5-mg treatment.

Comparison of efavirenz single dose pharmacokinetics when administered alone and in combination with cobicsistat

The pharmacokinetic parameters for single 600 mg doses of efavirenz when administered alone and in combination with cobicistat and the statistical analyses comparing the treatment arms are displayed in Table 9 and Table 10, respectively.

Based on the comparison of the pharmacokinetic data in Table 10, when all subjects were analyzed, cobicistat decreased the C_{max} of efavirenz, a sensitive CYP 2B6 substrate but the 90% confidence intervals for the decreases in $AUC_{(0-last)}$ and $AUC_{(0-inf)}$ were within 80%-125%. An additional statistical analyses was also conducted to exclude subject 3648-3018 that has a C_{max} of 5538.8 ng/mL with efavirenz when administered alone compared to 2859.9 ng/mL when coadministered with cobicistat. When subject 3648-3018 was excluded, the decreases in efavirenz $AUC_{(0-last)}$, C_{max} and $AUC_{(0-inf)}$ were within 80% to 125%.

There were minimal differences in efavirenz clearance and half life when efavirenz is administered alone compared to coadministration with cobicistat (see Table 9).

Table 9-Pharmacokinetic parameters for single 600 mg doses of efavirenz when administered alone and in combination with cobicistat

Efavirenz PK Parameter	Efavirenz 600 mg (N = 17)	Efavirenz/COBI 600/150 mg (N = 17)
AUClast (ng•h/mL), Mean (%CV)	139,120.4 (26.2)	129,806.8 (29.1)
AUCinf (ng•h/mL), Mean (%CV)	161,453.8 (25.5)	150,254.8 (27.2)
Cmax (ng/mL), Mean (%CV)	4400.2 (14.4)	3866.4 (20.6)
Clast (ng/mL), Mean (%CV)	108.6 (45.1)	102.4 (43.4)
Tmax (h), Median (Q1, Q3)	4.50 (4.50, 5.00)	4.50 (4.50, 5.50)
Tlast (h), Median (Q1, Q3)	336.00 (336.00, 336.00)	336.00 (336.00, 336.00)
T½ (h), Median (Q1, Q3)	133.15 (97.89, 149.85)	122.05 (103.95, 141.00)
CL/F (mL/h), Mean (%CV)	3971.7 (28.3)	4284.0 (28.6)
Weight-adjusted CL/F (mL/h/kg), Mean (CV%)	54.8 (28.8)	59.2 (31.2)

%CV, percentage coefficient of variation
Note: Subject 3648-3001 did not have PK data for a treatment pair of interest and was excluded from the summaries.

Table 10-Statistical analyses for single 600 mg doses of efavirenz when administered alone and in combination with cobicistat

Efavirenz PK Parameter	Geometric Least Squares Means		Geometric Least Squares Mean Ratio (%)	90% Confidence Interval
	Test Treatment Efavirenz + COBI	Reference Treatment Efavirenz		
All Evaluable Subjects, n	17	17		
AUC_{inf} (ng•h/mL)	144,706.5	155,804.5	92.88	89.22, 96.69
AUC_{last} (ng•h/mL)	125,146.8	134,798.4	92.84	89.70, 96.09
C_{max} (ng/mL)	3782.53	4366.99	86.62	79.59, 94.27
Excluding Outlier[a], n	16	16		
AUC_{inf} (ng•h/mL)	142,939.0	154,643.9	92.43	88.59, 96.44
AUC_{last} (ng•h/mL)	124,627.1	134,689.2	92.53	89.20, 95.98
C_{max} (ng/mL)	3850.72	4304.97	89.45	83.30, 96.05

Note: Subject 3648-3001 did not have PK data for a treatment pair of interest and was excluded from the summaries.

a   Analysis excludes data for Subject 3648-3018

Cobicistat multiple dosing pharmacokinetic data

The cobicistat pharmacokinetic parameters when coadministered with desipramine, digoxin, or efavirenz are displayed in Table 11. Overall, there appeared to be minimal differences in cobicistat $AUC_{(0-tau)}$, C_{max} and C_{tau} when comparing the exposure data between the three treatment arms. The trial report did not provide historical cobicistat pharmacokinetic data for comparison purposes.

Table 11-Pharmacokinetic parameters for multiple doses of 150 mg once daily of cobicistat when administered in combination with desipramine, digoxin, or efavirenz

COBI PK Parameter	Desipramine/COBI 50/150 mg (N = 9)	Digoxin/COBI 0.5/150 mg (N = 22)	Efavirenz/COBI 600/150 mg (N = 17)
AUC_{tau} (ng•h/mL), Mean (%CV)	17,850.4 (25.5)	15,637.7 (30.6)	14,784.4 (32.5)
C_{tau} (ng/mL), Mean (% CV)	146.1 (63.5)	116.2 (75.1)	103.8 (87.9)
C_{max} (ng/mL), Mean (% CV)	1905.3 (16.2)	1816.5 (22.1)	1692.5 (19.9)
C_{last} (ng/mL), Mean (% CV)	12.4 (64.6)	24.7 (83.3)	17.3 (98.2)
T_{max} (h), Median (Q1, Q3)	4.50 (3.50, 4.50)	3.50 (3.00, 4.50)	4.00 (3.50, 4.50)
T_{last} (h), Median (Q1, Q3)	36.00 (36.00, 48.00)	36.00 (24.00, 36.00)	36.00 (24.00, 36.00)
$T_{\frac{1}{2}}$ (h), Median (Q1, Q3)	4.44 (4.12, 4.70)	4.32 (3.95, 4.78)	4.03 (3.69, 4.63)
CL/F (mL/h), Mean (%CV)	9030.1 (31.4)	10,472.8 (30.6)	11,281.6 (36.6)

%CV, percentage coefficient of variation

Note: Subjects 3648-1007, 3648-2003, 3648-2006, 3648-2023, and 3648-3001 did not have PK data for any treatment and were excluded from the summaries.

11. Discussion and Conclusions

Based on the results from the GS-US-216-112 trial, the following conclusions can be made:

- Cobicistat increases the C_{max}, $AUC_{(0-last)}$, and $AUC_{(0-inf)}$ of desipramine, a sensitive CYP 2D6 substrate, by 24%, 58% and 65%, respectively. Based on the magnitude of increase in desipramine exposure, cobicistat is a weak inhibitor of CYP 2D6.

- Cobicistat increases digoxin C_{max}, $AUC_{(0-last)}$, and $AUC_{(0-inf)}$ of digoxin, a P-gp substrate, by 41%, 20% and 8%, respectively. The 90% confidence interval for the increase in $AUC_{(0-inf)}$ of digoxin was within 80%-125%. Overall, cobicistat's inhibitory effects on P-gp are not clinically significant. However, for subjects concurrently receiving digoxin with antiretroviral HIV-1 regimens that include cobicistat, the potential for increased digoxin exposure should be considered when monitoring digoxin concentrations.

- When all subjects were analyzed, cobicistat decreased the C_{max}, $AUC_{(0-last)}$, and $AUC_{(0-inf)}$ of efavirenz, a sensitive CYP 2B6 substrate, by 13%, 7%, and 7%. The 90% confidence intervals for the decreases in $AUC_{(0-last)}$ and $AUC_{(0-inf)}$ were within 80%-125%.

- When comparing the cobicistat exposure data when coadministered with desipramine, digoxin, or efavirenz, there appeared to be minimal differences in cobicistat $AUC_{(0-tau)}$, C_{max} and C_{tau}.

GS-US-216-113

1. Title

A Phase 1 Study to Evaluate the Pharmacokinetics, Safety and Tolerability of GS-9350 in Healthy Volunteers

2. Information Regarding the Clinical Trial Site and Duration of the Trial

The trial was conducted at SeaView Research, Miami FL from February 9, 2009 (first subject screened) to March 19, 2009 (last subject observation).

3. Objectives

The objective of the trial was to evaluate the single and multiple dosing pharmacokinetics of GS-9350 (cobicistat) after oral dosing.

4. Trial Design

GS-US-216-113 was a Phase I, open label, clinical trial that enrolled healthy male and female subjects between 18 and 45 years old. The overall trial design is displayed in Figure 1 and the trial designs for cohorts 1 and 2 are displayed in Figures 2 and 3, respectively. In cohort 1, 12 subjects were to receive 300 mg of cobicistat once daily for seven days. In cohort 2, 12 subjects were to receive a single 400 mg dose of cobicistat.

Figure 1-Overall GS-US-216-113 trial design

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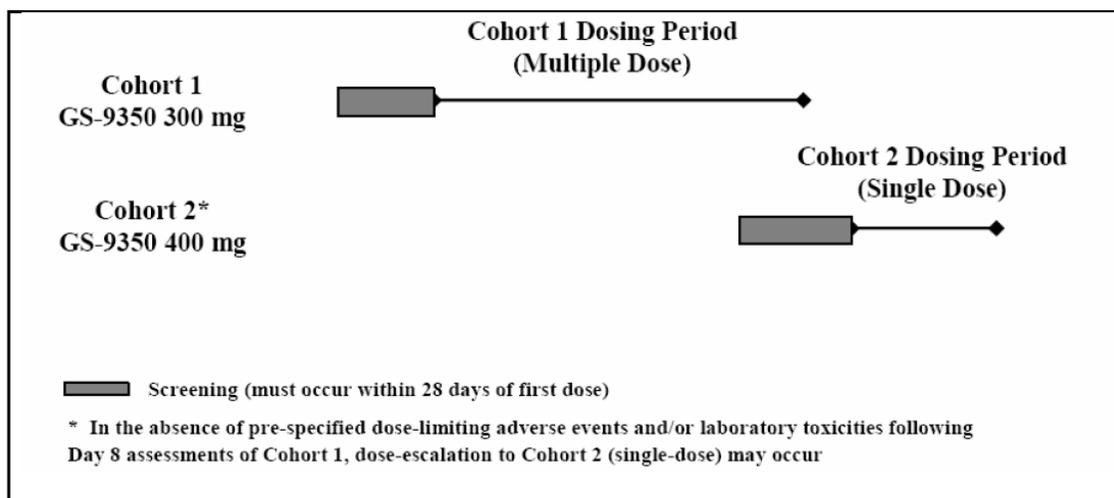
Figure 2-GS-US-216-113 cohort 1 trial design
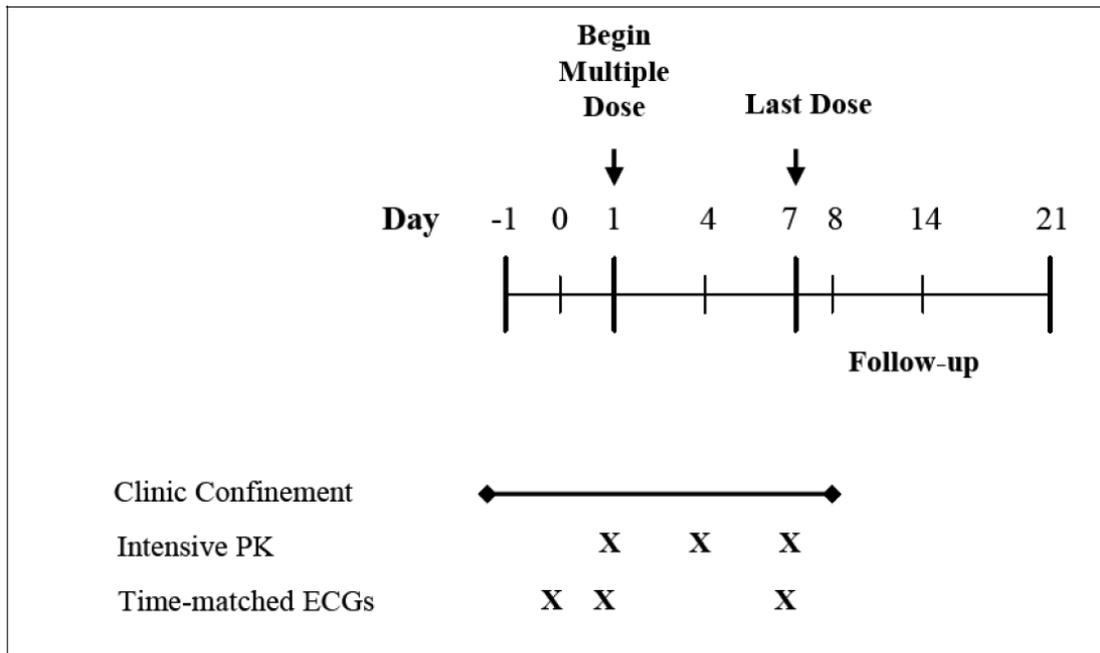
Figure 3-GS-US-216-113 cohort 2 trial design
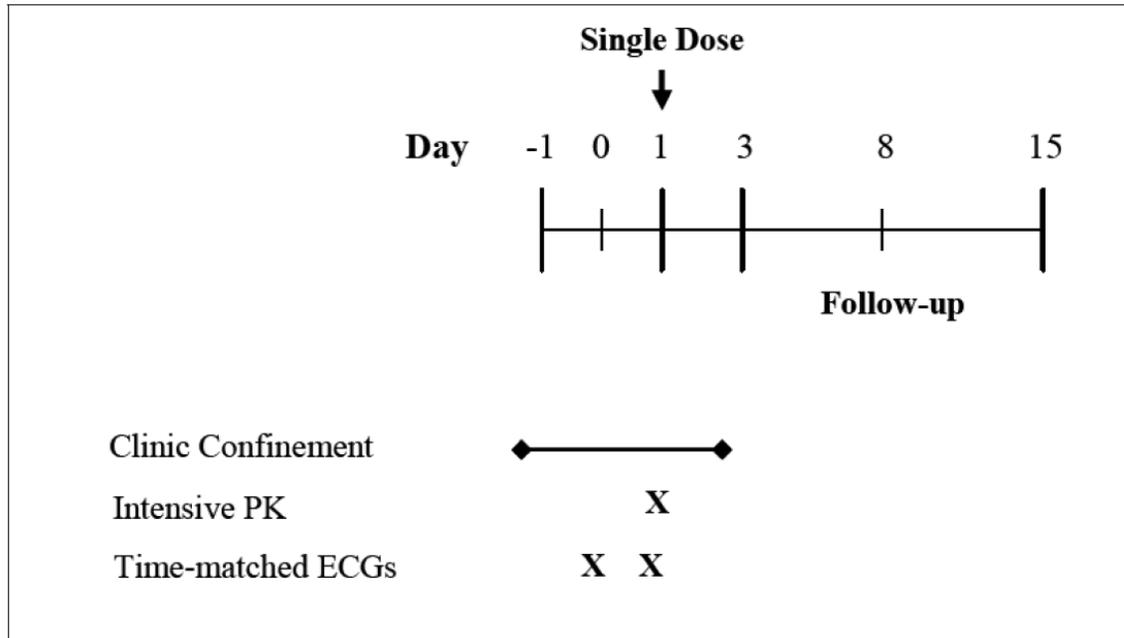
Reference ID: 3153809

5. Medication Restrictions and Exceptions

Use of vitamins, acetaminophen, ibuprofen, oral contraceptives, and topical hydrocortisone cream or A&D ointment to treat skin irritation from ECG leads was permitted during the trial. All other prescription and nonprescription medications were not permitted.

6. Dosage and Administration

In cohort 1, subjects were to receive 300 mg of cobicistat once daily for seven days. In cohort 2, subjects were to receive a single 400 mg dose of cobicistat. In cohort 1, on days 1, 4, 5, 6, and 7 and in cohort 2, day 1, after fasting overnight for a minimum of eight hours, cobicistat was administered within 5 minutes of finishing a standardized meal. Additionally, on days 1, 4, and 7 (cohort 1), and day 1 (cohort 2), water was not permitted from one hour before dosing to two hours after dosing, except with the 240 mL of water that was provided with the trial medication.

7. Rationale for Doses Used in the Trial

Cobicistat 150 mg once daily is the proposed dosage regimen for cobicistat when combined with elvitegravir (cobicistat is utilized to achieve therapeutic concentrations of elvitegravir). A specific reason for evaluating multiple doses of 300 mg cobicistat once daily and single doses of 400 mg cobicistat in the GS-US-216-113 trial was not provided in the trial report. However, one of the reasons citied in the trial protocol was to obtain information to determine the appropriate cobicistat doses and duration (single versus multiple doses) to achieve supratherapeutic cobicistat exposure for the thorough QT trial.

8. Drugs Used in the Trial

Cobicistat 100 mg tablets were administered in the trial. Information regarding the cobicistat formulation is displayed in Table 1.

Table 1-Cobcistat formulation administered in the GS-US-216-113 trial

	GS-9350
Strength	100 mg
Lot No.	BB0703A1
Expiration Date	31 January 2010
Manufacturer/Supplier	Gilead Sciences, Inc. 333 Lakeside Drive Foster City, CA 94404, USA

9. Sample Collection, Bioanalysis, Pharmacokinetic Assessments, and Statistical Analysis

Sample Collection

Blood samples for analysis of cobicistat plasma concentrations were obtained on days 1, 4, and 7 (cohort 1) at predose (time 0) and up to 24 hours postdose. In cohort 2, day 1, blood samples were collected at predose (time 0) and up to 48 hours postdose.

Bioanalysis

The method and bioanalysis of cobicistat are acceptable, with the exception of long term stability. Cobicistat plasma samples were analyzed using a validated LC/MS/MS method by Gilead Sciences using K_2EDTA anticoagulated plasma. The bioanalytical report does not specify the anticoagulant that was used in collecting blood samples from subjects in the GS-US-216-113 trial. The lower limit of quantification for cobicistat was 5 ng/mL and the upper limit of quantification was 2500 ng/mL. There were no precision or accuracy issues identified for cobicistat based on the bioanalytical report. However, out of the 26 analytical runs where samples were analyzed, 7 analytical runs failed. The bioanalytical report does not discuss whether an investigation was conducted to determine the cause(s) of the analytical run failures or whether the cause(s) of the run failures were identified and corrective actions taken.

For the GS-US-216-113 trial, cobicistat precision and accuracy were evaluated using the low (15 ng/mL), medium (500 ng/mL), and high (2000 ng/mL) QC samples. A dilution QC (2000 ng/mL) was also analyzed in most of the analytical runs.   The corresponding cobicistat inter-run accuracy values were 4% for the low QCs, 0.3% for the medium QCs, -7.1% for the high QCs, and -6.2% for the dilution QCs. The cobicistat inter-run precision values were 7.1% for the low QCs, 7.8% for the medium QCs, 5.6% for the high QCs and 4.1% for the dilution QCs. In runs 4, 5, and 6, both dilution QCs that were analyzed in each of the runs were excluded because of an error in preparing the samples and no reported concentration results using a dilution factor were reported for these runs.

Of the 60 samples selected for incurred sample reanalysis, 6 samples were not within 20% using the mean value of the repeat and original concentrations. However, it is not clear whether the total number of samples that were reanalyzed represents 5% to 10% of the total number of samples that were initially analyzed.

The submitted cobicistat long term stability data of 122 days at -80°C had multiple low QCs that exceeded 15% and additional stability data is necessary to characterize cobicistat long term sample stability at -80°C. At the Gilead
^{(b) (4)} subject samples from the GS-US-216-113 trial were

stored at -80°C but it is not clear whether samples were also stored at -80°C at the trial site or any intermediate storage facility, if applicable.

Therefore, the long term stability of the cobicistat subject samples has not been established for the GS-US-216-113 trial.

Pharmacokinetic Assessments

Noncompartmental analysis was performed to calculate cobicistat plasma pharmacokinetic parameters, including C_{max}, and $AUC_{(0-inf)}$ for single dosing, and C_{tau}, C_{max}, and $AUC_{(0-\tau)}$ for multiple dosing.

Statistical Analysis

Descriptive statistics were calculated for cobicistat plasma concentrations and pharmacokinetic parameters, including the number of subjects (n), mean, standard deviation, the coefficient of variation (CV%), median, and the minimum and maximum values. The geometric mean was also calculated for the individual pharmacokinetic parameters.

The following comparisons of plasma cobicistat natural log transformed pharmacokinetic parameters were also conducted that included the following:

a) The multiple dosing pharmacokinetics for day 7 compared to the multiple dosing pharmacokinetics for day 4 in Cohort 1.
b) The multiple dosing pharmacokinetics for day 7 compared to the single dosing pharmacokinetics for day 1 in Cohort 1.
c) The multiple dosing pharmacokinetics for day 4 compared to the single dosing pharmacokinetics for day 1.
d) The single dosing pharmacokinetics for day 1 (cohort 2) compared to the single dosing pharmacokinetics for day 1 (cohort 1).

The sponsor did not specify the "no effect" criterion in comparing the pharmacokinetic parameters. For the purposes of this review, it was assumed that the "no effect" criterion was a 90% confidence interval within 80% to 125%.

10. Results

10.1 Subject Demographics and Disposition

Table 2-GS-US-216-113 subject demographics

Characteristic	Cohort 1 (300 mg) n = 12	Cohort 2 (400 mg) n = 12
Age (years)		
Mean (SD)	33 (6.6)	29 (6.8)
Median	35	28
Min, Max	21, 45	19, 45
Sex (n, %)		
Male	6 (50.0%)	6 (50.0%)
Female	6 (50.0%)	6 (50.0%)
Race (n, %)		
White	9 (75.0%)	10 (83.3%)
Black	3 (25.0%)	2 (16.7%)
Ethnicity (n, %)		
Hispanic/Latino	12 (100.0%)	12 (100.0%)
Non-Hispanic/Latino	0	0
Height at Screening (centimeters)		
Mean (SD)	166 (8.5)	162 (10.8)
Median	164	162
Min, Max	154, 179	139, 179
Weight at Screening (kg)		
Mean (SD)	72.8 (13.64)	66.3 (12.41)
Median	75.6	64.4
Min, Max	52.8, 92.0	48.5, 88.2
Body Mass Index at Screening (kg/m^2)[a]		
Mean (SD)	26.2 (2.82)	25.1 (2.71)
Median	27.5	25.1
Min, Max	21.3, 29.2	20.4, 29.1
Estimated Creatinine Clearance (mL/minute)[b]		
Mean (SD)	127.9 (20.54)	130.8 (26.59)
Median	128.2	124.9
Min, Max	91.3, 158.2	84.7, 174.5

SD = standard deviation

a   Body Mass Index = (weight [kg]/height [cm]^2) × 10,000

b   calculated by the Cockcroft-Gault equation {2202} using actual body weight.

Figure 4-GS-US-216-113 subject disposition
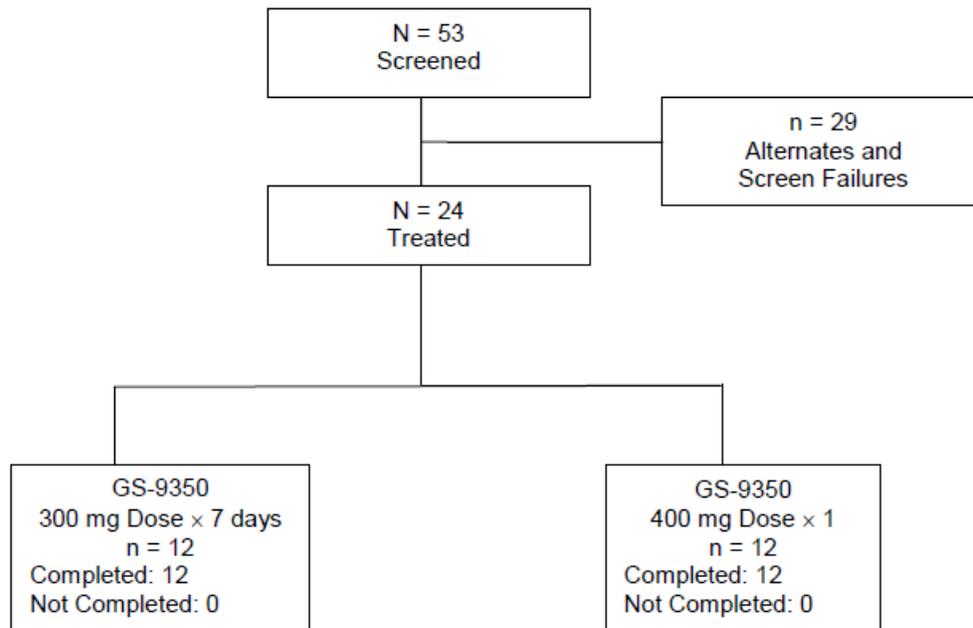
10.2 Concomitant Medications

No subjects received concomitant medications during the trial.

10.3 Pharmacokinetic and Statistical Analysis

There were no subjects with quantifiable predose cobicistat concentrations on Day 1 in either cohort 1 or cohort 2.

Comparison of the pharmacokinetics for 300 mg of cobicistat (Day 1) and the
single dose pharmacokinetics for 400 mg of cobicistat

The mean plasma-concentration time profiles for 300 mg of cobicistat (Day 1)
and a single 400 mg dose of cobicistat are displayed in Figure 5. The
pharmacokinetic parameters for 300 mg of cobicistat on Day 1 and a single 400
mg dose of cobicistat ae displayed in Table 3 and the statistical analyses
comparing 300 mg of cobicistat on day 1 to a single 400 mg dose of cobicistat
are displayed in Table 4.

Based on the comparison of the pharmacokinetics for 300 mg of cobicistat (day
1) and the single dose pharmacokinetics for 400 mg of cobicistat, greater than
dose proportional changes in cobicistat exposure were observed for both C_{max}
and AUC. However, the differences in the total duration of sampling (24 hours
for 300 mg cobicistat [day 1] dosing versus 48 hours for a single 400 mg dose of
cobicistat) also contributes to the observed difference in AUC values and a direct
comparison of the cobicistat elimination half life can not be made.

**Figure 5-Mean plasma-concentration time profiles for 300 mg of cobicistat
(day 1) and a single 400 mg dose of cobicistat**
*(Note: for cobicistat 300 mg [Day 1], samples were collected up to 24 hours, and
for a single 400 mg dose, samples were collected up to 48 hours)*
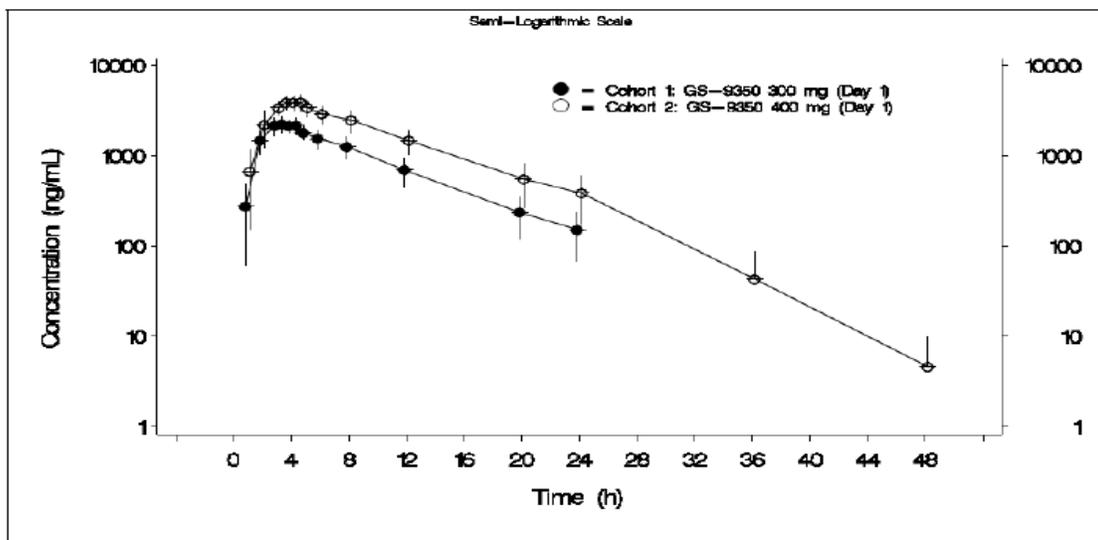
Values presented as mean ± standard deviation. The PK analysis set includes 24 subjects. Plasma concentrations BLQ were
treated as 0 for summary purposes and were excluded for log-normalized data.

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Table 3-Pharmacokinetic parameters for 300 mg of cobicistat (day 1) and a single 400 mg dose of cobicistat

GS-9350 PK Parameter	Cohort 1 (300 mg) n = 12	Cohort 2 (400 mg) n = 12
AUC_{inf} (ng•h/mL), mean (% CV)	20772.5 (27.7)	39898.6 (26.2)
C_{max} (ng/mL), mean (% CV)	2338.5 (16.9)	4113.0 (17.2)
T_{max} (h), median (Q1, Q3)	3.75 (3.50, 4.50)	4.25 (3.75, 4.50)
C_{last} (ng/mL), mean (% CV)	152.4 (55.5)	14.2 (58.3)
T_{last} (h), median (Q1, Q3)	23.95 (23.95, 23.95)	42.00 (36.00, 48.00)
$T_{\frac{1}{2}}$ (h), median (Q1, Q3)	5.20 (4.25, 5.83)	4.75 (4.13, 4.93)
CL/F (ml/hr), mean (% CV)	15525.9 (28.8)	10591.5 (23.2)

CV = coefficient of variation, h = hour(s), Q1 = first quartile, Q3 = third quartile

Table 4-Statistical analyses for 300 mg of cobicistat (day 1) and a single 400 mg dose of cobicistat

GS-9350 PK Parameter	Geometric Least Squares Means[a] Test[b] (n = 12)	Reference[b] (n = 12)	GLS Means Ratio: Test/Reference (%)	90% CI
AUC_{inf} (ng•h/mL)	38774.29	20043.37	193.45	160.78, 232.76
C_{max} (ng/mL)	4056.11	2306.14	175.88	155.39, 199.08

CI = confidence interval, h = hour(s)

a Geometric least squares (GLS) means were obtained by PROC MIXED model

b Test = GS-9350 400 mg , Reference = GS-9350 300 mg

Comparison of the pharmacokinetics for 300 mg of cobicistat on day 1, day 4 and day 7

The mean multiple dosing plasma-concentration time profiles for 300 mg of cobicistat on day 4 and day 7 are displayed in Figure 6. The pharmacokinetic parameters for 300 mg of cobicistat on Day 1, 4, and 7 are displayed in Table 5 and the statistical analyses comparing 300 mg of cobicistat on day 7 to day 4, day 4 to day 1, and day 7 to day 1 are displayed in Table 6.

Based on the comparison of the pharmacokinetics for 300 mg of cobicistat on day 7 and day 4, there was no significant difference in the C_{max} or AUC values but the C_{tau} value was higher on day 7. In general, it appears that steady state was achieved for cobicistat by day 4. In comparing the day 4 to day 1 and day 7 to day 1 cobicistat parameters, higher cobicistat exposure were observed for both C_{max} and AUC on days 4 and 7 as well as lower cobicistat clearance. This information supports the fact that cobicistat demonstrates nonlinear pharmacokinetics with respect to time.

A more appropriate comparison for changes in exposure and clearance would have evaluated the differences between the single dose $AUC_{(0-inf)}$ and the steady state AUC values for 300 mg of cobicistat instead of comparing Day 1 AUC and steady state AUC values for 300 mg. However, the mean percentage of the 300 mg Day 1 AUC that was extrapolated was 5.3%. The single dose $AUC_{(0-inf)}$ for 300 mg of cobicistat was not characterized for this trial.

Figure 6-Mean plasma-concentration time profiles for 300 mg of cobicistat on day 4 and day 7
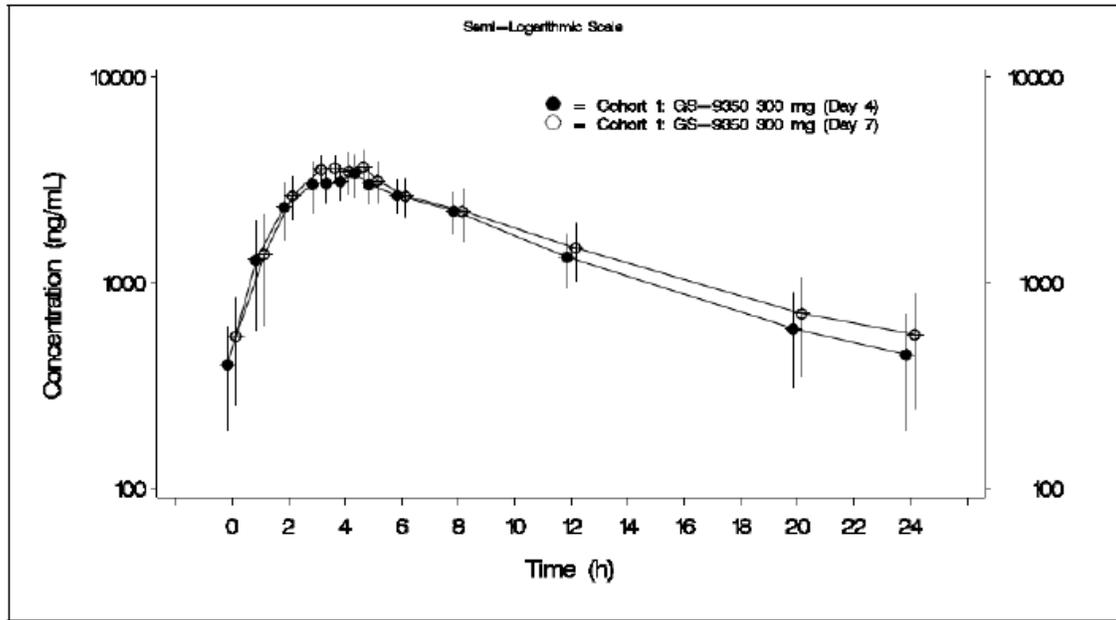
Values presented as mean ± standard deviation. The PK analysis set includes 24 subjects. Plasma concentrations BLQ were treated as 0 for summary purposes and were excluded for log-normalized data.

Table 5-Pharmacokinetic parameters for 300 mg of cobicistat on day 1 and day 4

GS-9350 PK Parameter	Day 1 (n = 12)	Day 4 (n = 12)	Day 6 Trough (n = 12)	Day 7 (n = 12)
AUC^a (ng•h/mL), mean (% CV)	20772.5 (27.7)	35798.7 (26.8)		39124.7 (27.6)
C_{max} (ng/mL), mean (% CV)	2338.5 (16.9)	3509.8 (18.9)		3837.3 (16.7)
T_{max} (h) median (Q1, Q3)	3.75 (3.50, 4.50)	4.50 (3.75, 4.50)		4.00 (3.25, 4.50)
C_{last}^b (ng/mL), mean (% CV)	152.4 (55.5)	449.1 (57.2)	469.8 (54.0)	563.3 (57.0)
T_{last} (h), median (Q1, Q3)	23.95 (23.95, 23.95)	24.00 (24.00, 24.00)		24.00 (24.00, 24.00)
$T_{½}$ (h), median (Q1, Q3)	5.20 (4.25, 5.83)	6.28 (5.35, 8.44)		8.07 (5.89, 9.01)
CL/F (mL/h), mean (% CV)	15525.9 (28.8)	9007.3 (28.7)		8281.9 (29.9)

CV = coefficient of variation, h = hour(s), Q1 = first quartile, Q3 = third quartile.

a   AUC represents AUC_{inf} for Day 1 and AUC_{tau} for Days 4 and 7.

b C_{tau} for Days 4 and 7.

Table 6-Statistical analyses for 300 mg of cobicistat comparing 300 mg of cobicistat on day 7 to day 4, day 4 to day 1 and day 7 to day 1

GS-9350 PK Parameter	Geometric Least Squares Means[a]		GLS Means Ratio: Test/Reference (%)	90% CI
Day 7/Day 4[b]	Test (n = 12)	Reference (n = 12)		
AUC$_{tau}$ (ng•h/mL)	37685.88	34558.76	109.05	105.65, 112.56
C$_{max}$ (ng/mL)	3787.49	3447.91	109.85	105.90, 113.95
C$_{tau}$ (ng/mL)	467.16	374.02	124.90	111.27, 140.21
Day 4/Day 1[c]	Test (n = 12)	Reference (n = 12)		
AUC[d] (ng•h/mL)	34558.76	20043.37	172.42	155.00, 191.79
C$_{max}$ (ng/mL)	3447.91	2306.14	149.51	139.57, 160.16
Day 7/Day 1[e]	Test (n = 12)	Reference (n = 12)		
AUC[d] (ng•h/mL)	37685.88	20043.37	188.02	167.02, 211.67
C$_{max}$ (ng/mL)	3787.49	2306.14	164.24	153.80, 175.38

CI = confidence interval, h = hour(s)

a   Geometric least squares (GLS) means were obtained by PROC MIXED model.

b   Test = GS-9350 300 mg (Day 7), Reference = GS-9350 300 mg (Day 4).

c   Test = GS-9350 300 mg (Day 4), Reference = GS-9350 300 mg (Day 1).

d   AUC represents AUC$_{inf}$ for Day 1 and AUC$_{tau}$ for Days 4 and 7.

e   Test = GS-9350 300 mg (Day 7), Reference = GS-9350 300 mg (Day 1).

GS-9350 PK Parameter			
Day 7/Day 4[a]	Test (n = 12)	Reference (n = 12)	p-value[b]
T$_{½}$ (h), mean (% CV)	7.6 (27.0)	6.7 (24.9)	0.29
CL/F (mL/hr), mean (% CV)	8281.9 (29.9)	9007.3 (28.7)	0.29
Day 4/Day 1[c]	Test (n = 12)	Reference (n = 12)	p-value
T$_{½}$ (h), mean (% CV)	6.7 (24.9)	5.1 (17.8)	<0.001
CL/F (mL/hr), mean (% CV)	9007.3 (28.7)	15525.9 (28.8)	0.037
Day 7/Day 1[d]	Test (n = 12)	Reference (n = 12)	p-value
T$_{½}$ (h), Mean (% CV)	7.6 (27.0)	5.1 (17.8)	<0.001
CL/F (mL/hr), mean (% CV)	8281.9 (29.9)	15525.9 (28.8)	0.002

CV = coefficient of variation, h = hour(s)

a   Test = GS-9350 300 mg (Day 7), Reference = GS-9350 300 mg (Day 4).

b   p-values were obtained from Wilcoxon signed rank exact test.

c   Test = GS-9350 300 mg (Day 4), Reference = GS-9350 300 mg (Day 1).

d   Test = GS-9350 300 mg (Day 7), Reference = GS-9350 300 mg (Day 1).

11. Discussion and Conclusions

Based on the results from the GS-US-216-113 trial, the following conclusions can be made:

- In comparing the pharmacokinetics for 300 mg of cobicistat (day 1) and the single dose pharmacokinetics for 400 mg of cobicistat, greater than dose proportional changes in cobicistat exposure were observed for both C_{max} (75.88 % higher) and AUC (93.45% higher). The differences in the total duration of sampling for the two arms also contributed to the observed difference in AUC values.
- In general, it appears that steady state was achieved for cobicistat by day 4. In comparing the pharmacokinetics for 300 mg of cobicistat on day 7 and day 4, there was no significant difference in the C_{max} or AUC values (increased by 9.85% and 9.05%, respectively on day 7) compared to day 4. The C_{tau} value was higher on day 7 compared to day 4 (increased by 24.9%).
- In comparing the day 4 to day 1 and day 7 to day 1 cobicistat parameters, higher cobicistat exposure were observed for both C_{max} and AUC on days 4 and 7 as well as lower cobicistat clearance. On day 4, C_{max} and AUC values were increased by 49.51% and 72.42%, respectively, and clearance was decreased by 42% compared to day 1. On Day 7, C_{max} and AUC values were increased by 64.24% and 88.02%, respectively, and clearance was decreased by 47% compared to Day 1. This information supports the fact that cobicistat demonstrates nonlinear pharmacokinetics with respect to time.
- More definitive conclusions regarding the nonlinear pharmacokinetics with respect to time for cobicistat could not be made because the differences between the single dose $AUC_{(0-inf)}$ and the steady state AUC values for 300 mg of cobicistat were not evaluated for this trial.

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1. Title

A Phase 1 Multiple Dose Study to Evaluate Two Formulations of GS-9350 Tablets and the Pharmacokinetics of Elvitegravir Tablets administered with GS-9350 Tablets

2. Information Regarding the Clinical Trial Site and Duration of the Trial

The trial was conducted at SeaView Research, Miami, FL. The trial was conducted from August 3, 2009 (first subject screened) to October 5, 2009 (last subject observation).

3. Objectives

The objective of the trial was to compare the pharmacokinetics of two cobicistat formulations (Formulation 1 and Formulation 2) and the multiple dose pharmacokinetics of elvitegravir using either ritonavir or cobicistat to increase plasma concentrations. At the time the trial was conducted, Formulation 1 was the cobicistat formulation that was studied in the Phase 2 trials, and Formulation 2 was a new cobicistat formulation.

4. Trial Design

The trial was a randomized, open label multiple dose trial in two parallel cohorts (cohort 1 and cohort 2). The trial design for cohort 1 and cohort 2 is displayed in Figure 1 and Figure 2. Cohort 1 evaluated the two cobicistat formulations and cohort 2 evaluated evitegravir using either ritonavir or cobicistat to increase plasma concentrations. There was no washout period between the two arms in each cohort.

Figure 1-GS-US-216-116 cohort 1 trial design
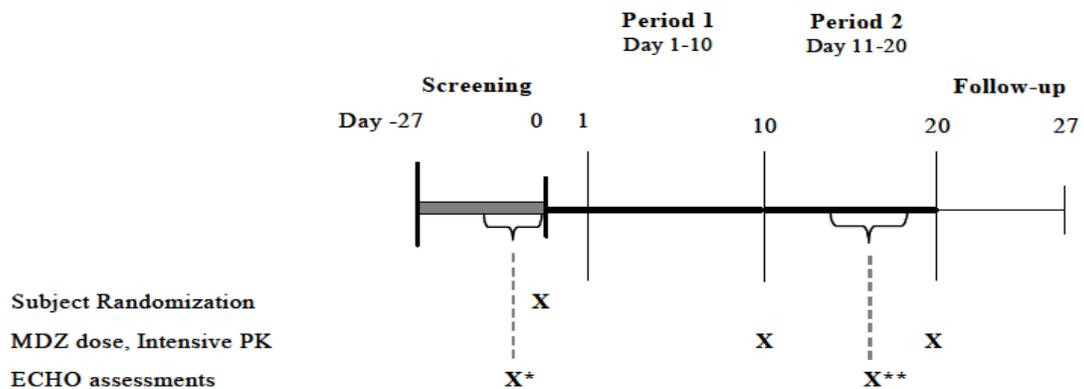
* 1 echocardiogram/ electrocardiogram performed within 7 days prior to Day 1.
** 1 echocardiogram/ electrocardiogram performed on Day 14, 15, 16, 17, 18, or 19.

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Figure 2-GS-US-216-116 cohort 2 trial design
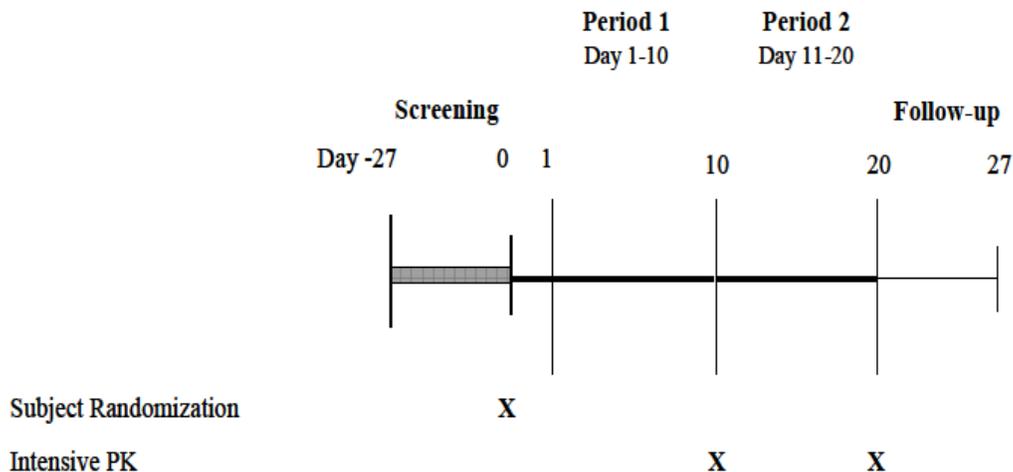
5. Medication Restrictions and Exceptions

Use of vitamins, acetaminophen, ibuprofen, oral contraceptives, and topical hydrocortisone cream or A&D ointment to treat skin irritation from ECG leads was permitted during the trial. All other prescription and nonprescription medications, including herbal medications, were not permitted during the trial.

6. Dosage and Administration

Within each cohort, subjects were randomized to a specific treatment sequence (AB or BA for cohort 1, and CD or DC for cohort 2).

In cohort 1, subjects received the following two treatments for ten days: a) formulation 2-cobicistat 150 mg once daily (treatment A), and b) formulation 1-cobicistat 150 mg once daily (treatment B). 5 mg of midazolam was also administered on days 10 and 20. In cohort 2, subjects received the following two treatments for ten days: a) elvitegravir 150 mg coadministered with formulation 2-cobicistat 150 mg once daily (treatment C), and b) elvitegravir 150 mg coadministered with ritonavir 100 mg once daily (treatment D).

Trial medications were administered within 5 minutes of completion of a standardized meal. Subjects fasted overnight for a minimum of 8 hours prior to days 10 and 20. Water was not permitted from one hour before dosing to two hours after dosing, except with the 240 mL of water that was provided with the trial medication.

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7. Rationale for Doses Used in the Trial

The selected cobicistat dosage regimen for the GS-US-216-116 trial, 150 mg once daily, is the proposed dosage regimen for cobicistat when combined with elvitegravir (cobicistat is utilized to achieve therapeutic concentrations of elvitegravir).

The selected elvitegravir dosage regimen for the GS-US-216-116 trial, 150 mg once daily, is the proposed dosage regimen for elvitegravir when combined with cobicistat.

The selected ritonavir dosage regimen for the GS-US-216-116 trial, 100 mg once daily, is used to increase the systemic exposure of concurrently administered HIV-1 protease inhibitors such as darunavir or atazanavir.

There was no specific rationale stated for selecting a 5 mg oral midazolam dose. However, the GS-216-101 trial report states that a 5 mg oral midazolam dose is commonly used in trials where midazolam is used as a CYP3A substrate.

8. Drugs Used in the Trial

A summary of the cobicistat, ritonavir, elvitegravir and midazolam formulations that were administered in the GS-US-216-116 trial is displayed in Table 1 below.

Table 1-Cobcistat, ritonavir, midazolam and elvitegravir formulations administered in the GS-US-216-116 trial

	GS-9350 Tablet Form. 1 and 2	MDZ Syrup	EVG Tablet	RTV Capsule
Strength (mg)	150	5	150	100
Lot No.	BB0902A1, BB0904B1	8454780	AJ0705E1	706752E1
Expiration Date	April 2011, July 2011	October 2010	November 2010	December 2010
Manufacturer/ Supplier	Gilead Sciences, Inc. Foster City, CA 94404	(b) (4)	Gilead Sciences, Inc. Foster City, CA 94404	Abbott Laboratories Chicago, IL 60064
Site of Release in Europe	Not applicable	Not applicable	Not applicable	Not applicable

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9. Sample Collection, Bioanalysis, Pharmacokinetic Assessments, and Statistical Analysis

Sample Collection

On days 10 and 20, blood samples for analysis of cobicistat, ritonavir, elvitegravir and midazolam and 1-hydroxymidazolam plasma concentrations were collected up to 24 hours.

Bioanalysis

Reviewer note: only the method and bioanalysis of cobicistat are discussed below.

With the exception of long term stability, the method and bioanalysis of the cobicistat analyte are acceptable.

Blood samples for the GS-US-216-116 trial for the cobicistat elvitegravir, ritonavir and midazolam analytes were collected in tubes containing K_2EDTA as the anticoagulant according to the bioanalytical reports for the respective analytes.

For the method analyzing cobicistat in combination with elvitegravir, cobicistat plasma samples were analyzed using a validated LC/MS/MS method with K_2EDTA anticoagulated plasma by ⬛ (b) (4) The lower limit of quantification for cobicistat was 5 ng/mL and the upper limit of quantification was 2500 ng/mL.

For the GS-US-216-116 trial, cobicistat precision and accuracy were evaluated using the low (15 ng/mL), medium (200 ng/mL), and high (2000 ng/mL) QC samples. There were no dilution QCs analyzed in the analytical runs. Two separate bioanalytical reports using the same validated method were generated: one for treatments A and B and a second one for treatment C. There were no precision or accuracy issues identified for cobicistat based on the bioanalytical reports. For the analysis of samples from treatments A and B, out of the twenty two analytical runs where cobicistat samples were analyzed, one run was not completed and for the rest of the runs, there were no analytical runs that failed for the cobicistat analysis. However, there were multiple runs where one of the low cobicistat QCs failed or two or more calibration curve standards were not accepted. For treatments A and B, the cobicistat inter-run accuracy values were -0.3% for the low QCs, -6.5% for the medium QCs, and -4.2% for the high QCs. The cobicistat inter-run precision values were 17.1% for the low QCs, 5.1% for the medium QCs, and 5% for the high QCs. For the analysis of samples from treatment C, out of the ten analytical runs where cobicistat samples were analyzed, two analytical runs failed for the cobicistat analysis. Both failed runs analyzed the same set of samples. The reasons for the run failures were included in the bioanalytical report but did not specify whether any specific corrective actions were made. The first run (run 8) was rejected ⬛ (b) (4) ⬛ and the same samples were reanalyzed in run 10 that was also rejected ⬛ (b) (4)

(b) (4) The samples were reanalyzed again in run 11 which was accepted. For treatments C, the cobicistat inter-run accuracy values were -5.5% for the low QCs, -7.5% for the medium QCs, and -5.2% for the high QCs. The cobicistat inter-run precision values were 8.4% for the low QCs, 6.3% for the medium QCs, and 6.2% for the high QCs.

For treatments A and B, a total of 111 samples were selected for incurred sample reanalysis. Of these 111 samples, 4 samples were not within 20% of the original value. For treatment C, a total of 40 samples were selected for incurred sample reanalysis. Of these 40 samples, 6 samples were not within 20% of the original value. However, it is not clear whether the total number of samples that were reanalyzed represents 5% to 10% of the total number of samples that were initially analyzed.

(b) (4) did not conduct long term stability experiments for cobicistat. Instead, (b) (4) references the long term stability data for cobicistat that was generated by (b) (4). The cobicistat analytical method that was used by (b) (4) is not identical to the cobicistat analytical method used by (b) (4). The submitted cobicistat long term sample stability data demonstrated that cobicistat was stable for 121 days at -10°C to -30°C and 365 days at -60°C to -80°C in K_2EDTA anticoagulated plasma. At the (b) (4) bioanalytical laboratory, subject samples from the GS-US-216-116 trial were stored at -70°C and the bioanalytical report states that subject samples were also stored at -70°C at the trial site until they were shipped to (b) (4). Overall, while the submitted cobicistat long term stability data of 365 days at-70°C covers the duration of long term cobicistat stability data necessary for treatments A , B, and C for the GS-US-216-116 trial, the impact of the differences in the cobicistat analytical methods used by (b) (4) compared to (b) (4) is unknown.

One additional issue that was identified based on the method validation results for cobicistat was the (b) (4)

Pharmacokinetic Assessments

Noncompartmental analysis was performed to calculate cobicistat, ritonavir, elvitegravir and midazolam and 1-hydroxymidazolam plasma pharmacokinetic parameters, including C_{tau}, C_{max}, and $AUC_{(0-\tau)}$.

Statistical Analysis

Descriptive statistics were calculated for cobicistat, ritonavir, elvitegravir and midazolam and 1-hydroxymidazolam plasma concentrations and pharmacokinetic parameters, including the number of subjects (n), mean, standard deviation, the coefficient of variation (CV%), median, and the minimum and maximum values. The geometric mean was also calculated for the individual pharmacokinetic parameters.

Statistical analysis involved comparison of cobicistat, ritonavir, elvitegravir and midazolam and 1-hydroxymidazolam natural log transformed pharmacokinetic parameters for the relevant test arms (treatments A and C) compared to the reference arms (treatments B and D) and 90% confidence intervals were derived. Bioequivalence was concluded if the 90% confidence intervals were within 80%-125%. A second analysis was also conducted to determine if there was no difference in the carryover effect between the trial arms. A p value of less than 0.05 was needed to conclude that the carryover effect was not equal between the trial arms.

The Wilcoxon rank sum test was used to compare the clearance and half life values for cobicistat, midazolam and 1-hydroxymidazolam in cohort 1 and elvitegravir in cohort 2.

10. Results

10.1 Subject Demographics and Disposition

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Table 2-GS-US-216-116 subject demographics

Characteristic	Cohort 1 (N = 38)	Cohort 2 (N = 24)
Sex (n, %)		
Male	19 (50%)	13 (54.2%)
Female	19 (50%)	11 (45.8%)
Age at baseline (years)		
Mean (SD)	35 (7.7)	35 (6.3)
Median (Q1, Q3)	37 (30, 42)	36 (30, 41)
Min, Max	19, 45	25, 44
Ethnicity (n, %)		
Hispanic/Latino	37 (97.4%)	23 (95.8%)
Non-Hispanic/Latino	1 (2.6%)	1 (4.2%)
Race (n, %)		
White	35 (92.1%)	20 (83.3%)
Black	3 (7.9%)	4 (16.7%)
Weight (kg)		
Mean (SD)	73.6 (12.81)	70.8 (11.75)
Median (Q1, Q3)	72.9 (62.0, 83.0)	69.7 (62.9, 79.5)
Min, Max	51.3, 99.2	53.2, 100.0
Height (cm)		
Mean (SD)	168.2 (10.16)	167.1 (9.78)
Median (Q1, Q3)	170.5 (161.5, 176.0)	167.0 (159.0, 174.5)
Min, Max	144.0, 184.0	149.5, 183.5
BMI (kg/m^2)^a		
Mean (SD)	25.8 (2.50)	25.3 (2.93)
Median (Q1, Q3)	26.2 (23.7, 27.8)	25.8 (22.9, 27.8)
Min, Max	20.9, 29.6	20.1, 29.7
Serum creatinine clearance (mL/min)^b		
Mean (SD)	128.6 (18.95)	123.9 (18.92)
Median (Q1, Q3)	128.2 (115.2, 143.5)	123.3 (111.9, 135.9)
Min, Max	79.4, 162.0	83.6, 166.7

BMI: body mass index, SD: standard deviation, Q1: first quartile, Q3: third quartile
a   BMI = (weight [kg]/height [m]^2)

Figure 3-GS-US-216-116 subject disposition
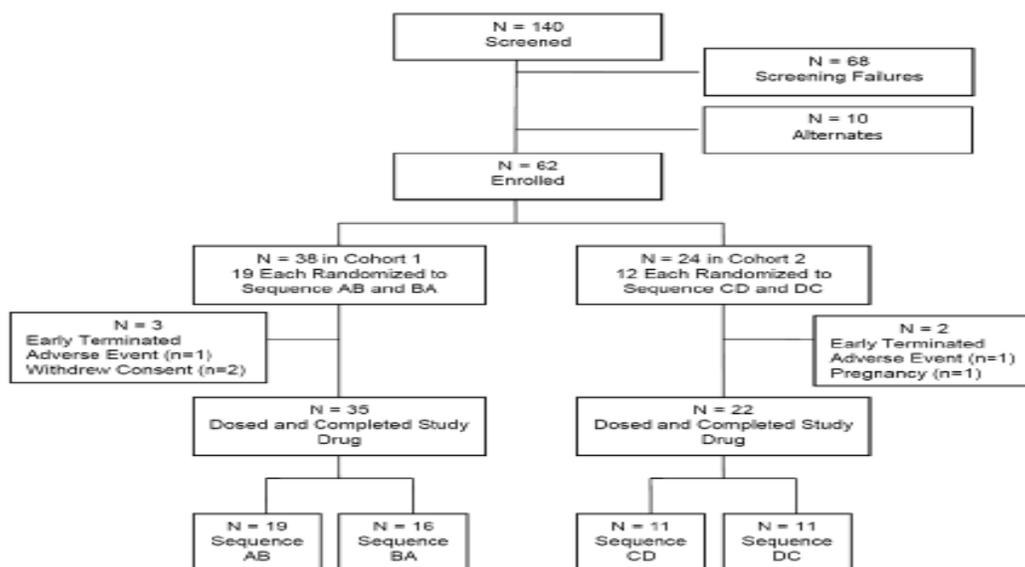
10.2 Concomitant Medications

Subjects received a variety of concomitant medications during the trial including ibuprofen. None of the administered concomitant medications are anticipated to significantly impact the results of the trial.

10.3 Pharmacokinetic and Statistical Analysis

Carry over analysis for cohort 1 and cohort 2

For both cohort 1 and cohort 2, the carryover over analysis did not result in p values of less than 0.05 for any of the trial arm comparisons. Therefore, the lack of a washout period between trial arms did not appear to impact the results of the trial's results.

Comparison of cobicistat pharmacokinetic data for formulation 1 and formulation 2

The pharmacokinetic parameters for cobicistat 150 mg once daily for formulation 1 and formulation 2 are displayed in Table 3.

Based on the comparison of the pharmacokinetic data for formulation 1 and formulation 2 in Table 4, bioequivalence was demonstrated for cobicistat C_{max}, C_{tau}, and $AUC_{(0-tau)}$.

Table 3-Pharmacokinetic parameters for cobicistat 150 mg on daily (formulation 1 and formulation 2)

GS-9350 PK Parameter (N = 35)	GS-9350 Formulation 2	GS-9350 Formulation 1
AUC$_{tau}$ (ng·h/mL)	12,429.5 (30.6)	12,577.3 (28.5)
C$_{max}$ (ng/mL)	1597.6 (21.7)	1578.5 (22.0)
C$_{tau}$ (ng/mL)	82.5 (81.9)	80.0 (69.9)
T$_{max}$ (h)	4.50 (3.50, 4.50)	4.50 (4.00, 4.50)
T$_{1/2}$ (h)	4.53 (3.98, 5.62)	4.77 (3.95, 5.69)

CV: coefficient of variation, PK: pharmacokinetic, Q1: first quartile, Q3: third quartile
Data are presented as mean (%CV), except for T$_{max}$ and T$_{½}$, which are presented as median (Q1, Q3).

Table 4-Statistical analyses for cobicistat 150 mg once daily (formulation 1 and formulation 2)

PK Parameter (N = 35)	GLSMs		GLSM Ratio (%) (90% CI)
	Test Treatment: GS-9350 Formulation 2	Reference Treatment: GS-9350 Formulation 1	
AUC$_{tau}$ (ng·h/mL)	11,788.86	12,004.30	98.21 (94.61, 101.94)
C$_{tau}$ (ng/mL)	58.29	60.52	96.31 (87.12, 106.47)
C$_{max}$ (ng/mL)	1557.73	1538.26	101.27 (97.47, 105.21)

CI: confidence interval, GLSM: geometric least-squares mean, PK: pharmacokinetic
GLSMs were obtained using a mixed-effects model. The model included treatment, sequence, and period as fixed effects, and subject-within-sequence as a random effect.

<u>Comparison of midazolam and 1-hydroxymidazolam pharmacokinetic data for formulation 1 and formulation 2</u>

The pharmacokinetic parameters for midazolam and 1-hydroxymidazolam when 5 mg of midazolam was coadministered with either cobicistat formulation 1 or cobicistat formulation 2 are displayed in Table 5 and 7, respectively. For 1-hydroxymidazolam, the plasma concentrations were converted to pg/mL. It was noted that the 1-hydroxymidazolam concentration were clustered around the lower end of the calibration curve range, however, a revised method was not developed to reflect the lower observed concentrations.

Based on the comparison of the midazolam and 1-hydroxymidazolam pharmacokinetic data when midazolam was coadministered with either cobicistat formulation 1 or cobicistat formulation 2 in Table 6 and Table 8, bioequivalence was demonstrated for midazolam and 1-hydroxymidazolam C$_{max}$, C$_{tau}$, and AUC$_{(0-tau)}$. Therefore, no clinically significant differences in the CYP3A inhibition effects were demonstrated in comparing the two cobcistat formulations.

Table 5-Midazolam pharmacokinetic parameters for 5 mg of midazolam when coadministered with either cobicistat formulation 1 or cobicistat formulation 2

MDZ PK Parameter (N = 35)	GS-9350 Formulation 2	GS-9350 Formulation 1
AUC_{inf} (ng·h/mL)	1181.4 (28.9)	1219.9 (32.3)
AUC_{last} (ng·h/mL)	617.1 (13.5)	624.6 (14.9)
C_{max} (ng/mL)	51.0 (13.7)	52.6 (16.8)
C_{last} (ng/mL)	16.4 (19.1)	17.2 (23.9)
T_{max} (h)	2.00 (2.00, 3.50)	3.00 (2.00, 4.00)
$T_{1/2}$ (h)	22.14 (17.64, 26.28)	21.33 (17.60, 24.78)

CV: coefficient of variation, MDZ: midazolam, PK: pharmacokinetic, Q1: first quartile, Q3: third quartile

Data are presented as mean (%CV), except for T_{max} and $T_{1/2}$, which are presented as median (Q1, Q3).

Table 6-Midazolam statistical analyses for 5 mg of midazolam when coadministered with either cobicistat formulation 1 or cobicistat formulation 2

PK Parameter (N = 35)	GLSMs		GLSM Ratio (%) (90% CI)
	Test Treatment: GS-9350 Formulation 2	Reference Treatment: GS-9350 Formulation 1	
AUC_{inf} (ng·h/mL)	1137.27	1167.79	97.39 (92.67, 102.35)
AUC_{last} (ng·h/mL)	610.57	619.03	98.63 (97.40, 99.88)
C_{max} (ng/mL)	50.45	52.14	96.76 (93.59, 100.04)
C_{last} (ng/mL)	16.12	16.70	96.51 (93.79, 99.31)

CI: confidence interval, GLSM: geometric least-squares mean, MDZ: midazolam, PK: pharmacokinetic

GLSMs were obtained using a mixed-effects model. The model included treatment, sequence, and period as fixed effects, and subject-within-sequence as a random effect.

Table 7-1-hydroxymidazolam pharmacokinetic parameters for 5 mg of midazolam when coadministered with either cobicistat formulation 1 or cobicistat formulation 2

1-OH MDZ PK Parameter	GS-9350 Formulation 2	GS-9350 Formulation 1
AUC_{inf} (pg·h/mL)	11,069.8 (26.9)	10,766.1 (29.3)
AUC_{last} (pg·h/mL)	6092.9 (36.8)	5872.6 (32.0)
C_{max} (pg/mL)	579.6 (32.1)	540.2 (27.9)
C_{last} (pg/mL)	199.1 (66.2)	198.9 (64.5)
T_{max} (h)	2.00 (2.00, 3.50)	2.00 (2.00, 3.53)
$T_{1/2}$ (h)	11.60 (10.42, 14.45)	14.28 (11.08, 16.64)

CV: coefficient of variation, 1-OH MDZ: 1-hydroxymidazolam, PK: pharmacokinetic, Q1: first quartile, Q3: third quartile
Sample size was 35 subjects, except for AUC_{inf} and $T_{1/2}$, which each had 8 subjects for Formulation 2 and 11 subjects for Formulation 1.

Data are presented as mean (%CV), except for T_{max} and $T_{1/2}$, which are presented as median (Q1, Q3).

Table 8-1-hydroxymidazolam statistical analyses for 5 mg of midazolam when coadministered with either cobicistat formulation 1 or cobicistat formulation 2

| PK Parameter | GLSMs | | GLSM Ratio (%) (90% CI) |
	Test Treatment: GS-9350 Formulation 2	Reference Treatment: GS-9350 Formulation 1	
AUC_{inf} (pg·h/mL)	9660.98	10,065.20	95.98 (90.16, 102.18)
AUC_{last} (pg·h/mL)	5725.51	5602.66	102.19 (97.35, 107.27)
C_{max} (pg/mL)	552.71	521.02	106.08 (100.29, 112.20)
C_{last} (pg/mL)	180.16	179.75	100.23 (95.01, 105.73)

CI: confidence interval, GLSM: geometric least squares mean, 1-OH MDZ: 1-hydroxymidazolam, PK: pharmacokinetic
Sample size was 35 subjects, except for AUC_{inf}, which had 8 subjects for Formulation 2 and 11 subjects for Formulation 1.
GLSMs were obtained using a mixed-effects model. The model included treatment, sequence, and period as fixed effects, and subject-within-sequence as a random effect.

<u>Comparison of elvitegravir pharmacokinetic data when coadministered with formulation 2-cobicistat 150 mg once daily or ritonavir 100 mg once daily</u>

The pharmacokinetic parameters for elvitegravir when coadministered with formulation 2-cobicistat 150 mg once daily or ritonavir 100 mg once daily are displayed in Table 9.

Based on the comparison of the elvitegravir pharmacokinetic data coadministered with formulation 2-cobicistat 150 mg once daily or ritonavir 100 mg once daily in Table 10, bioequivalence was demonstrated for elvitegravir C_{max}, C_{tau}, and $AUC_{(0-tau)}$. Therefore, in comparing the CYP3A inhibition effects for cobicistat 150 mg once daily or ritonavir 100 mg once daily, no clinically significant differences in elvitegravir exposure were observed.

Table 9-Pharmacokinetic parameters for elvitegravir 150 mg once daily when coadministered with formulation 2-cobicistat 150 mg once daily or ritonavir 100 mg once daily

EVG PK Parameter (N = 22)	EVG/GS-9350 Formulation 2	EVG/r
AUC_{tau} (ng·h/mL)	22,246.5 (18.2)	20,270.3 (23.1)
C_{max} (ng/mL)	2253.0 (18.4)	2048.3 (24.1)
C_{tau} (ng/mL)	379.4 (40.7)	397.2 (38.0)
T_{max} (h)	4.00 (3.98, 4.50)	4.00 (3.50, 4.00)
$T_{1/2}$ (h)	8.28 (7.30, 12.19)	11.64 (9.99, 14.39)

CV: coefficient of variation, EVG: elvitegravir, PK: pharmacokinetic, Q1: first quartile, Q3: third quartile, /r: boosted with ritonavir
Data are presented as mean (%CV), except for T_{max} and $T_{1/2}$, which are presented as median (Q1, Q3).

Table 10-Statistical analyses for elvitegravir 150 mg once daily when coadministered with formulation 2-cobicistat 150 mg once daily or ritonavir 100 mg once daily

PK Parameter (N = 22)	GLSMs		GLSM Ratio (%) (90% CI)
	Test Treatment: EVG/GS-9350 Formulation 2	Reference Treatment: EVG/r	
AUC$_{tau}$ (ng·h/mL)	21,905.69	19,794.22	110.67 (104.47, 117.23)
C$_{tau}$ (ng/mL)	349.53	372.90	93.73 (81.40, 107.93)
C$_{max}$ (ng/mL)	2215.92	1991.58	111.26 (103.30, 119.85)

CI: confidence interval, EVG: elvitegravir, GLSM: geometric least-squares mean, PK: pharmacokinetic, /r: boosted with ritonavir

GLSMs were obtained using a mixed-effects model. The model included treatment, sequence, and period as fixed effects, and subject-within-sequence as a random effect.

<u>Ritonavir pharmacokinetic data when coadministered with elvitegravir 150 mg once daily</u>

The pharmacokinetic parameters for ritonavir 100 mg once daily when coadministered with elvitegravir 150 mg once daily are displayed in Table 11. The applicant did not provide comparative ritonavir exposure data when coadministered with elvitegravir 150 mg once daily from other trials.

Table 11-Pharmacokinetic parameters for ritonavir 100 mg once daily with elvitegravir 150 mg once daily

RTV PK Parameter (N = 23)	EVG/r
AUC$_{tau}$ (ng·h/mL)	4942.8 (27.4)
C$_{max}$ (ng/mL)	908.1 (32.2)
C$_{tau}$ (ng/mL)	33.9 (51.1)
T$_{max}$ (h)	4.50 (4.00, 4.50)
T$_{1/2}$ (h)	5.74 (5.38, 6.74)

CV: coefficient of variation, EVG: elvitegravir, PK: pharmacokinetic, Q1: first quartile, Q3: third quartile, /r: boosted with ritonavir, RTV: ritonavir

Data are presented as mean (%CV), except for T$_{max}$ and T$_{1/2}$, which are presented as median (Q1, Q3).

<u>Cobcistat pharmacokinetic data when coadministered with elvitegravir 150 mg once daily</u>

The pharmacokinetic parameters for cobicistat 150 mg once daily when coadministered with elvitegravir 150 mg once daily are displayed in Table 12. The applicant did not provide comparative cobcistat exposure data when coadministered with elvitegravir 150 mg once daily from other trials. When compared to the cobicistat data in Table 4, the cobcistat C$_{max}$ was similar with a minimal decrease in the AUC$_{(0-tau)}$; however the C$_{tau}$ was significantly lower.

Table 12-Pharmacokinetic parameters for cobicistat 150 mg on daily with elvitegravir 150 mg once daily

GS-9350 PK Parameter (N = 23)	EVG/GS-9350 Formulation 2
AUC_{tau} (ng·h/mL)	10,054.9 (24.2)
C_{max} (ng/mL)	1584.5 (24.4)
C_{tau} (ng/mL)	21.8 (62.1)
T_{max} (h)	3.50 (3.00, 4.50)
$T_{1/2}$ (h)	3.12 (2.86, 3.61)

CV: coefficient of variation, PK: pharmacokinetic, Q1: first quartile, Q3: third quartile
Data are presented as mean (%CV), except for T_{max} and $T_{½}$, which are presented as median (Q1, Q3).

11. Discussion and Conclusions

Based on the results from the GS-US-216-116 trial, the following conclusions can be made:

- For the two cobicistat formulations (formulation 1 and formulation 2), bioequivalence was demonstrated for cobicistat C_{max}, C_{tau}, and $AUC_{(0-tau)}$.

- Bioequivalence was demonstrated for midazolam and 1-hydroxymidazolam C_{max}, C_{tau}, and $AUC_{(0-tau)}$. Therefore, no clinically significant differences in the CYP3A inhibition effects were demonstrated in comparing the two cobcistat formulations.

- Bioequivalence was demonstrated for elvitegravir C_{max}, C_{tau}, and $AUC_{(0-tau)}$. Therefore, in comparing the CYP3A inhibition effects for cobicistat 150 mg once daily or ritonavir 100 mg once daily, no clinically significant differences in elvitegravir exposure were observed.

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GS-US-216-121

1. Title

A Randomized, Blinded, Placebo-Controlled Phase 1 Study Evaluating the Effect of GS-9350 and Ritonavir on Renal Function as Assessed by Markers of Glomerular Filtration Rate

2. Information Regarding the Clinical Trial Site and Duration of the Trial

The trial was conducted at two sites: a) cohort 1 was enrolled at SeaView Research, Miami, FL, and b) cohort 2 was enrolled at New Orleans Center for Clinical Research, Knoxville, TN. The trial was conducted from December 28, 2009 (first subject screened) to December 7, 2010 (last subject observation).

3. Objectives

The objective of the trial was to evaluate renal function prior to, during, and after administration of cobicistat or ritonavir in addition to the pharmacokinetics of cobicistat or ritonavir.

4. Trial Design

Cohort 1 was the randomized, double blind, multiple dosing, parallel group, portion of the trial that enrolled subjects with normal renal function (defined as subjects with creatinine clearances ≥ 80 mL/min using the Cockroft-Gault formula. Subjects in cohort 1 received multiple doses of placebo, cobicistat or ritonavir along with an intravenous (IV) dose of iohexol on days 0, 7 and 14. 36 subjects in total (12 subjects per treatment arm) were to be enrolled. The trial design for cohort 1 is displayed in Figure 1.

Figure 1-GS-US-216-121 cohort 1 trial design
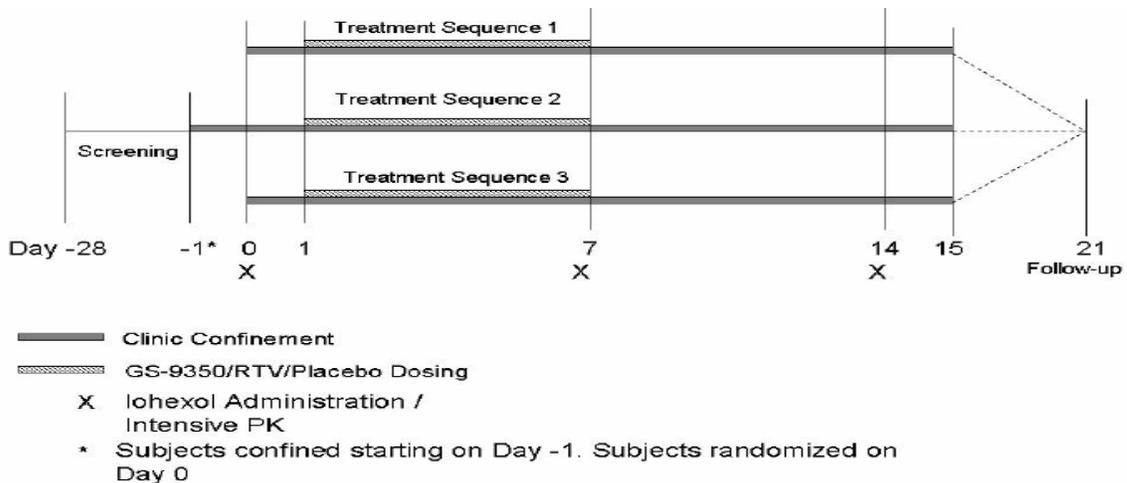
Cohort 2 was the open label, multiple dosing portion of the trial that enrolled subjects with mild or moderate renal impairment (defined in the protocol as subjects with creatinine clearance ≥ 50-79 mL/min using the Cockroft-Gault formula). Subjects in cohort 2 received multiple doses of cobicistat along with an intravenous dose of iohexol on days 0, 7 and 14. 18 subjects were to be enrolled. The trial design for cohort 2 is displayed in Figure 2.

Figure 2-GS-US-216-121 cohort 2 trial design
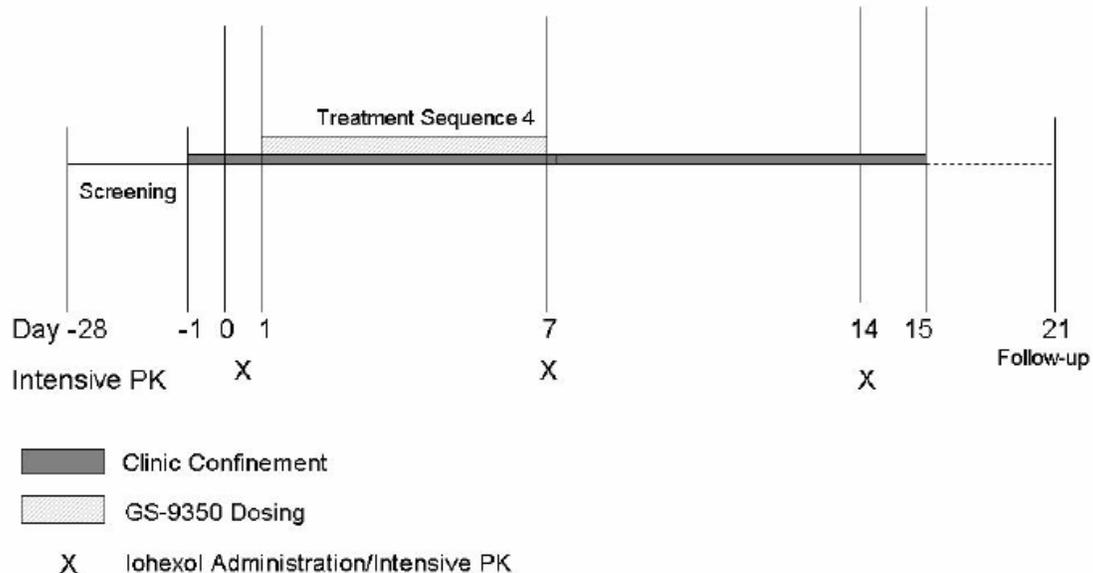
5. Medication Restrictions and Exceptions

In cohort 1, use of vitamins, acetaminophen, ibuprofen, oral contraceptives, and topical hydrocortisone cream or A&D ointment to treat skin irritation from ECG leads was permitted during the trial. All other prescription and nonprescription medications, including herbal medications, were not permitted during the trial. In cohort 2, a table was included in the protocol with a specific list of medications that were to be used with caution or were not permitted.

6. Dosage and Administration

In cohort 1, subjects received one of the following three treatments for seven days: a) cobicistat 150 mg plus one placebo capsule once daily (treatment 1), b) ritonavir 100 mg plus one placebo tablet once daily (treatment 2), or c) one placebo tablet and one placebo capsule once daily (treatment 3). In cohort 2, subjects received cobicistat 150 mg once daily for seven days.

In both cohorts, 1500 mg of iohexol (Omnipaque 300) was administered as a 5 mL IV bolus dose over 1 to 2 minutes of a 300 mg iodine/mL solution on days 0,

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7 and 14. Iohexol was administered within 5 minutes after administration of oral medications on day 7.

Trial medications were administered within 5 minutes of completion of a standardized meal. During the period when subjects were confined to the trial site, subjects were required to drink 240 mL of water when waking up and every 6 hours while awake.

7. Rationale for Doses Used in the Trial

The selected cobicistat dosage regimen for the GS-US-216-121 trial, 150 mg once daily, is the proposed dosage regimen for cobicistat when combined with elvitegravir (cobicistat is utilized to achieve therapeutic concentrations of elvitegravir).

The selected ritonavir dosage regimen for the GS-US-216-121 trial, 100 mg once daily, is used to increase the systemic exposure of concurrently administered HIV-1 protease inhibitors such as darunavir or atazanavir.

The applicant stated that 1500 mg of iohexol administered intravenously is the dose that is used to evaluate the glomerular filtration rate.

8. Drugs Used in the Trial

A summary of the cobicistat, ritonavir, iohexol and placebo formulations that were administered in the GS-US-216-121 trial is displayed in Table 1 below.

Table 1-Cobcistat, ritonavir, iohexol and placebo formulations administered in the GS-US-216-121 trial

	COBI Tablet	Placebo to Match COBI Tablet	RTV Soft Gelatin Capsule	Placebo to Match RTV Soft Gelatin Capsule	Iohexol Solution[a]
Strength (mg)	150	—	100	—	300 mg iodine/mL
Lot no.	BB0904B1 BB1004B1	BB0905B1	BP0902A1	BP0901A1	10954088 11105064 81552H
Expiration date	July 2011 April 2012	September 2014	August 2010	March 2012	June 2012 March 2013 March 2013
Manufacturer/ supplier	Lot BB0904B1 Gilead Sciences, Inc. 333 Lakeside Drive, Foster City, CA 94404, USA (b) (4)				(b) (4)
Site of release in Europe	Lot BB0904B1 Not applicable Lot BB1004B1 Gilead Sciences Ltd (b) (4)	Not applicable	Not applicable	Not applicable	Not applicable

a   Commercial drug product

9. Sample Collection, Bioanalysis, Pharmacokinetic Assessments, and Statistical Analysis

Sample Collection

On days 0, 7 and 14, blood samples for analysis of cobicistat, ritonavir and iohexol plasma concentrations were collected up to 24 hours.

On days 0, 7 and 14, urine was collected at 0-4, 4-8, 8-12, and 12-24 hours and pooled at the end of the 24 hour collection period. On all other days, the urine was pooled over the 24 hour collection period

Bioanalysis

Reviewer note: only the method and bioanalysis of cobicistat and iohexol are discussed below.

With the exception of long term stability for cobicistat, the method and bioanalysis of the cobicistat, and iohexol analytes are acceptable.

Blood samples for the GS-US-216-112 trial for the cobicistat and ritonavir analytes were collected in tubes containing K_2EDTA as the anticoagulant according to the bioanalytical reports for the cobicistat and ritonavir analytes. This information was not provided for the iohexol analyte.

Cobicistat plasma samples were analyzed using a validated LC/MS/MS method with K_2EDTA anticoagulated plasma by ⁽ᵇ⁾ ⁽⁴⁾. There was insufficient information in the bioanalytical report to determine whether the reference standards for cobicistat and the cobicistat internal standard were used either prior to the expiration date or within the established stock solution stability period in analyzing cobicistat samples in the GS-US-216-121 trial. However, there were no precision or accuracy issues identified for cobicistat based on the bioanalytical report. The lower limit of quantification for cobicistat was 5 ng/mL and the upper limit of quantification was 2500 ng/mL. Out of the ten analytical runs where cobicistat samples were analyzed, no analytical runs failed for the cobicistat analysis. For the GS-US-216-121 trial, cobicistat precision and accuracy were evaluated using the low (15 ng/mL), medium (200 ng/mL), and high (2000 ng/mL) QC samples. A dilution QC (2000 ng/mL with a dilution factor of 2) was also analyzed in two analytical runs. The cobicistat inter-run accuracy values were 0.7% for the low QCs, -0.8% for the medium QCs, -3.5% for the high QCs, and 0.8% for the dilution QCs. The cobicistat inter-run precision values were 5.3% for the low QCs, 3.4% for the medium QCs, 3.8% for the high QCs, and 1.9% for the dilution QCs. Incurred sample reanalysis for cobicistat was not conducted for the GS-US-216-121 trial.

(b) (4) did not conduct long term stability experiments for cobicistat. Instead, (b) (4) references the long term stability data for cobicistat that was generated by (b) (4). The cobicistat analytical method that was used by (b) (4) is not identical to the cobicistat analytical method used by (b) (4). The submitted cobicistat long term sample stability data demonstrated that cobicistat was stable for 121 days at -10°C to -30°C and 365 days at -60°C to -80°C in K_2EDTA anticoagulated plasma. At the (b) (4) bioanalytical laboratory, subject samples from the GS-US-216-121 trial were stored at -70°C and the bioanalytical report states that subject samples were also stored at -70°C at the trial site until they were shipped to (b) (4). Overall, while the submitted cobicistat long term stability data of 365 days at-70°C covers the duration of long term cobicistat stability data necessary for the GS-US-216-121 trial, the impact of the differences in the cobicistat analytical methods used by (b) (4) compared to (b) (4) is unknown.

One additional issue that was identified based on the method validation results for cobicistat was the multiple QCs at 200 ng/mL that exceeded 15% for the post preparative reinjection stability experiment at 4ºC.

Iohexol plasma samples were analyzed using a validated LC/MS/MS method with K_2EDTA anticoagulated plasma by (b) (4) at the (b) (4) site. The lower limit of quantification for iohexol was 1 µg/mL and the upper limit of quantification was 500 µg/mL. There were no precision or accuracy issues identified for iohexol based on the bioanalytical report. Out of the 39 and 21 analytical runs where iohexol samples were analyzed in cohort 1 and cohort 2, respectively, no analytical run failed for the iohexol analysis.

For the GS-US-216-121 trial, iohexol precision and accuracy were evaluated at the following concentrations: 3 µg/mL, 40 µg/mL, and 400 µg/mL for both cohort 1 and cohort 2. There were no dilution QCs analyzed in the analytical runs for cohort 1 but a 2500 µg/mL dilution QC (dilution factor of 50) was analyzed in cohort 2. For cohort 1, the iohexol inter-run accuracy values were 2% for 3 µg/mL, 2% for 40 µg/mL, and 0.8% for 400 µg/mL and the iohexol inter-run precision values were 3.6% for 3 µg/mL, 2.1% for 40 µg/mL, and 2.1% for 400 µg/mL. For cohort 2, the iohexol inter-run accuracy values were 0% for 3 µg/mL, 0.3% for 40 µg/mL, -1.5% for 400 µg/mL, and 3.2% for 2500 µg/mL, and the iohexol inter-run precision values were 2.8% for 3 µg/mL, 2.5% for 40 µg/mL, 2.3% for 400 µg/mL, and 1.8% for 2500 µg/mL.

A total of 152 and 100 samples from cohorts 1 and 2, respectively, were selected for incurred sample reanalysis. All of the reanalyzed samples in both cohorts were within 20% of the original value. However, it is not clear whether the total number of samples that were reanalyzed represents 5% to 10% of the total number of samples that were initially analyzed.

The long term iohexol sample stability experiments were conducted at the (b) (4) site and not at the (b) (4)

site. A partial validation of the iohexol method was conducted at the site. The submitted iohexol long term sample stability data demonstrated that iohexol was stable for 497 days at -60ºC to -80ºC and 725 days at-10ºC to -30ºC in K_2EDTA anticoagulated plasma. The submitted data sufficiently establishes the long term stability of the iohexol subject samples for the GS-US-216-121 trial.

Pharmacokinetic Assessments

Noncompartmental analysis was performed to calculate the following pharmacokinetic parameters:

Table 2-Pharmacokinetic parameters derived for the GS-US-216-121 trial

Analyte	Pharmacokinetic Parameter
COBI	AUC_{tau}, AUC_{last}, C_{tau}, C_{max}, C_{last}, T_{max}, T_{last}, $t_{\frac{1}{2}}$, λ_z, CL/F, weight-adjusted CL/F
RTV	AUC_{tau}, AUC_{last}, C_{tau}, C_{max}, C_{last}, T_{max}, T_{last}, $t_{\frac{1}{2}}$, λ_z, CL/F, weight-adjusted CL/F
Iohexol	AUC_{inf}, $\%AUC_{exp}$

Statistical Analysis

Descriptive statistics were calculated for cobicistat, ritonavir and iohexol plasma concentrations and pharmacokinetic parameters, including the number of subjects (n), mean, standard deviation, the coefficient of variation (CV%), median, and the minimum and maximum values. The geometric mean was also calculated for the individual pharmacokinetic parameters.

Using a mixed effects model, geometric least squares means were calculated and 90% confidence intervals were derived in comparing the cobicistat $AUC_{(0-tau)}$, C_{tau}, and C_{max} for cohort 1 and cohort 2.

The Wilcoxon rank sum test was used to compare the cobicistat clearance and half life values for cohort 1 and cohort 2.

Pharmacodynamic Assessments

Using serum creatinine and the Cockcroft-Gault and the Modification of Diet in Renal Disease (MDRD) equations, the calculated GFR was derived for cobicistat, ritonavir, and placebo. The GFR was also derived using iohexol and cystatin C as markers of GFR and using 24 hour urine collection.

Reference ID: 3153809

10. Results

10.1 Subject Demographics and Disposition

Table 3-GS-US-216-121 subject demographics

Characteristics[a,b,c,d]	Cohort 1 eGFR$_{CG}$ > 80 mL/min			Cohort 2 eGFR$_{CG}$ 50-79 mL/min	
	COBI (N=12)	RTV (N=12)	Placebo (N=12)	COBI (N=18)	Total (N=54)
Age at Day 0 (Years)					
Mean (SD)	32 (5.8)	34 (5.5)	32 (7.3)	58 (5.8)	41 (13.4)
Median	32	34	33	59	37
Min, Max	22, 39	24, 43	19, 43	46, 65	19, 65
Sex (n, %)					
Female	6 (50.0%)	6 (50.0%)	6 (50.0%)	10 (55.6%)	28 (51.9%)
Male	6 (50.0%)	6 (50.0%)	6 (50.0%)	8 (44.4%)	26 (48.1%)
Race (n, %)					
Black or African Heritage	5 (41.7%)	6 (50.0%)	7 (58.3%)	3 (16.7%)	21 (38.9%)
White	6 (50.0%)	6 (50.0%)	5 (41.7%)	14 (77.8%)	31 (57.4%)
Other	1 (8.3%)	0	0	1 (5.6%)	2 (3.7%)
Ethnicity (n, %)					
Hispanic/Latino	10 (83.3%)	8 (66.7%)	8 (66.7%)	1 (5.6%)	27 (50.0%)
Non-Hispanic/Latino	2 (16.7%)	4 (33.3%)	4 (33.3%)	17 (94.4%)	27 (50.0%)
Weight (kg)					
Mean (SD)	72.8 (13.99)	71.3 (10.79)	69.9 (10.51)	69.3 (12.56)	70.7 (11.84)
Median	77.3	73.6	69.6	67.2	71.5
Min, Max	50.6, 87.9	53.2, 85.9	54.9, 89.5	50.3, 99.9	50.3, 99.9
BMI (kg/m^2)					
Mean (SD)	25.3 (3.34)	26.0 (2.39)	24.7 (3.45)	25.1 (3.16)	25.2 (3.06)
Median	25.8	26.9	25.1	25.6	26.0
Min, Max	19.7, 30.1	20.8, 28.2	18.5, 28.7	19.6, 32.3	18.5, 32.3
eGFR$_{CG}$ (mL/min)					
Mean (SD)	121.3 (22.88)	116.9 (17.53)	113.8 (26.72)	68.7 (9.67)	101.1 (29.86)
Median	124.7	114.7	110.4	70.0	98.9
Min, Max	88.1, 166.8	96.1, 142.8	76.2, 159.1	54.1, 86.8	54.1, 166.8
eGFR$_{MDRD}$ (mL/min/1.73m^2)					
Mean (SD)	108.7 (18.58)	108.8 (19.09)	108.4 (24.03)	68.9 (13.01)	95.4 (26.04)
Median	105.9	104.2	104.2	66.5	92.8
Min, Max	89.4, 145.5	85.3, 151.5	76.5, 153.3	47.5, 98.6	47.5, 153.3

a   The safety analysis set includes all enrolled subjects who received at least 1 dose of study drug (COBI, RTV or placebo).

b   The denominator for percentages was the number of subjects in the safety analysis set.

c   Baseline value was generally defined as the last available value collected prior to the first dose of the study drug (COBI, RTV or placebo).

d   Baseline estimated GFR using the Cockcroft-Gault or MDRD method was calculated using Day 0 serum creatinine concentration at 12 hours postdose.

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Figure 3-GS-US-216-121 subject disposition

Cohort 1
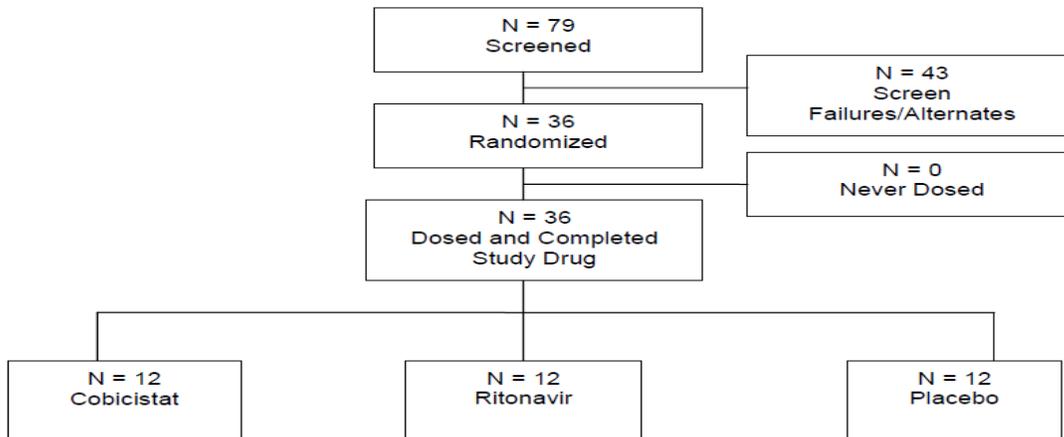
Cohort 2
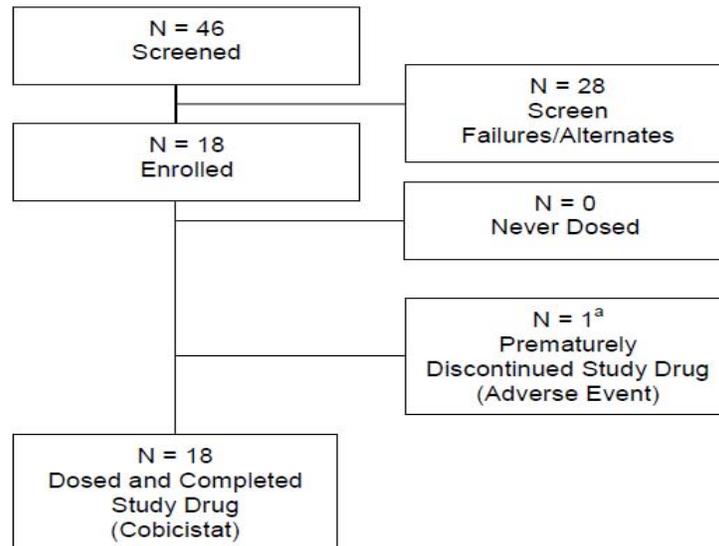
a   Subject 5291-2014 received all doses of COBI but discontinued before receiving iohexol on Day 14.

10.2 Concomitant Medications

Subjects received a variety of concomitant medications during the trial; however there were no specific medications that were commonly used. None of the administered concomitant medications are anticipated to significantly impact the results of the trial.

10.3 Pharmacokinetic and Statistical Analysis

Reviewer note: the actual pharmacokinetic data that was generated included the following information: cobicistat day 7(cohort 1 and 2), ritonavir day 7(cohort 1) and iohexol for day 0, day 7, day 14 for the cobicistat (cohorts 1 and 2) and the ritonavir and placebo arms (cohort 1).

There were no subjects on day 0 receiving the iohexol intravenous dose with quantifiable predose iohexol concentrations.

Cobicistat pharmacokinetic data for cohort 1 and cohort 2

The pharmacokinetic parameters for cobicistat 150 mg once daily on day 7 with cohort 1 and cohort 2 are displayed in Table 4.

Based on the comparison of the pharmacokinetic data in Table 5, increased cobicistat C_{max}, C_{tau}, and $AUC_{(0-tau)}$ were observed in subjects with mild to moderate renal impairment. The results are not consistent with the results from the GS-US-216-0124 trial where the increases in cobicistat C_{max}, C_{tau}, and $AUC_{(0-tau)}$ in subjects with severe renal impairment were 25% or less.

There was no specific potential explanation(s) discussed in the trial report regarding the differences in the reported results.

Table 4-Pharmacokinetic parameters for cobicistat 150 mg on daily with cohort 1 and cohort 2

PK Parameter[a]	Cohort 1 $eGFR_{CG}$ > 80 mL/min N = 12	Cohort 2 $eGFR_{CG}$ 50-79 mL/min N = 18
AUC_{tau} (ng•h/mL)	14,545.3 (28.2)	20,819.7 (38.5)
C_{max} (ng/mL)	1719.3 (19.8)	2028.6 (31.6)
C_{tau} (ng/mL)	99.8 (59.2)	302.7 (70.6)
T_{max} (h)	2.83 (2.58, 3.83)	3.07 (2.08, 4.03)
T_{last} (h)	24.08 (24.08, 24.08)	24.05 (24.03, 24.08)
$t_{½}$ (h)	4.97 (3.54, 5.74)	7.78 (6.58, 9.11)
CL/F (mL/min)	187.7 (35.1)	146.8 (59.6)

a   Data are presented as mean (%CV), except T_{max}, T_{last}, and $t_{½}$ which are presented as median (Q1, Q3).

Table 5-Statistical analyses for or cobicistat 150 mg once daily on day 7 with cohort 1 and cohort 2

PK Parameter	Geometric Least-Squares Mean		Geometric Least-Squares Means Ratio (%) (90% CI)
	Test Cohort 2 (N = 18)	Reference Cohort 1 (N = 12)	
AUC$_{tau}$ (ng•h/mL)	19104.61	13962.97	136.82 (107.78,173.69)
C$_{max}$ (ng/mL)	1907.92	1689.17	112.95 (93.93,135.82)
C$_{tau}$ (ng/mL)	230.29	83.44	275.98 (171.56,443.95)

Ritonavir pharmacokinetic data for cohort 1

The pharmacokinetic parameters for ritonavir 100 mg once daily once daily on day 7 with cohort 1 are displayed in Table 6. The reported results are similar to the reported ritonavir multiple dosing pharmacokinetic data from the GS-US-216-101 trial (data not shown).

Table 6-Pharmacokinetic parameters for ritonavir 100 mg on daily with cohort 1

PK Parameter[a]	Cohort 1 eGFR$_{CG}$ ≥ 80 mL/min N = 12
AUC$_{tau}$ (ng•h/mL)	8745.7 (42.8)
C$_{max}$ (ng/mL)	1598.8 (39.3)
C$_{tau}$ (ng/mL)	75.2 (75.3)
T$_{max}$ (h)	4.58 (4.33, 4.58)
T$_{last}$ (h)	24.08 (24.08, 24.08)
t$_{½}$ (h)	5.91 (5.16, 6.24)
CL/F (mL/min)	226.1 (41.7)

a   Data are presented as mean (%CV), except T$_{max}$, T$_{last}$, and t$_{½}$ which are presented as median (Q1, Q3).

Evaluation of glomerular filtration rate (GFR) using the Cockcroft-Gault equation

Using serum creatinine and the Cockcroft-Gault equation, the calculated GFR was derived for placebo, cobicistat and ritonavir in addition to the change from baseline for Days 7 and 14. The results are displayed in Table 7.

No statistically significant changes (p <.05) in GFR from baseline were observed on days 7 and 14 for placebo. For both subgroups (creatinine clearance ≥ 80 mL/min and creatinine clearance of 50 to 79 mL/min), a statistically significant change in GFR from baseline was observed on day 7 but no statistically

significant changes in GFR from baseline was observed on day 14 for cobicistat. In both groups, the GFR decreased on days 7 and 14 with the exception of the subgroup with creatinine clearance ≥ 80 mL/min on day 14. In comparing the changes from baseline, no statistically significant change in GFR was observed on day 7 but a statistically significant change in GFR on day 14 was observed for ritonavir. The GFR increased on both days for ritonavir.

Table 7-Calculated GFR for placebo, cobicistat and ritonavir using serum creatinine and the Cockcroft-Gault equation

| Treatment | Estimated GFR$_{CG}$ (mL/min; 12 h postdose) Mean (SD) | | |
	Day 0	Day 7	Day 14
Placebo			
eGFR ≥ 80 mL/min (n = 12)	113.8 (26.72)	116.0 (23.87)	136.6 (59.04)
Change from Day 0 (mL/min)	——	2.2 (9.10) ($p = 0.42$)	22.8 (59.86) ($p = 0.21$)
COBI			
eGFR ≥ 80 mL/min (n = 12)	121.3 (22.88)	111.4 (26.62)	122.6 (28.03)
Change from Day 0 (mL/min)	——	-9.9 (13.14) ($p = 0.024$)	1.4 (11.5) ($p = 0.69$)
eGFR 50-79 mL/min (n = 18)	68.7 (9.67)	56.8 (10.98)	66.5 (10.59)
Change from Day 0 (mL/min)	——	-11.9 (6.97) ($p < 0.001$)	-2.2 (5.61) ($p = 0.12$)
RTV			
eGFR ≥ 80 mL/min (n = 12)	116.9 (17.53)	117.9 (18.46)	122.6 (21.24)
Change from Day 0 (mL/min)	——	1.0 (8.62) ($p = 0.70$)	5.7 (8.21) ($p = 0.035$)

The applicant also compared the calculated GFR using the Cockcroft-Gault equation on Days 7 and 14 for cobicistat compared to either ritonavir or placebo and for ritonavir compared to placebo. A statistically difference was observed for cobicistat compared to either ritonavir or placebo on day 7.

Evaluation of glomerular filtration rate (GFR) using the MDRD equation

Using serum creatinine and the MDRD equation, the calculated GFR was derived for placebo, cobicistat and ritonavir in addition to the change from baseline for Days 7 and 14. The results are displayed in Table 8 and are consistent with the results obtained using the Cockcroft-Gault equation.

No statistically significant changes ($p < .05$) in GFR from baseline were observed on days 7 and 14 for placebo. For both subgroups (creatinine clearance ≥ 80 mL/min and creatinine clearance of 50 to 79 mL/min), a statistically significant change in GFR from baseline was observed on day 7 but no statistically significant changes in GFR from baseline was observed on day 14 for cobicistat.

In both groups, the GFR decreased on days 7 and 14 with the exception of the subgroup with creatinine clearance ≥ 80 mL/min on day 14. In comparing the changes from baseline, no statistically significant change in GFR was observed on day 7 but a statistically significant change in GFR on day 14 was observed for ritonavir. The GFR increased on both days for ritonavir.

Table 8-Calculated GFR for placebo, cobicistat and ritonavir using serum creatinine and the MDRD equation

Treatment	Estimated GFR$_{MDRD}$ (mL/min/1.73 m^2; 12 h postdose) Mean (SD)		
	Day 0	Day 7	Day 14
Placebo			
eGFR ≥ 80 mL/min (n = 12)	108.4 (24.03)	110.5 (25.27)	132.1 (60.04)
Change from Day 0 (mL/min)	———	2.2 (11.29) (p = 0.52)	23.7 (64.98) (p = 0.23)
COBI			
eGFR ≥ 80 mL/min (n = 12)	108.7 (18.58)	98.8 (25.78)	109.8 (26.65)
Change from Day 0 (mL/min)	———	-9.9 (12.20) (p = 0.017)	1.1 (11.31) (p = 0.74)
eGFR 50-79 mL/min (n = 18)	68.9 (13.01)	55.1 (12.75)	66.4 (14.53)
Change from Day 0 (mL/min)		-13.9 (8.22) (p < 0.001)	-2.6 (5.24) (p = 0.053)
RTV			
eGFR ≥ 80 mL/min (n = 12)	108.8 (19.09)	109.2 (20.48)	114.1 (21.16)
Change from Day 0 (mL/min)	———	0.4 (8.68) (p = 0.88)	5.3 (7.99) (p = 0.041)

<u>Evaluation of glomerular filtration rate (GFR) using iohexol plasma clearance</u>

Iohexol primarily undergoes glomerular filtration by the kidneys. Using iohexol plasma clearance, the GFR was derived for placebo, cobicistat and ritonavir in addition to the change from baseline for Days 7 and 14. The results are displayed in Table 9.

In contrast to the results obtained using the Cockcroft-Gault and MDRD equations, no statistically significant changes (p <.05) in GFR from baseline were observed on days 7 and 14 for placebo, cobicistat or ritonavir.

Table 9-Calculated GFR for placebo, cobicistat and ritonavir using iohexol plasma clearance

Treatment	Actual GFR (mL/min) Mean (SD)		
	Day 0	Day 7	Day 14
Placebo			
eGFR ≥ 80 mL/min (n = 12)	129.8 (24.23)	134.6 (26.68)	129.8 (23.54)
Change from Day 0 (mL/min)	——	4.7 (15.11) (p = 0.30)	0.0 (12.04) (p = 0.99)
COBI			
eGFR ≥ 80 mL/min (n = 12)	133.8 (14.29)	131.1 (16.23)	131.3 (15.96)
Change from Day 0 (mL/min)	——	-2.7 (8.71) (p = 0.30)	-2.5 (5.50) (p = 0.14)
eGFR 50-79 mL/min (n = 18)	76.8 (15.36)	73.2 (16.56)	70.8 (21.74)[a]
Change from Day 0 (mL/min)	——	-3.6 (7.63) (p = 0.062)	-5.8 (13.18) (p = 0.087)
RTV			
eGFR ≥ 80 mL/min (n = 12)	141.5 (22.42)	143.7 (23.82)	140.7 (20.09)
Change from Day 0 (mL/min)	——	2.2 (9.69) (p = 0.45)	-0.8 (6.28) (p = 0.67)

a   n = 17 (Subject 5291-2014 did not receive iohexol on Day 14)

The applicant also compared the GFR that was derived using iohexol plasma clearance on Days 7 and 14 for cobicistat compared to either ritonavir or placebo and for ritonavir compared to placebo. No statistically differences on days 7 or 14 were observed for any of the comparisons.

<u>Evaluation of glomerular filtration rate (GFR) using 24 hour urine collection</u>

Using serum creatinine and 24 hour urine collection, the GFR was derived for placebo, cobicistat and ritonavir in addition to the change from baseline for Days 7 and 14. The results are displayed in Table 10.

No statistically significant changes (p <.05) in GFR from baseline were observed on days 7 and 14 for placebo. For cobicistat, in both subgroups (creatinine clearance ≥ 80 mL/min and creatinine clearance of 50 to 79 mL/min), a statistically significant change in GFR from baseline was observed on day 7 and a statistically significant change in GFR from baseline was observed for the 50 to 79 mL/min subgroup on day 14. In both groups, the GFR decreased on both days. No statistically significant change in GFR from baseline on day 7 and day 14 were observed for ritonavir.

Table 10-Calculated GFR for placebo, cobicistat and ritonavir using serum creatinine and 24 hour urine collection

Treatment	Measured GFR (mL/min) Mean (SD)		
	Day 0	**Day 7**	**Day 14**
Placebo			
eGFR ≥ 80 mL/min (n = 12)	111.7 (33.51)	111.5 (22.29)	137.2 (59.89)
Change from Day 0 (mL/min)	——	-0.2 (31.05) (p = 0.98)	25.5 (72.74) (p = 0.25)
COBI			
eGFR ≥ 80 mL/min (n = 12)	98.3 (38.90)	79.8 (29.36)	93.1 (35.67)
Change from Day 0 (mL/min)	——	-18.4 (23.90) (p = 0.022)	-5.1 (31.46) (p = 0.58)
eGFR 50-79 mL/min (n = 18)	76.7 (24.88)	65.4 (19.38)	70.5 (22.32)
Change from Day 0 (mL/min)	——	-11.2 (18.03) (p = 0.017)	-6.1 (11.95) (p = 0.044)
RTV			
eGFR ≥ 80 mL/min (n = 12)	106.5 (43.11)	109.3 (31.68)	111.1 (40.22)
Change from Day 0 (mL/min)	——	2.8 (34.95) (p = 0.79)	4.6 (22.83) (p = 0.50)

The applicant also compared the GFR that was derived using serum creatinine and 24 hour urine collection on Days 7 and 14 for cobicistat compared to either ritonavir or placebo and for ritonavir compared to placebo. No statistically differences on days 7 or 14 were observed for any of the comparisons.

<u>Evaluation of glomerular filtration rate (GFR) using cystatin C</u>

Cystatin C undergoes glomerular filtration but is also reabsorbed by the kidneys. Using cystatin C, the GFR was derived for placebo, cobicistat and ritonavir in addition to the change from baseline for Days 7 and 14. The results are displayed in Table 11.

No statistically significant changes (p <.05) in GFR from baseline were observed on days 7 and 14 for placebo, cobicistat or ritonavir.

Table 11-Calculated GFR for placebo, cobicistat and ritonavir using cystatin C

Treatment	cysGFR (mL/min/1.73 m^2) (Adjusted) Mean (SD)		
	Day 0	Day 7	Day 14
Placebo			
eGFR ≥ 80 mL/min (n = 12)	143.4 (20.32)	139.4 (19.28)	142.6 (24.17)
Change from Day 0 (mL/min)	———	-4.0 (9.46) (p = 0.17)	-0.7 (12.15) (p = 0.83)
COBI			
eGFR ≥ 80 mL/min (n = 12)	130.3 (19.59)	127.9 (17.49)	129.8 (16.75)
Change from Day 0 (mL/min)	———	-2.4 (7.55) (p = 0.29)	-0.5 (12.26) (p = 0.90)
eGFR 50-79 mL/min (n = 18)	91.0 (21.52)	86.5 (19.41)	86.2 (19.30)
Change from Day 0 (mL/min)	———	-4.5 (11.91) (p = 0.13)	-4.8 (12.18) (p = 0.11)
RTV			
eGFR ≥ 80 mL/min (n = 12)	151.8 (27.67)	146.6 (20.91)	149.6 (23.34)
Change from Day 0 (mL/min)	———	-5.2 (17.03) (p = 0.31)	-2.2 (12.41) (p = 0.55)

The applicant also compared the GFR that was derived using cystatin C on Days 7 and 14 for cobicistat compared to either ritonavir or placebo and for ritonavir compared to placebo. No statistically differences on days 7 or 14 were observed for any of the comparisons.

11. Discussion and Conclusions

Based on the results from the GS-US-216-121 trial, the following conclusions can be made:

- Cobicistat C_{max}, C_{tau}, and $AUC_{(0-tau)}$ were increased by 13%, 176%, and 37% in subjects with mild to moderate renal impairment compared to subjects with normal renal function. In comparison, in the GS-US-216-0124 trial, the increases in cobicistat C_{max}, C_{tau}, and $AUC_{(0-tau)}$ in subjects with severe renal impairment were 25% or less.

- The following results were obtained from the various methods used to evaluate GFR:

  -Cockcroft-Gault and MDRD equations-A statistically significant change in GFR from baseline was observed on day 7 for cobicistat for both subgroups and day 14 for ritonavir.

-Plasma iohexol clearance and cystatin C-No statistically significant changes in GFR from baseline were observed on days 7 and 14 for placebo, cobicistat or ritonavir.

-24 hour urine collection-For cobicistat, a statistically significant change in GFR from baseline was observed on day 7 for both subgroups (creatinine clearance ≥ 80 mL/min and creatinine clearance of 50 to 79 mL/min), and a statistically significant change in GFR from baseline was observed for the 50 to 79 mL/min subgroup on day 14.

- Clinically significant changes in GFR from baseline for cobicistat or ritonavir on day 7 or day 14 were not observed when evaluating the changes in plasma iohexol clearance or cystatin C, the two renal markers that provide more accurate assessments of GFR.

In Vivo Trials of Elvitegravir and Cobicistat administered together

S. No.	Study Number	Description of the Trial	Page Number
1	GS-US-183-0133	A Phase 1, Open Label, Parallel Group, Multiple Dose Study to Evaluate the Pharmacokinetics of Cobicistat (Cobi)-Boosted Elvitegravir (EVG) in Subjects with Normal and Impaired Hepatic Function.	287
2	GS-US-216-0120	A Phase 1 Study to Evaluate the Relative Bioavailability and Pharmacokinetics of GS-9350-boosted Elvitegravir upon Co-administration with a Representative H2-Receptor Antagonist or Proton Pump Inhibitor.	296
3	GS-US-216-0122	A Phase 1 Study to Evaluate the Relative Bioavailability and Pharmacokinetics of Cobicistat-boosted Elvitegravir upon Simultaneous Co-administration with a Representative H2-Receptor Antagonist.	303
4	GS-US-216-0123	A Phase 1 Study Evaluating the Drug Interaction Potential Between Cobicistat-Boosted Elvitegravir (EVG/co) Administered Once-Daily and Atazanavir (ATV), Rosuvastatin (ROS), or Rifabutin (RIF)	309
5	GS-US-216-0124	A Phase 1, Open Label, Parallel Design, Multiple Dose Study to Evaluate the Pharmacokinetics of Cobicistat (Cobi)-Boosted Elvitegravir (EVG) in Subjects with Severe Renal Impairment	323

GS-US-183-0133

1. Title

A Phase 1, Open Label, Parallel Group, Multiple Dose Study to Evaluate the Pharmacokinetics of Cobicistat (Cobi)-Boosted Elvitegravir (EVG) in Subjects with Normal and Impaired Hepatic Function.

2. Objectives

The primary objective of the study was to evaluate the steady state pharmacokinetics of cobicistat-boosted elvitegravir (EVG/co) in subjects with normal and impaired hepatic function (determined by Child-Pugh-Turcotte [CPT] classification). The secondary objective of the study was to evaluate the safety of EVG/co in subjects with normal and impaired hepatic function

Reviewer's Note:

EVG and Cobi are predominantly metabolized by CYP3A4. EVG also undergoes glucuronic acid conjugation (via GT1A1/1A3) and CYP2D6 is involved in the metabolism of cobicistat. Therefore, the applicant conducted this trial to evaluate the effect of altered enzyme activity (potentially due to impaired hepatic function) on the pharmacokinetics of EVG/co.

Per the applicant, subjects with severe hepatic impairment (CPT Category C) were not enrolled due to "complexities involved with this condition and difficulties in enrolling this subject population".

3. Trial Design

Open Label, Parallel Group, Multiple Dose Study. The study planned to enroll 20 subjects (10 subjects in the normal matched control group and 10 subjects in the moderate hepatic impairment group) with a target of 8 evaluable subjects per group. Each subject in the normal matched control group was matched for age (± 5 years), gender, and body mass index (BMI; ± 15 %) with a subject in the moderate hepatic impairment group. Subjects with stable hepatic impairment with a CPT score of 7-9 (CPT Classification B) were enrolled in the trial.

The subjects received a single 150 mg EVG tablet plus a single 150 mg Cobi tablet, co-administered once daily in the morning for 10 days, following a standard meal. On days of PK assessments, subjects took EVG and cobi with the standard meal but were restricted from food intake until after collection of the 4-hour blood draw, relative to EVG and Cobi dosing. Water consumption was also restricted 1 hour before- and 2 hours after dosing with EVG/cobi, except for the 240 mL water given with the study drugs.

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The doses of EVG and Cobi selected for this trial are the doses of EVG and Cobi in the QUAD (EVG/Cobi/Emtricitabine/Tenofovir) tablet.

Reviewer's Note:

As cobicistat has been shown to be a mechanism-based - and time-dependant inhibitor, multiple doses of EVG/co were administered in this trial.

Serial blood samples to measure plasma concentrations were collected pre-dose on days 7, 9, 10. Intensive PK sampling was conducted up to 96 hours post dosing on day 10.

Aliquots of blood samples were retained to determine the protein binding of EVG on day 10 at pre-dose and post-dose (4 and 5 hours). For Cobi, protein binding was determined at 4.5 and 24 hours post dose on day 10.

5. Drugs Used in the Trial

The trial was conducted between October 2010 (first subject screened) and Feb 2011 (last subject observation) using the following drugs:

EVG: 150 mg tablets (Lot # AJ0802D1 and Expiration Date September 2011).
Cobi: 150 mg tablets (Lot # BB1004B1 and Expiration Date April 2012).

6. Pharmacokinetic Assessments and Statistical Analysis

Pharmacokinetic Assessments

Non-compartmental pharmacokinetic parameters were computed for EVG and Cobi. The primary pharmacokinetic parameters were AUC_{tau} and C_{max} of EVG. The secondary pharmacokinetic parameters were C_{tau} of EVG and AUC_{tau}, C_{max}, and C_{tau} of Cobi.

Statistical Analysis

90 % confidence intervals were constructed for the ratio of geometric means of each of the pharmacokinetic parameters (AUC and C_{max}) of EVG in the moderate hepatic impairment group versus the normal matched control group. Similar analysis was conducted for the other computed pharmacokinetic parameters of EVG and Cobi

7. Results

Subject Disposition and Demographics

All 20 subjects completed the study and were included in the pharmacokinetic and safety analysis data sets for EVG and Cobi.

Of the 10 subjects in the normal matched control group, 9 (90 %) were male, mean age of 56 years (range 41-70 years). 8 (80 %) subjects were white and 2 (20 %) were black. Mean values for weight and BMI (body mass index; computed as weight X [703/height^2]) were 86.9 kg (min-max 70.3-99.1 kg) and 28.2 kg/m^2 (min-max 21.1-32.2 kg/m^2). The median creatinine clearance (CrCL) was 102.9 mL/min (Q_1-Q_3 87.3-107.1 mL/min).

Of the 10 subjects in the moderate hepatic impairment group, 9 (90 %) were male, mean age of 56 years (range 41-68 years). 9 (90 %) subjects were white and 1 (10 %) was black. Mean values for weight and BMI were 85.5 kg (min-max 58.6-108.6 kg) and 27.7 kg/m^2 (min-max 22.2-33.1 kg/m^2). The median creatinine clearance (CrCL) was 118.7 mL/min (Q_1-Q_3 95.7-133.9 mL/min).

There were 3 (30 %) subjects with a CPT score of 7, 4 (40 %) subjects with a CPT score of 8, and 3 (30 %) subjects with a CPT score of 9.

Protocol deviations

A total of 3 protocol deviations (defined as violation of inclusion/exclusion criteria and subject taking prohibited concomitant medication) occurred in 2 subjects in the trial. Both subjects were in the moderate hepatic impairment group.

One subject took propoxyphene (CYP3A inhibitor) within 30 days prior to dosing and hence was in violation of the inclusion/exclusion criteria. Urine tests performed at screening and throughout the study were negative for propoxyphene. The second subject was taking propoxyphene at screening and continued to take propoxyphene until study day 9 when it was discontinued.

These deviations were not found to impact the overall conclusions of the trial, hence the subjects were included in the analysis.

Bioanalysis

Plasma concentrations of EVG and Cobi were determined using LC/MS/MS. All samples were analyzed in the time frame supported by frozen stability storage data.

Table 1 shows the performance characteristics of the assay used to quantify the concentrations of EVG and Cobi.

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Table 1: Performance characteristics of the assay used to quantify the concentrations of EVG and Cobi

Parameter	EVG	COBI
Linear range (ng/mL)	20–10,000	5–2,500
LLQ (ng/mL)	20	5
Interassay precision range (% CV)	2.8 to 8.1	3.9 to 8.3
Interassay accuracy range	−8.0 to 5.7	−0.3 to 9.7
Stability in frozen matrix (days)	585 days at -70°C	121 days at −10°C to −30°C 365 days at −60°C to −80°C

Pharmacokinetics

EVG

Fig 1 shows the mean steady state pharmacokinetic profile of EVG in subjects with moderate hepatic impairment and subjects with normal hepatic function.

Fig 1: Mean steady state pharmacokinetic profile of EVG in subjects with moderate hepatic impairment and subjects with normal hepatic function
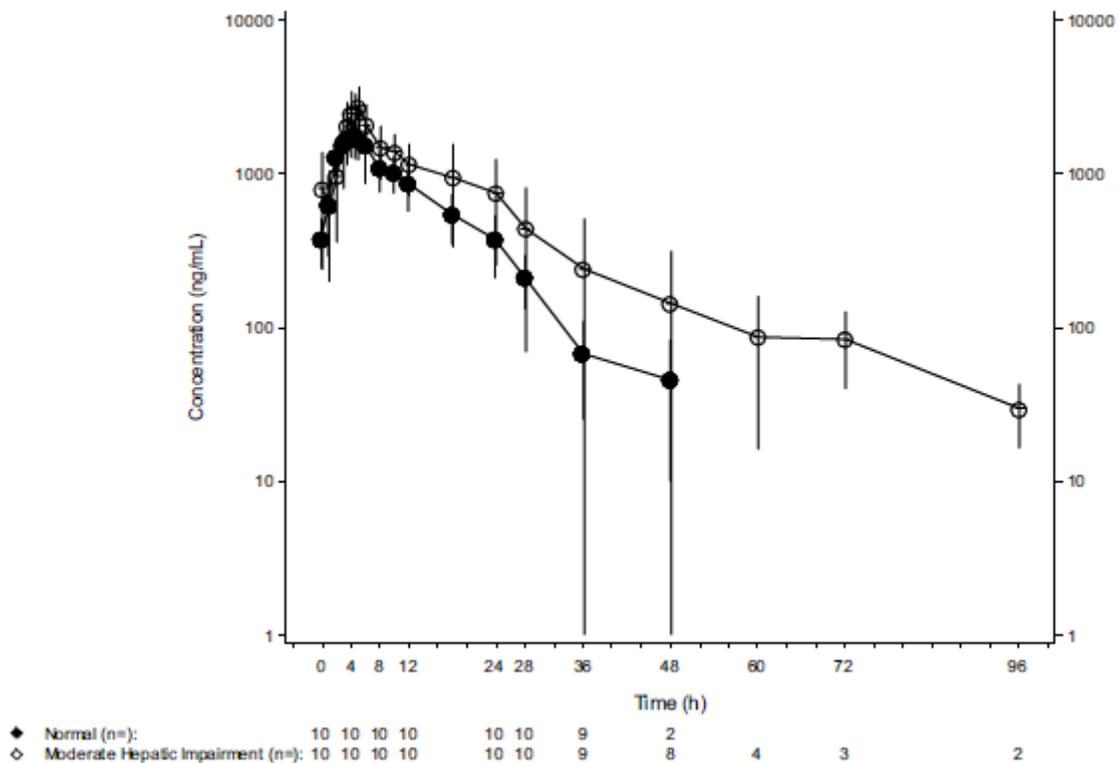
Fig 1 shows that 48 hours post dosing on day 10, there were more subjects in the moderate hepatic impairment group with detectable EVG concentrations, thereby suggesting decreased apparent clearance of EVG in subjects with moderate hepatic impairment.

Fig 2 shows the plot of individual AUC (panel A) and Cmax (panel B) of EVG in subjects with moderate hepatic impairment as compared with subjects with normal hepatic function

Fig 2: Plot of individual AUC (panel A) and C_{max} (panel B) of EVG in subjects with moderate hepatic impairment as compared with subjects with normal hepatic function

Panel A

Panel B
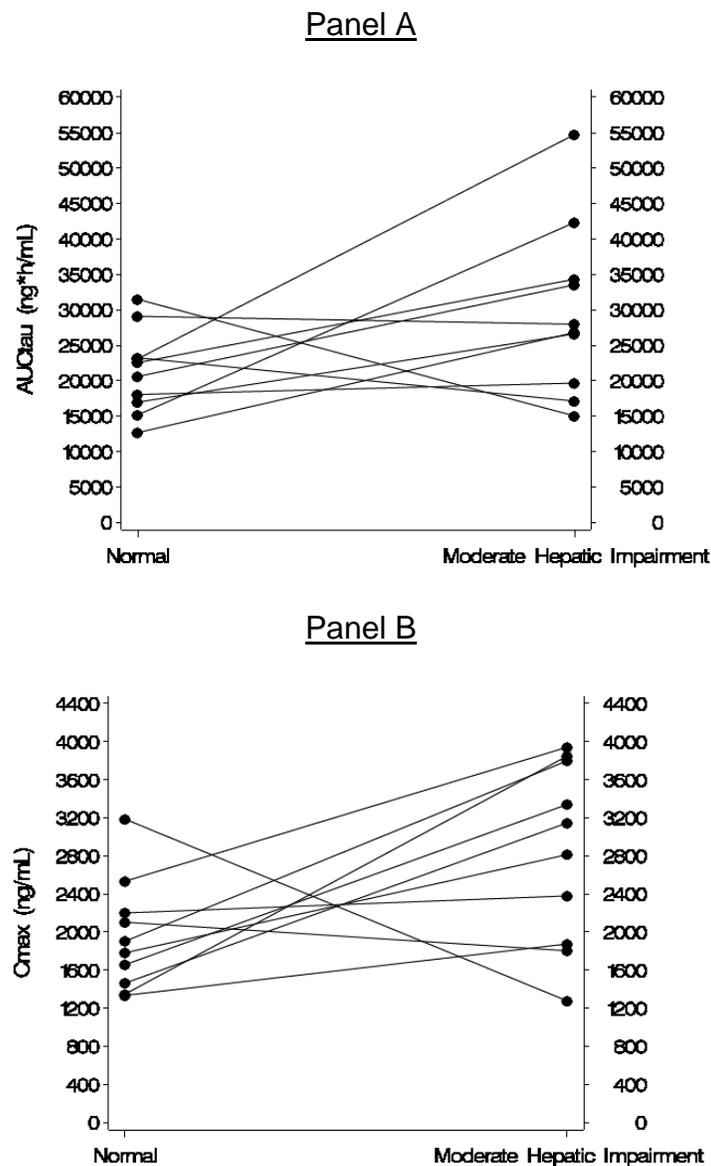
Table 2 shows the mean pharmacokinetic parameters of EVG in subjects with moderate hepatic impairment and subjects with normal hepatic function

Table 2 : Mean pharmacokinetic parameters of EVG in subjects with moderate hepatic impairment and subjects with normal hepatic function

EVG PK Parameter	Normal Matched Control Group (N = 10)	Moderate Hepatic Impairment Group (N = 10)
AUC_{tau} (ng·h/mL)	21,278.5 (27.8)	29,802.7 (40.6)
C_{max} (ng/mL)	1951.5 (29.8)	2822.9 (33.7)
C_{tau} (ng/mL)	370.2 (43.7)	740.9 (65.3)
T_{max} (h)	4.00 (3.50, 5.00)	4.75 (4.00, 5.00)
$T_{1/2}$ (h)	7.56 (6.74, 8.68)	8.24 (7.12, 11.42)
Vz/F (mL)	81,797.0 (22.3)	73,641.5 (28.6)
CL/F (mL/h)	7574.0 (28.8)	5812.4 (39.8)

Data are presented as mean (%CV), except for T_{max} and $T_{1/2}$, which are presented as median (Q1, Q3).

Mean CL/F was approximately 23 % lower in subjects with moderate hepatic impairment as compared with subjects with normal hepatic function. Mean V_z/F was approximately 10 % lower in subjects with moderate hepatic impairment as compared with subjects with normal hepatic function. Hence, longer half life in subjects with moderate hepatic impairment can primarily be explained based on lower CL/F in subjects with moderate hepatic impairment.

Table 3 shows the statistical comparison of the mean pharmacokinetic parameters of EVG in subjects with moderate hepatic impairment and subjects with normal hepatic function.

Table 3: Statistical comparison of the mean pharmacokinetic parameters of EVG in subjects with moderate hepatic impairment and subjects with normal hepatic function.

PK Parameter	GLSMs		GLSM Ratio (%) (90% CI)
	Reference Treatment: Normal Matched Control Group (N=10)	Test Treatment: Moderate Hepatic Impairment Group (N=10)	
AUC_{tau} (ng·h/mL)	20537.29	27722.39	134.99 (103.09, 176.75)
C_{tau} (ng/mL)	335.30	602.29	179.63 (111.03, 290.60)
C_{max} (ng/mL)	1880.67	2657.00	141.28 (108.80, 183.45)

GLSMs were obtained using a mixed-effects model. The model included treatment, sequence, and period as fixed effects, and subject-within-sequence as a random effect.

Cobi

Table 4 shows the mean pharmacokinetic parameters of Cobi in subjects with moderate hepatic impairment and subjects with normal hepatic function.

Table 4: Mean pharmacokinetic parameters of Cobi in subjects with moderate hepatic impairment and subjects with normal hepatic function

COBI PK Parameter	Normal Matched Control Group (N=10)	Moderate Hepatic Impairment Group (N=10)
AUC_{tau} (ng·h/mL)	9844.4 (36.8)	9903.8 (33.9)
C_{max} (ng/mL)	1293.8 (29.6)	1152.1 (33.4)
C_{tau} (ng/mL)	41.0 (75.2)	90.7 (75.5)
T_{max} (h)	3.00 (2.00, 3.00)	4.03 (3.50, 4.50)
$T_{\frac{1}{2}}$ (h)	3.99 (3.72, 4.94)	6.05 (4.79, 6.69)
Vz/F (mL)	103029.0 (34.3)	140179.5 (44.7)
Cl/F (mL/h)	16717.0 (28.0)	17204.6 (41.9)

Data are presented as mean (%CV), except for T_{max} and $T_{\frac{1}{2}}$, which are presented as median (Q1, Q3).

Mean systemic exposure of Cobi was similar in subjects with moderate hepatic impairment and subjects with normal hepatic function. Mean CL/F was also similar between the two groups, however, the mean V_z/F was 36 % higher in subjects with moderate hepatic impairment group as compared with subjects with normal hepatic function. Higher V_z/F of Cobi in subjects with moderate hepatic impairment can potentially explain the longer half life, lower C_{max}, and higher C_{tau} as compared with subjects with normal hepatic function.

Table 5 shows the statistical comparison of the mean pharmacokinetic parameters of Cobi in subjects with moderate hepatic impairment and subjects with normal hepatic function.

Table 5: Statistical comparison of the mean pharmacokinetic parameters of Cobi in subjects with moderate hepatic impairment and subjects with normal hepatic function

PK Parameter	GLSMs		GLSM Ratio (%) (90% CI)
	Reference Treatment: Normal Matched Control Group (N=10)	Test Treatment: Moderate Hepatic Impairment Group (N=10)	
AUC_{tau} (ng•h/mL)	9358.61	9334.67	99.74 (76.01, 130.89)
C_{tau} (ng/mL)	33.30	69.17	207.70 (117.13, 368.31)
C_{max} (ng/mL)	1250.46	1076.63	86.10 (65.35, 113.43)

GLSMs were obtained using a mixed-effects model. The model included treatment, sequence, and period as fixed effects, and subject-within-sequence as a random effect.

Plasma Protein Binding

The mean (SD) % free fraction (unbound concentration) of EVG in normal matched control subjects and subjects with moderate hepatic impairment was 1.15 (0.14) and 1.22 (0.23), respectively. These findings were consistent with the estimates of free fraction determined in other trials (GS-US-183-0101 and GS-US-183-0120) and suggest that hepatic impairment did not have a significant impact on the protein binding of EVG.

The mean (SD) % free fraction (unbound concentration) of Cobi in normal matched control subjects and subjects with moderate hepatic impairment was 2.71 (0.56) and 3.23 (0.63), respectively.

8. Results

Compared with subjects with normal hepatic function:

- Mean AUC, C_{tau}, and C_{max} of EVG increased by 35 %, 80 %, and 41 % in subjects with moderate hepatic impairment.
- Mean AUC of Cobi was not significantly (< 10 %) altered in subjects with moderate hepatic impairment, however, mean C_{tau} was 107 % higher and mean C_{max} was 14 % lower in subjects with moderate hepatic impairment.

9. Discussion and Conclusion

The range of systemic exposures of EVG observed in this trial in subjects with moderate hepatic impairment are within the range of EVG exposures observed in pivotal trials. EVG exposures in Phase 3 trials have not been associated with any major adverse events.

The dosing recommendations based on the hepatic impairment trial conducted with EVG/Cobi can be extended to the QUAD tablet (EVG/Cobi/Emtricitabine/Tenofovir) based on the following considerations:

- Similarity in exposures of EVG and Cobi when given either as EVG/Cobi or the QUAD tablet.
- Emtricitabine and Tenofovir are primarily renally excreted, hence, hepatic impairment is not expected to significantly alter either emtricitabine or tenofovir concentrations.

QUAD tablet can be given to subjects with either mild- or moderate hepatic impairment without any dose adjustments. The pharmacokinetics of QUAD tablet in subjects with severe hepatic impairment has not been evaluated.

GS-US-216-0120

1. Title

A Phase 1 Study to Evaluate the Relative Bioavailability and Pharmacokinetics of GS-9350-boosted Elvitegravir upon Co-administration with a Representative H2-Receptor Antagonist or Proton Pump Inhibitor.

2. Objectives

The primary objective of the study was to evaluate the relative bioavailability and pharmacokinetics of GS-9350 (cobicistat; Cobi) boosted Elvitegravir (EVG) upon co-administration with a representative H2-Receptor Antagonist (H2RA) or Proton Pump Inhibitor (PPI).

3. Trial Design

Phase I, randomized, open-label, single-center, multiple-dose, crossover study. The study consisted of a 16-day treatment phase and 7 day follow up phase. Subjects enrolled in the study were randomized to one of the three treatment sequences (sequence AB, AC, or AD).

Treatment Sequence AB:

Day 1-8: EVG (1 X 150 mg tablet) + Cobi (1 X 150 mg tablet) once daily, administered in the morning with a meal. **(treatment A)**

Day 9-15: EVG (1 X 150 mg tablet) + Cobi (1 X 150 mg tablet) once daily, administered in the morning with a meal; famotidine 40 mg administered daily in the evening with a meal 12 hours after EVG/cobi administration. **(treatment B)**

Day 16: EVG (1 X 150 mg tablet) + Cobi (1 X 150 mg tablet) administered in the morning with a meal.

Treatment Sequence AC:

Day 1-8: EVG (1 X 150 mg tablet) + Cobi (1 X 150 mg tablet) once daily, administered in the morning with a meal. **(treatment A)**

Day 9-16: EVG (1 X 150 mg tablet) + Cobi (1 X 150 mg tablet) once daily, administered in the morning with a meal; omeprazole 20 mg administered once daily in the morning in the fasted state 2 hours before EVG/co. **(treatment C)**

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Treatment Sequence AD:

Day 1-8: EVG (1 X 150 mg tablet) + Cobi (1 X 150 mg tablet) once daily, administered in the morning with a meal. **(treatment A)**

Day 9-15: EVG (1 X 150 mg tablet) + Cobi (1 X 150 mg tablet) once daily, administered in the morning with a meal; omeprazole 20 mg once daily administered in the evening in the fasted state 12 hours after EVG/co. **(treatment D)**

Day 16: EVG (1 X 150 mg tablet) + Cobi (1 X 150 mg tablet) administered in the morning with a meal.

All doses of the study drug were administered at the study center at approximately the same time with 240 mL of water. All study drugs, with the exception of omeprazole as a part of treatment C and D, were administered to subjects within 5 minutes of completing a meal. Subjects receiving omeprazole as part of treatment C and treatment D were required to fast for ≥ 2 hours prior to the dose of omeprazole.

On days 7 and 16, after an overnight fast (no food or liquids, except water, for at least 8 hours prior to dosing with EVG + Cobi), EVG + Cobi was administered within 5 minutes of completing 100 % of a standardized breakfast. Subjects enrolled in sequence AB also had to complete 100 % of a standardized meal given with the evening dose of famotidine on day 15.

On days 7 and 16, all subjects were restricted from water consumption 1 hour before or 2 hours after dosing with EVG + Cobi, except for the 240 mL water given with the study drugs. For subjects randomized to sequence AC, subjects were also restricted from water 1 hour before the omeprazole dose. Water could be freely consumed by all subjects following the 2-hour blood draw for the remainder of the collection period. Subjects were also restricted from food consumption for approximately 4 hours after administration of EVG + Cobi. A meal (standardized lunch) was provided to subjects after the 4 hour post-dose blood draw.

4. Dose Selection

The dose of EVG (150 mg QD) and Cobi (150 mg QD) selected for this trial are the doses of EVG and Cobi in the QUAD (EVG/Cobi/Emtricitabine/Tenofovir) tablet.

The dose of famotidine (Pepcid[®]; 40 mg QD) and omeprazole (Prilosec® or Prilosec OTC® 20 mg QD) are representative of the doses typically used for management of acid-related diseases.

5. Timing of Dose for Each Subject

H2As (such as famotidine) are recommended to be taken at night to control nocturnal acid secretion. Hence, administration of famotidine in the evening, 12 hours after administration of EVG/Cobi, is appropriate.

Omeprazole is generally recommended to be administered in the morning on an empty stomach. Further, it has been shown that night time dosing can prevent nocturnal breakthrough. Therefore, this trial investigated the use of the PPI with EVG/Cobi under two different clinically relevant scenarios: 1) per the labeled PPI use in the morning on an empty stomach 2 hours prior to EVG/Cobi and 2) PPI administered in the evening to treat overnight acid breakthrough, allowing for a 12-hour staggering from the morning dose of EVG/Cobi.

6. Drugs Used in the Trial

The trial was conducted between November 2009 (first subject screened) and January 2010 (last subject observation) using the following drugs:

EVG: 150 mg tablets (Lot # AJ0705E1 and Expiration Date November 2011).
Cobi: 150 mg tablets (Lot # BB0904B1 and Expiration Date July 2011).
Omeprazole: 20 mg capsule (Lot # D014772 and Expiration Date May 2011).
Famotidine: 40 mg tablet (Lot # Y1803 and Expiration Date May 2011).

7. Plasma sampling and PK/Statistical Analysis

Serial blood samples to measure plasma concentrations of EVG and Cobi were collected up to 24 hours on days 7 and 16 and the standard non-compartmental pharmacokinetic parameters were computed. The applicant did not collect plasma samples to determine concentration of famotidine and omeprazole.

The primary end points were AUC_{tau} and C_{max} for EVG. 90 % CIs were constructed for the ratio of the geometric least square (GLS) means (test treatment: reference treatment) for each of the three test treatments (treatment B, C, and D) versus reference treatment (treatment A)

Similar evaluation was also performed for the secondary parameters of interest (AUC_{tau}, C_{max} and C_{tau} of GS-9350 and C_{tau} of EVG).

8. Results

Subject Disposition and Demographics

33 eligible subjects were enrolled and randomized (11 subjects in each of the three treatment sequences AB, AC, and AD). A total of 32 subjects completed the study (one subject {3648-1023} in treatment sequence AB discontinued the

study due to pregnancy). Subject 3648-1023 did not have evaluable PK profile for the treatment pair of interest and was excluded from the PK analysis. Hence, the PK analysis datasets for EVG and cobicistat included a total of 32 subjects (n = 10 subjects in sequence AB and n = 11 subjects each in sequence AC and AD)

Of the 33 enrolled subjects, 18 (54.5 %) subjects were male, 25 (75.8 %) were white, and 8 (24.2 %) were black. The mean age was 34 years (range 19-45 years), mean BMI was 25.2 kg/m^2 (range 20.1 to 30 kg/m^2) and the mean CL_{cr} was 116.6 mL/min (range 71.9 mL/min to 154.1 mL/min).

Bioanalysis

Plasma concentrations of EVG and Cobi were determined using validated LC/MS/MS methods. All samples were analyzed within the storage stability window (135 days at -70^{0}C).

<u>EVG</u>

The calibration samples for EVG ranged from 20 ng/mL to 10,000 ng/mL. The quality control samples were prepared at three concentration levels (60, 800, and 8000 ng/mL).

The % CV of the QCs ranged from 3 % - 9.1 % and the % RE (relative error) ranged from -2.2 % to 7.2 %.

<u>Cobi</u>

The calibration samples for Cobi ranged from 5 ng/mL to 2500 ng/mL. The quality control samples were prepared at three concentration levels (15, 200, and 2000 ng/mL).

The % CV of the QCs ranged from 4.8 % - 18.6 % and the % RE ranged from -0.8 % to 7.4 %.

Pharmacokinetics

<u>EVG</u>

Table 1 shows the steady state mean pharmacokinetic parameters of EVG in the various treatment sequences.

Table 1: Mean steady state mean pharmacokinetic parameters of EVG in the various treatment sequences

EVG PK Parameter	Sequence AB (n = 10)[a]		Sequence AC (n = 11)		Sequence AD (n = 11)	
	Treatment A	Treatment B	Treatment A	Treatment C	Treatment A	Treatment D
AUC_{tau} (ng•h/mL) Mean (%CV)	22,726.1 (18.9)	23,536.8 (20.4)	22,874.6 (22.9)	25,115.5 (22.5)	23,557.7 (21.7)	24,871.1 (26.9)
C_{max} (ng/mL) Mean (%CV)	2138.8 (23.0)	2200.1 (23.6)	2392.2 (27.8)	2766.6 (24.5)	2331.8 (26.8)	2413.8 (28.5)
C_{tau} (ng/mL) Mean (%CV)	440.5 (33.5)	525.7 (37.1)	418.9 (48.1)	474.9 (46.6)	387.9 (35.2)	429.4 (37.5)
T_{max} (h) Median (Q1, Q3)	4.00 (4.00, 4.50)	4.25 (3.50, 4.50)	4.00 (4.00, 4.00)	4.00 (3.50, 4.00)	3.50 (3.50, 4.50)	4.50 (4.00, 5.00)
$T_{1/2}$ (h) Median (Q1, Q3)	11.35 (8.62, 13.67)	11.02 (9.05, 13.64)	10.63 (8.15, 12.42)	9.78 (8.62, 13.27)	9.29 (6.75, 11.00)	8.74 (7.95, 10.49)

CV = coefficient of variation, PK = pharmacokinetic, Q1 = first quartile, Q3 = third quartile

Mean PK parameters of EVG (when given as EVG/Cobi) were similar across the three treatment sequences. Of note, the mean systemic exposures of EVG are in the range of the mean systemic exposures of EVG observed in some of the other healthy volunteer trials.

Table 2 shows the statistical comparison of the mean pharmacokinetic parameters of EVG in the various treatment sequences.

Table 2: Statistical comparison of the mean pharmacokinetic parameters of EVG in the various treatment sequences

EVG PK Parameter	Sequence AB Test (B) vs. Reference (A) GLS Means Ratio as % (90% CI) (n = 10)[a]	Sequence AC Test (C) vs. Reference (A) GLS Means Ratio as % (90% CI) (n = 11)	Sequence AD Test (D) vs. Reference (A) GLS Means Ratio as % (90% CI) (n = 11)
AUC_{tau} (ng•h/mL)	103.39 (94.87, 112.68)	109.95 (101.64, 118.94)	104.57 (92.85, 117.78)
C_{max} (ng/mL)	102.31 (89.42, 117.06)	116.35 (103.76, 130.46)	102.99 (91.88, 115.45)
C_{tau} (ng/mL)	117.90 (105.13, 132.23)	113.16 (95.85, 133.59)	110.18 (91.90, 132.10)

The results of the statistical analysis suggest that the mean change in EVG AUC_{tau} was not significant (< 10 %) when the PK of EVG (co-administered with Cobi) was determined either 12 hours after famotidine (treatment B), 2 hours after omeprazole (treatment C) or 12 hours after omeprazole (treatment D).

GS-9350

Table 3 shows the steady state mean pharmacokinetic parameters of Cobi in the various treatment sequences.

Table 3: Mean steady state mean pharmacokinetic parameters of Cobi in the various treatment sequences

GS-9350 PK Parameter	Sequence AB (n = 10)[a]		Sequence AC (n = 11)		Sequence AD (n = 11)	
	Treatment A	Treatment B	Treatment A	Treatment C	Treatment A	Treatment D
AUC_{tau} (ng•h/mL) Mean (%CV)	12,318.0 (51.3)	12,691.5 (45.1)	11,567.0 (29.8)	10,816.9 (35.9)	10,132.6 (20.2)	10,135.9 (26.2)
C_{max} (ng/mL) Mean (%CV)	1727.9 (37.2)	1767.0 (30.5)	1736.9 (21.4)	1569.3 (24.4)	1572.8 (15.1)	1516.0 (25.8)
C_{tau} (ng/mL) Mean (%CV)	35.3 (140.4)	39.3 (131.2)	34.6 (109.7)	28.5 (96.1)	27.0 (81.9)[b]	25.9 (69.0)
T_{max} (h) Median (Q1, Q3)	3.00 (2.50, 4.50)	4.00 (3.00, 4.50)	3.00 (2.50, 4.50)	4.50 (3.50, 4.50)	3.00 (2.50, 4.50)	4.50 (4.00, 4.50)
$T_{1/2}$ (h) Median (Q1, Q3)	3.30 (2.86, 3.48)	3.41 (2.83, 3.61)	3.11 (2.99, 3.89)	3.13 (3.00, 3.30)	3.45 (2.79, 3.68)	3.33 (2.95, 3.74)

Table 4 shows the statistical comparison of the mean pharmacokinetic parameters of Cobi in the various treatment sequences.

Table 4: Statistical comparison of the mean pharmacokinetic parameters of Cobi in the various treatment sequences

GS-9350 PK Parameter	Sequence AB Test (B) vs. Reference (A) GLS Mean Ratio (%) (90% CI) (n = 10)[a]	Sequence AC Test (C) vs. Reference (A) GLS Mean Ratio (%) (90% CI) (n = 11)	Sequence AD Test (D) vs. Reference (A) GLS Mean Ratio (%) (90% CI) (n = 11)
AUC_{tau} (ng•h/mL)	104.54 (101.51, 107.65)	92.36 (84.79, 100.59)	98.70 (88.98, 109.49)
C_{max} (ng/mL)	103.60 (99.07, 108.34)	90.16 (81.82, 99.34)	94.39 (85.10, 104.70)
C_{tau} (ng/mL)	115.41 (105.70, 126.00)	92.76 (73.56, 116.98)	102.04 (81.58, 127.64)[b]

CI = confidence interval, GLS = geometric least squares, PK = pharmacokinetic

The results of the statistical analysis suggest that the mean change in Cobi (co-administered with EVG) AUC_{tau} was not significant (< 10 %) in all the three treatment sequences.

9. Results

- Mean change in EVG AUC_{tau} was not clinically significant when the PK of EVG (co-administered with Cobi) was determined either 12 hours after famotidine (treatment B), 2 hours after omeprazole (treatment C) or 12 hours after omeprazole (treatment D).

- Mean change in Cobi AUC_{tau} was not clinically significant when the PK of Cobi (co-administered with EVG) was determined either 12 hours after famotidine (treatment B), 2 hours after omeprazole (treatment C) or 12 hours after omeprazole (treatment D).

10. Conclusion

The results of the trial suggest that:

- Omeprazole can either be administered 2 hours before- or staggered by 12 hours when given with EVG/Cobi.
- Famotidine can be staggered by 12 hours when given with EVG/Cobi.

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<u>GS-US-216-0122</u>

1. Title

A Phase 1 Study to Evaluate the Relative Bioavailability and Pharmacokinetics of Cobicistat-boosted Elvitegravir upon Simultaneous Co-administration with a Representative H2-Receptor Antagonist.

2. Objectives

The study was designed to determine whether simultaneous co-administration of EVG and cobi and H2 receptor antagonist reduces the relative bioavailability of EVG and cobi.

Reviewer's Note:

The results of trial GS-US-216-0120 show that the pharmacokinetics of EVG and Cobi are not significantly altered when the administration EVG and cobi and famotidine, an H2RA, is staggered by 12 hours.

3. Trial Design

Phase I, randomized, open-label, single-center, multiple-dose, 2-way crossover study in healthy HIV-uninfected male and female volunteers.

Subjects were randomized to 1 of 2 treatment sequences:

Treatment Sequence AB:

Day 1-8: EVG (1 X 150 mg tablet) + Cobi (1 X 150 mg tablet) once daily, administered in the morning with a meal.

Day 9-16: EVG (1 X 150 mg tablet) + Cobi (1 X 150 mg tablet) once daily + famotidine (1 X 40 mg tablet) once daily; all administered simultaneously in the morning with a meal

Treatment Sequence BA:

Day 1-8: EVG (1 X 150 mg tablet) + Cobi (1 X 150 mg tablet) once daily + famotidine (1 X 40 mg tablet) once daily; all administered simultaneously in the morning with a meal.

Day 9-16: EVG (1 X 150 mg tablet) + Cobi (1 X 150 mg tablet) once daily, administered in the morning with a meal.

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Subjects were confined to the study center starting on day 0 and remained there until completion of assessments on day 17. Subjects received study medication from days 1 through day 16.

All doses of the study drugs were administered at the study center at approximately the same time with 240 mL of water and within 5 minutes of consuming a meal. On days of PK assessment, the meal provided was standardized and subjects were restricted from food intake until after collection of the four hour blood draw, relative to EVG and Cobi dosing. Subjects were restricted from water consumption 1 hour before and 2 hours after dosing with EVG and Cobi, except for the 240 mL of water given with the study drugs.

4. Dose Selection

The dose of EVG (150 mg QD) and Cobi (150 mg QD) selected for this trial are the doses of EVG and Cobi in the QUAD (EVG/Cobi/Emtricitabine/Tenofovir) tablet.

The dose of famotidine (Pepcid$^{®}$; 40 mg QD) is the approved dose of famotidine.

5. Drugs Used in the Trial

The trial was conducted between July 2010 (first subject screened) and August 2010 (last subject observation) using the following drugs:

EVG: 150 mg tablets (Lot # AJ0705E1 and Expiration Date November 2010).
Cobi: 150 mg tablets (Lot # BB1004B1 and Expiration Date April 2012).
Famotidine: 40 mg tablet (Lot # LK10100 and Expiration Date December 2011).

6. Plasma sampling and PK/Statistical Analysis

Serial blood samples to measure plasma concentrations of EVG and Cobi were collected upto 24 hours on days 8 and 16 and the standard non-compartmental pharmacokinetic parameters were computed. The applicant did not collect plasma samples to determine the concentration of famotidine.

The primary pharmacokinetic parameters were AUC_{tau} and C_{max} for EVG. 90 % CIs were constructed for the ratio of the geometric means (test treatment/reference treatment) of the AUC and C_{max} for EVG.

Similar evaluation was also performed for the secondary parameters of interest (AUC_{tau}, C_{max} and C_{tau} of Cobi, and C_{tau} of EVG).

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Bioanalysis

Plasma concentrations of EVG and Cobi were determined using validated LC/MS/MS methods. All samples were analyzed within the storage stability window for EVG (135 days at -70^{0}C) and Cobi (135 days at -70^{0}C).

EVG

The calibration samples for EVG ranged from 20 ng/mL to 10,000 ng/mL. The quality control samples were prepared at three concentration levels (60, 800, and 8000 ng/mL).

The % CV of the QCs ranged from 4.3 % - 7.5 % and the % RE (relative error) ranged from -6.1 % to 6.1 %.

Cobi

The calibration samples for Cobi ranged from 5 ng/mL to 2500 ng/mL. The quality control samples were prepared at three concentration levels (15, 200, and 2000 ng/mL).

The % CV of the QCs ranged from 5.2 % - 8.6 % and the % RE ranged from -1.2 % to 2.8 %.

7. Results

Subject Disposition and Demographics

16 subjects were enrolled and randomized in the trial. All 16 subjects completed study drug dosing as well as all study visits.

Of the 16 subjects enrolled, 8 subjects (50 %) were male, 10 (62.5 %) were white, and 6 (37.5 %) were black or of African heritage. The median age for all enrolled subjects was 33 years and the median body mass index (BMI) was 25.8 kg/m^2.

Pharmacokinetics

Table 1 shows the summary of the steady state mean EVG pharmacokinetic parameters after administration of either EVG/co or EVG/co co-administered with famotidine.

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Table 1: Mean EVG pharmacokinetic parameters after administration of either EVG/co or EVG/co co-administered with famotidine

EVG PK Parameter	Reference Treatment A (n = 16)	Test Treatment B (n = 16)
AUC_{tau} (ng•h/mL) Mean (%CV)	23995.8 (15.8)	24947.1 (20.6)
C_{max} (ng/mL) Mean (%CV)	2238.9 (26.2)	2268.7 (28.6)
C_{tau} (ng/mL) Mean (%CV)	420.2 (32.1)	458.3 (38.5)
T_{max} (h) Median (Q1, Q3)	4.00 (3.75, 4.50)	4.00 (3.25, 4.25)
$T_{1/2}$ (h) Median (Q1, Q3)	8.21 (6.67, 9.41)	8.40 (7.59, 10.45)

Reference Treatment A = EVG 150 mg + COBI 150 mg
Test Treatment B = EVG 150 mg + COBI 150 mg + Famotidine 40 mg

Table 2 shows the statistical comparison of the mean pharmacokinetic parameters of EVG after administration of EVG/co and EVG/co co-administered with famotidine.

Table 2: Statistical comparison of the mean pharmacokinetic parameters of EVG after administration of EVG/co and EVG/co co-administered with famotidine

EVG Plasma PK Parameters	Geometric Least-Squares Mean		Geometric Least-Squares Mean Ratio % of Test/Reference (B/A) (90%CI)
	Test Treatment B	Reference Treatment A	
AUC_{tau} (ng•h/mL) n = 16	24,430.18	23,703.52	103.07 (98.10, 108.29)
C_{max} (ng/mL) n = 16	2183.55	2173.15	100.48 (91.66, 110.15)
C_{tau} (ng/mL) n = 16	427.70	399.24	107.13 (98.45, 116.57)

Reference Treatment A = EVG 150 mg + COBI 150 mg
Test Treatment B = EVG 150 mg + COBI 150 mg + famotidine 40 mg

The results of the statistical analysis suggest that the mean change in EVG AUC_{tau} and C_{max} was not clinically significant when EVG/co was co-administered with famotidine vs when EVG/co was administered alone.

Cobicistat

Table 3 shows the steady state mean pharmacokinetic parameters of cobicistat after administration of EVG/co and EVG/co co-administered with famotidine.

Table 3: Mean pharmacokinetic parameters of cobicistat after administration of EVG/co and EVG/co co-administered with famotidine

COBI PK Parameter	Reference Treatment A (n = 16)	Test Treatment B (n = 16)
AUC_{tau} (ng•h/mL) Mean (%CV)	10001.8 (19.9)	10464.6 (24.2)
C_{max} (ng/mL) Mean (%CV)	1533.4 (20.7)	1631.7 (21.7)
C_{tau} (ng/mL) Mean (%CV)	22.1 (72.7)	25.7 (78.4)
T_{max} (h) Median (Q1, Q3)	3.50 (3.25, 4.00)	3.50 (3.00, 4.50)
$T_{1/2}$ (h) Median (Q1, Q3)	2.99 (2.83, 3.54)	3.08 (2.75, 3.66)

Reference Treatment A = EVG 150 mg + COBI 150 mg

Test Treatment B = EVG 150 mg + COBI 150 mg + Famotidine 40 mg

Table 4 shows the statistical comparison of the mean pharmacokinetic parameters of cobicistat after administration of EVG/co and EVG/co co-administered with famotidine.

Table 4: Statistical comparison of the mean pharmacokinetic parameters of cobicistat after administration of EVG/co and EVG/co co-administered with famotidine

COBI Plasma PK Parameters	Geometric Least-Squares Mean		Geometric Least-Squares Mean Ratio % of Test/Reference (B/A) (90%CI)
	Test Treatment B	Reference Treatment A	
AUC_{tau} (ng•h/mL) n = 16	10,168.69	9825.88	103.49 (96.77, 110.68)
C_{max} (ng/mL) n = 16	1594.56	1503.76	106.04 (99.40, 113.12)
C_{tau} (ng/mL) n = 16	20.07	18.03	111.34 (99.81, 124.20)

Reference Treatment A = EVG 150 mg + COBI 150 mg

Test Treatment B = EVG 150 mg + COBI 150 mg + Famotidine 40 mg

The results of the statistical analysis suggest that the mean change in Cobi (co-administered with EVG) AUC_{tau} and C_{max} was not significant (< 10 %) when EVG/co was co-administered with famotidine vs when EVG/co was administered alone.

8. Results

- Mean change in EVG AUC_{tau} and C_{max} was not significant (< 10 %) when EVG/co was co-administered with famotidine vs when EVG/co was administered alone.
- Mean change in Cobi (co-administered with EVG) AUC_{tau} and C_{max} was not significant (< 10 %) when EVG/co was co-administered with famotidine vs when EVG/co was administered alone.

9. Conclusion

Famotidine can be co-administered with EVG/Cobi without any dose adjustments.

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1. Title

A Phase 1 Study Evaluating the Drug Interaction Potential Between Cobicistat-Boosted Elvitegravir (EVG/co) Administered Once-Daily and Atazanavir (ATV), Rosuvastatin (ROS), or Rifabutin (RIF)

2. Objectives

The primary objectives of the trial were:

- Evaluate the pharmacokinetics of EVG and ATV following once-daily co-administration of EVG and ATV co-boosted with Cobi relative to EVG/co or ATV/r administration.
- Evaluate the pharmacokinetics of EVG and ROS following once-daily co-administration of EVG/co and ROS relative to EVG/co or ROS administration.
- Evaluate the pharmacokinetics of EVG and RIF following once-daily co-administration of EVG/co and RIF relative to EVG/co or RIF administration.

Reviewer's Note:

One of the objectives of the trial was to evaluate the pharmacokinetics of EVG and ATV following co-administration of EVG and ATV co-boosted with Cobi. As the QUAD tablet will not be combined with other antiretroviral drugs and will only be recommended to be used with non-antiretroviral drugs, this review will focus on the drug-drug interaction results from EVG/co and ROS and EVG/co and RIF; results from the EVG/co and ATV DDI evaluation are included for information purposes only.

3. Trial Design

Phase I, open-label, crossover, partially-randomized, multiple and single dose, multiple-cohort study. Following screening procedures and day 0 assessments, eligible subjects were enrolled and received study drug treatments beginning on day 1 (except Cohort II which began treatment on day 4).

The study had 3 cohorts. Following completion of screening and baseline, subjects were randomized to treatment cohort I or II, or assigned to treatment cohort III.

Fig 1 shows the treatment schema.

Fig 1: Treatment schema

	Day 1	Day 2-3	Day 4-13	Day 14	Day 15-24	Day 25	Day 32
Cohort I	Treatment A	washout	Treatment B	washout	Treatment C	Treatment D	7-day follow up

	Day 1-3	Day 4-13	Day 14	Day 15-24	Day 31
Cohort II	-	Treatment B	washout	Treatment E	7-day follow up

	Day 1-10	Day 11-23	Day 24-33	Day 34-46	Day 53
Cohort III	Treatment C	Treatment F	washout	Treatment G	7-day follow up

Cohort I:

Treatment A: ROS 10 mg (1 X 10 mg tablet) single dose administered in the morning with food.

Treatment B: EVG 85 mg (1 X 85 mg tablet) + Cobi 150 mg (1 X 150 mg tablet) + ATV 300 mg (1 X 300 mg capsule) administered once daily in the morning with food.

Treatment C: EVG 150 mg (1 X 150 mg tablet) + Cobi 150 mg (1 X 150 mg tablet) administered once daily in the morning with food.

Treatment D: EVG 150 mg (1 X 150 mg tablet) + Cobi 150 mg (1 X 150 mg tablet) + ROS 10 mg (1 X 10 mg tablet) administered in the morning with food.

Cohort II:

Treatment B: EVG 85 mg (1 X 85 mg tablet) + Cobi 150 mg (1 X 150 mg tablet) + ATV 300 mg (1 X 300 mg capsule) administered once daily in the morning with food.

Treatment E: ATV 300 mg (1 X 300 mg capsule) + RTV 100 mg (1 X 100 mg capsule) administered once daily in the morning with food.

Cohort III:

Treatment C: EVG 150 mg (1 X 150 mg tablet) + Cobi 150 mg (1 X 150 mg tablet) administered once daily in the morning with food.

Treatment F: EVG 150 mg (1 X 150 mg tablet) + Cobi 150 mg (1 X 150 mg tablet) administered once daily + RIF 150 mg (1 X 150 mg capsule) administered every other day in the morning with food.

Treatment G: RIF 300 mg (2 X 150 mg capsules) administered once daily in the morning with food.

All study drugs were administered with 240 mL of water within 5 minutes of completion of a standardized meal (400 calories; approximately 13 gms fat). On days of intensive PK sampling (which followed an overnight fast), study treatments were administered in the morning within 5 minutes of completion of a meal. Subjects then refrained from food until after collection of the 4 hour PK sample, relative to drug dosing. Subjects were restricted from water consumption for 1 hour before and 2 hours after the morning dosing, except for the 240 mL water given with the study treatment.

4. Dose Selection

The dose of EVG (150 mg QD) and Cobi (150 mg QD) selected for this trial are the doses of EVG and Cobi in the QUAD (EVG/Cobi/Emtricitabine/Tenofovir) tablet.

The dose of ROS (10 mg), ATV (300 mg when co-administered with 100 mg ritonavir) and RIF (300 mg) are the approved doses. Due to the CYP3A inhibitory effect of Cobi, the evaluation of RIF 150 mg every other day is consistent with other drug-drug interaction trials of RIF and CYP3A inhibitor combinations such as ritonavir-boosted Protease Inhibitors.

5. Drugs Used in the Trial

The trial was conducted between July 2010 (first subject screened) and August 2010 (last subject observation) using the following drugs:

EVG: 150 mg tablets (Lot # AJ0802D1 and Expiration Date September 2012).
EVG: 85 mg tablets (Lot # AJ0802C1 and Expiration Date September 2012)
Cobi: 150 mg tablets (Lot # BB1004B1-A and Expiration Date April 2012).
ATV: 300 mg capsules (Lot # 0H5007A and Expiration Date August 2012)
ROS: 10 mg tablets (Lot # 112910 and Expiration Date July 2013)
RIF: 150 mg capsules (Lot # M002D and Expiration Date August 2013)
RTV: 100 mg capsule (Lot # 879672E21 and Expiration date June 2012).

6. Plasma sampling and PK/Statistical Analysis

Cohort I: Serial PK samples were collected on days 1, 13, 24 and 25. On days 1, 13, and 25, samples were collected upto 48 hours and on day 24, samples were collected up to 24 hours.

Cohort II: Serial PK samples were collected on days 13 and 24. On day 13, samples were collected up to 48 hours and on day 24, samples were collected up to 24 hours.

Cohort III: Serial PK samples were collected on days 10, 23, and 46. Samples were collected up to 24 hours on days 10 and 46; on day 23, samples were collected up to 48 hours.

Blood samples were collected to determine the pharmacokinetic profiles of ATV, EVG, ROS, RIF (and its metabolite 25-O-desacetylrifabutin), Cobi, and RTV.

The following treatment pairs were evaluated:

- EVG/co + ATV (treatment B in Cohort I) vs EVG/co (treatment C in Cohort I)
- EVG/co + ROS (treatment D in Cohort I) vs EVG/co (treatment C in Cohort I)
- EVG/co + RIF (treatment F in Cohort III) vs EVG/co (treatment C in Cohort III)
- EVG/co + ATV (treatment B in Cohort II) vs ATV/r (treatment E in Cohort II)
- EVG/co +ROS (treatment D in Cohort I) vs ROS (treatment A in Cohort I)
- EVG/co + RIF (treatment F in Cohort III) vs RIF (treatment G in Cohort III)

The primary pharmacokinetic end points were AUC_{tau} and C_{max} of EVG, ATV, ROS, RIF (and its metabolite 25-O-desacetylrifabutin). The secondary PK end points were C_{tau} of EVG, ATV, ROS, RIF, and 25-O-desacetylrifabutin.

Bioanalysis

Plasma concentrations of all analytes were determined using validated LC/MS/MS methods. All samples were analyzed within the storage stability period.

<u>EVG</u>

The calibration samples for EVG ranged from 20 ng/mL to 10,000 ng/mL. The quality control samples were prepared at three concentration levels (60, 800, and 8000 ng/mL).

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The % CV of the QCs ranged from 3.6 % - 4.7 % and the % RE (relative error) ranged from -0.1 % to 1.4 %.

Cobi

The calibration samples for Cobi ranged from 5 ng/mL to 2500 ng/mL. The quality control samples were prepared at three concentration levels (15, 200, and 2000 ng/mL).

The % CV of the QCs ranged from 3.7 % - 6 % and the % RE ranged from -1.5 % to 0.9 %.

ROS

The calibration samples for ROS ranged from 0.5 ng/mL to 50 ng/mL. The quality control samples were prepared at three concentration levels (0.15, 10, and 40 ng/mL).

The % CV of the QCs ranged from 2.6 % - 3.2 % and the % RE ranged from 0.9 % to 4 %.

RIF and 25-O-desacetylrifabutin

The calibration samples ranged from 1 to 1000 ng/mL. The quality control samples were prepared at four concentration levels (3, 50, 200, and 800 ng/mL).

The % CV of the QCs for rifabutin ranged from 5.6 % - 13.8 % and the % RE ranged from -2.2 % to 7.1 %. The % CV of the QCs for 25-O-desacetylrifabutin ranged from 2.1 % - 4.8 % and the % RE ranged from -0.2 % to 2.2%.

7. Results

Subject Disposition and Demographics

34 subjects were enrolled in the study. 22 subjects were randomized to cohorts 1 and 2 (12 subjects in Cohort I and 10 subjects in Cohort II), and 12 subjects were assigned to Cohort III. 29 subjects completed the study and 5 subjects discontinued due to an adverse event (2 subjects in Cohort I and 3 subjects in Cohort II). Hence, 10 subjects in Cohort I, 7 subjects in Cohort II, and 12 subjects in Cohort III completed all assessments.

Table 1 shows the demographics (based on 34 subjects enrolled) of the trial.

Table1: Demographics in trial GS-US-216-0123

Characteristic	Cohort I/ ABCD (N=12)	Cohort II/ BE (N=10)	Cohort III/ CFG (N=12)	Total (N=34)
Sex (n, %)				
Male	6 (50.0%)	5 (50.0%)	6 (50.0%)	17 (50.0%)
Female	6 (50.0%)	5 (50.0%)	6 (50.0%)	17 (50.0%)
Age at Day -1 (Years)				
Mean (SD)	35 (6.1)	32 (7.9)	35 (6.3)	34 (6.7)
Median	37	33	35	35
Min, Max	27, 42	19, 43	25, 44	19, 44
Race (n, %)				
American Indian or Alaska Native	0	0	0	0
Asian	0	0	0	0
Black or African Heritage	2 (16.7%)	2 (20.0%)	3 (25.0%)	7 (20.6%)
Native Hawaiian or Pacific Islander	0	0	0	0
White	10 (83.3%)	8 (80.0%)	9 (75.0%)	27 (79.4%)
Other	0	0	0	0
Ethnicity				
Hispanic/Latino	12 (100.0%)	8 (80.0%)	12 (100.0%)	32 (94.1%)
Nonhispanic/Latino	0	2 (20.0%)	0	2 (5.9%)
Weight (kg)				
Mean (SD)	76.3 (10.82)	75.6 (10.14)	75.0 (8.26)	75.6 (9.49)
Median	74.4	76.8	77.8	76.6
Min, Max	57.0, 95.1	59.7, 93.4	56.9, 88.5	56.9, 95.1
Height (cm)				
Mean (SD)	168.0 (9.70)	167.7 (7.15)	165.8 (6.42)	167.1 (7.75)
Median	167.5	167.5	166.3	167.0
Min, Max	152.0, 187.0	152.5, 178.0	156.0, 178.0	152.0, 187.0
Body Mass Index (kg/m^2)				
Mean (SD)	27.0 (2.57)	26.9 (3.18)	27.3 (2.25)	27.1 (2.58)
Median	27.8	27.9	28.0	27.9
Min, Max	22.3, 29.6	20.2, 30.0	22.0, 29.6	20.2, 30.0
Creatinine Clearance: Cockcroft-Gault (mL/min)				
Mean (SD)	129.1 (21.18)	138.6 (21.58)	139.5 (21.59)	135.6 (21.34)
Median	123.5	139.6	136.8	133.6
Min, Max	105.1, 172.7	102.6, 180.4	112.3, 178.2	102.6, 180.4

Note: Treatment A = ROS 10 mg; B = EVG 85 mg + COBI 150 mg + ATV 300 mg (EVG/co + ATV); C = EVG 150 mg + COBI 150 mg (EVG/co); D = EVG 150 mg + COBI 150 mg + ROS 10 mg (EVG/co + ROS); E = ATV 300 mg + RTV 100 mg (ATV/r); F = EVG 150 mg + COBI 150 mg + RIF 150 mg (EVG/co + RIF); G = RIF 300 mg.

Pharmacokinetics

The PK analysis sets for Cohort I (EVG, ATV, ROS, and COBI) included 10 subjects. Two subjects (subject 3648-4005 and 3648-4014) did not have

evaluable PK profiles for ATV, EVG, COBI or ROS and hence were excluded from the data listings.

The PK analysis for Cohort II included 8 subjects in the EVG, ATV, and Cobi analysis sets and 7 subjects in the RTV analysis sets. Two subjects (subject 3648-4013 and 3648-4022) in Cohort II did not have evaluable PK profiles for ATV, EVG, COBI, or RTV and were excluded from data listings and summaries. Subject 3648-4009 in Cohort II did not have evaluable PK profiles for RTV and was excluded from the RTV PK analysis datasets. This subject also did not have evaluable paired PK profiles for ATV and was excluded from summaries for paired comparisons of ATV between treatment B and treatment E.

No subjects in Cohort III were excluded from the PK analysis and the PK analysis sets for Cohort III included 12 subjects.

EVG:

Table 2 shows the mean pharmacokinetic parameters of EVG after administration of various treatments.

Table 2: Mean pharmacokinetic parameters of EVG after administration of various treatments

EVG PK Parameter	EVG/COBI + ROS in Cohort I (N=10)	EVG/COBI in Cohort I (N=10)	EVG/COBI + ATV in Cohort I (N=10)	EVG/COBI + ATV in Cohort II (N=8)	EVG/COBI + ATV in Cohorts I and II (N=18)	EVG/COBI + RIF in Cohort III (N=12)	EVG/COBI in Cohort III (N=12)
AUC_{tau} (ng·h/mL), Mean (%CV)	18,848.6 (30.5)	18695.8 (35.1)	21,577.0 (38.0)	21,668.8 (32.4)	21,617.8 (34.6)	18,397.4 (26.4)	23,041.7 (24.0)
C_{max} (ng/mL), Mean (%CV)	1992.9 (36.2)	2150.6 (40.9)	1844.3 (47.6)	1728.7 (37.8)	1792.9 (42.7)	2051.5 (25.4)	2250.9 (25.7)
C_{tau} (ng/mL), Mean (%CV)	306.4 (45.3)	318.2 (49.4)	595.0 (52.8)	499.8 (42.8)	552.7 (49.0)	164.1 (60.7)	452.1 (45.7)
T_{max} (h), Median (Q1, Q3)	4.50 (4.00, 4.50)	4.49 (4.00, 4.50)	4.50 (4.00, 4.50)	4.25 (4.00, 4.50)	4.50 (4.00, 4.50)	4.00 (4.00, 4.04)	4.25 (4.00, 4.50)
$T_{½}$ (h), Median (Q1, Q3)	8.72 (7.09, 9.30)	8.42 (7.65, 10.27)	12.04 (9.80, 23.16)	12.26 (10.01, 14.29)	12.04 (9.80, 14.74)	5.47 (4.62, 6.26)	9.99 (8.77, 13.07)

%CV=percentage coefficient of variation; Q1=first quartile; Q2=second quartile

Note: Subjects 4005 and 4014 in Cohort I and Subjects 4013 and 4022 in Cohort II did not have evaluable PK profiles for EVG and were excluded from data listings and summaries.

In cohort I, the mean systemic exposures of EVG were comparable following administration of EVG/co + ROS relative to EVG/co. In Cohort III, mean AUC_{tau} of EVG was lower and $T_{1/2}$ shorter when EVG/co was co-administered with dose

adjusted RIF (150 mg every other day) as compared with the mean pharmacokinetic parameters of EVG when EVG/co was administered alone.

Table 3 shows the statistical comparison of the pharmacokinetic parameters of EVG after administration of various treatments.

Table 3 : Statistical comparison of the pharmacokinetic parameters of EVG after administration of various treatments

Elvitegravir PK Parameter	Geometric Least Squares Means		Geometric Least Squares Mean Ratio (%)	90% Confidence Interval
	Test Treatment	Reference Treatment		
EVG/co + ROS in Cohort I (Test) vs EVG/co in Cohort I (Ref)	N=10	N=10		
AUC$_{tau}$ (ng•h/mL)	17,723.54	17,347.84	102.17	91.44, 114.15
C$_{max}$ (ng/mL)	1842.85	1961.73	93.94	82.61, 106.82
C$_{tau}$ (ng/mL)	261.96	266.76	98.20	83.42, 115.60
EVG/co + ATV in Cohort I (Test) vs EVG/co in Cohort I (Ref)	N=10	N=10		
AUC$_{tau}$ (ng•h/mL)	19,938.25	17,347.84	114.93	108.14, 122.16
C$_{max}$ (ng/mL)	1661.29	1961.73	84.68	75.34, 95.18
C$_{tau}$ (ng/mL)	511.64	266.76	191.80	163.43, 225.10
EVG/co + ATV in Cohort I and II (Test) vs EVG/co in Cohort I (Ref)	N=18	N=10		
AUC$_{tau}$ (ng•h/mL)	20,329.22	17,347.84	117.19	88.30, 155.53
C$_{max}$ (ng/mL)	1650.87	1961.73	84.15	61.79, 114.61
C$_{tau}$ (ng/mL)	489.08	266.76	183.34	117.38, 286.37
EVG/co + RIF in Cohort III (Test) vs EVG/co in Cohort III (Ref)	N=12	N=12		
AUC$_{tau}$ (ng•h/mL)	17,726.19	22,325.62	79.40	74.10, 85.07
C$_{max}$ (ng/mL)	1981.26	2173.93	91.14	83.56, 99.40
C$_{tau}$ (ng/mL)	133.69	406.97	32.85	26.93, 40.07

Ref=reference

Cobi:

Table 4 shows the mean pharmacokinetic parameters of Cobi after administration of the various treatments.

Table 4: Mean pharmacokinetic parameters of Cobi after administration of the various treatments

COBI PK Parameter	EVG/COBI + ROS in Cohort I (N=10)	EVG/COBI in Cohort I (N=10)	EVG/COBI + ATV in Cohort I (N=10)	EVG/COBI + ATV in Cohort II (N=8)	EVG/COBI + RIF in Cohort III (N=12)	EVG/COBI in Cohort III (N=12)
AUC_{tau} (ng•h/mL), Mean (%CV)	11,065.7 (38.1)	10,389.4 (38.6)	12,147.5 (30.7)	12,941.9 (20.7)	11,409.7 (29.2)	11,215.2 (18.5)
C_{max} (ng/mL), Mean (%CV)	1451.1 (25.2)	1399.7 (32.3)	1612.0 (24.3)	1836.2 (21.7)	1877.3 (28.8)	1642.2 (19.0)
C_{tau} (ng/mL), Mean (%CV)	34.2 (113.4)	32.3 (123.2)	46.6 (106.2)	32.3 (38.0)	6.5 (71.7)	22.8 (61.2)
T_{max} (h), Median (Q1, Q3)	3.00 (2.00, 4.50)	4.25 (3.00, 4.50)	3.00 (3.00, 3.50)	2.00 (2.00, 3.75)	3.00 (2.00, 3.25)	3.00 (3.00, 4.00)
$T_{½}$ (h), Median (Q1, Q3)	3.19 (3.07, 3.87)	3.10 (3.02, 3.40)	3.68 (3.32, 3.82)	3.51 (3.33, 3.60)	2.49 (2.06, 2.60)	3.33 (2.81, 3.67)

%CV=percentage coefficient of variation; Q1=first quartile; Q2=second quartile

Note: Subjects 4005 and 4014 in Cohort I and Subjects 4013 and 4022 in Cohort II did not have evaluable PK profiles for COBI and were excluded from data listings and summaries.

ROS:

Table 5 shows the mean steady state pharmacokinetic parameters of ROS, with- and without co-administration with EVG/co.

Table 5: Mean steady state pharmacokinetic parameters of ROS, with- and without co-administration with EVG/co

ROS PK Parameter	EVG/co + ROS in Cohort I (N=10)	ROS in Cohort I (N=10)
AUC_{inf} (ng•h/mL), Mean (%CV)	38.2 (39.9)	27.2 (40.6)
AUC_{last} (ng•h/mL), Mean (%CV)	34.2 (39.7)	23.9 (48.2)
C_{last} (ng/mL), Mean (%CV)	0.2 (52.7)	0.1 (0.0)
C_{max} (ng/mL), Mean (%CV)	5.0 (40.9)	2.7 (54.6)
T_{max} (h), Median (Q1, Q3)	4.50 (4.50, 4.50)	4.50 (4.50, 4.50)
$T_{½}$ (h), Median (Q1, Q3)	18.03 (16.69, 20.47)	21.15 (14.28, 26.96)

Table 6 shows the statistical comparison of the pharmacokinetic parameters of ROS, with- and without co-administration with EVG/co

Table 6: Statistical comparison of the pharmacokinetic parameters of ROS, with- and without co-administration with EVG/co

ROS PK Parameter	Geometric Least Squares Means		Geometric Least Squares Mean Ratio (%)	90% Confidence Interval
	Test Treatment	Reference Treatment		
EVG/co + ROS (Test) vs ROS (Ref) in Cohort I	N=10	N=10		
AUC_{inf} (ng•h/mL)	34.99	25.36	137.98	113.83, 167.25
C_{max} (ng/mL)	4.46	2.35	189.31	148.19, 241.84
C_{last} (ng/mL)	0.14	0.10	143.10	108.22, 189.22

Ref=reference

RIF:

Table 7 shows the mean steady state pharmacokinetic parameters of RIF, with- and without co-administration with EVG/co.

Table 7: Mean steady state pharmacokinetic parameters of RIF, with- and without co-administration with EVG/co

RIF PK Parameter	EVG/co + RIF in Cohort III (N=12)	RIF in Cohort III (N=12)
AUC_{tau} (ng•h/mL), Mean (%CV)	8369.6 (13.1)	9321.1 (29.3)
C_{max} (ng/mL), Mean (%CV)	565.7 (17.2)	528.9 (26.8)
C_{tau} (ng/mL), Mean (%CV)	73.5 (30.0)	80.1 (39.2)
T_{max} (h), Median (Q1, Q3)	4.50 (3.25, 4.50)	3.75 (3.50, 4.50)
$T_{\frac{1}{2}}$ (h), Median (Q1, Q3)	28.60 (22.06, 30.59)	11.71 (9.63, 13.58)

%CV=percentage coefficient of variation; Q1=first quartile; Q2=second quartile

Note: For RIF and 25-*O*-desacetylrifabutin, AUC_{tau} represents AUC(0-48) for test treatment F and doubled AUC(0-24) for reference treatment G.

Table 8 shows the statistical comparison of the pharmacokinetic parameters of RIF, with- and without co-administration with EVG/co.

Table 8: Statistical comparison of the pharmacokinetic parameters of RIF, with- and without co-administration with EVG/co

RIF PK Parameter	Geometric Least Squares Means		Geometric Least Squares Mean Ratio (%)	90% Confidence Interval
	Test Treatment	Reference Treatment		
EVG/co + RIF (Test) vs RIF (Ref) in Cohort III	N=12	N=12		
AUC$_{tau}$ (ng•h/mL)	8301.95	8984.81	92.40	82.86, 103.04
C$_{max}$ (ng/mL)	558.29	512.79	108.87	98.48, 120.37
C$_{tau}$ (ng/mL)	70.16	74.72	93.90	84.77, 104.01

Ref=reference

Note: For RIF and 25-*O*-desacetylrifabutin, AUC$_{tau}$ represents AUC(0-48) for test treatment F and doubled AUC(0-24) for reference treatment G.

25-*O*-desacetylrifabutin:

Table 9 shows the mean steady state pharmacokinetic parameters of 25-O-desacetylrifabutin after administration of rifabutin alone or co-administration of rifabutin with EVG/co

Table 9: Mean steady state pharmacokinetic parameters of 25-O-desacetylrifabutin after administration of rifabutin alone or co-administration of rifabutin with EVG/co

25-*O*-desacetylrifabutin PK Parameter	EVG/co + RIF in Cohort III (N=12)	RIF in Cohort III (N=12)
AUC$_{tau}$ (ng•h/mL), Mean (%CV)	3014.9 (20.6)	517.9 (47.4)
C$_{max}$ (ng/mL), Mean (%CV)	149.6 (22.4)	32.2 (39.3)
C$_{tau}$ (ng/mL), Mean (%CV)	18.4 (38.9)	4.0 (60.7)
T$_{max}$ (h), Median (Q1, Q3)	5.00 (4.50, 5.00)	4.00 (3.50, 4.50)
T$_{\frac{1}{2}}$ (h), Median (Q1, Q3)	15.71 (11.87, 17.34)	10.48 (8.63, 12.59)

%CV=percentage coefficient of variation; Q1=first quartile; Q2=second quartile

Note: For RIF and 25-*O*-desacetylrifabutin, AUC$_{tau}$ represents AUC(0-48) for test treatment F and doubled AUC(0-24) for reference treatment G.

Table 10 shows the statistical analysis of the pharmacokinetic parameters of 25-*O*-desacetylrifabutin after administration of rifabutin alone or co-administration of rifabutin with EVG/co.

Table 10: Statistical analysis of the pharmacokinetic parameters of 25-O-desacetylrifabutin after administration of rifabutin alone or co-administration of rifabutin with EVG/co

25-*O*-desacetylrifabutin PK Parameter	Geometric Least Squares Means		Geometric Least Squares Mean Ratio (%)	90% Confidence Interval
	Test Treatment	Reference Treatment		
EVG/co + RIF (Test) vs RIF (Ref) in Cohort III	N=12	N=12		
AUC$_{tau}$ (ng•h/mL)	2954.91	472.73	625.07	508.37, 768.57
C$_{max}$ (ng/mL)	146.28	30.20	484.43	408.94, 573.85
C$_{tau}$ (ng/mL)	16.94	3.43	494.20	404.20, 604.24

Ref=reference

Note: For RIF and 25-*O*-desacetylrifabutin, AUC$_{tau}$ represents AUC(0-48) for test treatment F and doubled AUC(0-24) for reference treatment G.

Rifabutin and 25-*O*-desacetylrifabutin total activity

Statistical comparison of the total antimycobacterial activity (represented by AUC$_{tau}$ in µM*hr) was conducted following administration of RIF alone (reference treatment; treatment G in Cohort III) and when rifabutin was co-administered with EVG/co (test treatment' treatment F in Cohort III). The total antimycobacterial activity was calculated by adding AUC$_{tau}$ of rifabutin (normalized by m.wt of 847.02 g/mol) and AUC$_{tau}$ of 25-*O*-desacetylrifabutin (normalized by m.wt of 804.97 g/mol).

Table 11 shows the total antimycobacterial activity when rifabutin was administered alone and co-administered with EVG/co.

Table 11: Total antimycobacterial activity when rifabutin was administered alone and co-administered with EVG/co

RIF and 25-*O*-desacetylrifabutin PK Parameter	EVG/co + RIF in Cohort III (N=12)	RIF in Cohort III (N=12)
Total Activity (uM•h), Mean (%CV)	13.6 (11.0)	11.6 (29.9)

%CV=percentage coefficient of variation

Note: Molecular weights used in the conversion to micromoles are as follows: RIF: 847.02 g/mole; 25-*O*-desacetylrifabutin: 804.97 g/mole.

Table 12 shows the statistical comparison of the total antimycobacterial activity when rifabutin was administered alone and co-administered with EVG/co.

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Table 12: Statistical comparison of the total antimycobacterial activity when rifabutin was administered alone and co-administered with EVG/co

RIF and 25-*O*-desacetylrifabutin PK Parameter	Geometric Least Squares Means		Geometric Least Squares Mean Ratio (%)	90% Confidence Interval
	Test Treatment	Reference Treatment		
EVG/co + RIF (Test) vs RIF (Ref) in Cohort III	N=12	N=12		
Total Activity (uM•h)	13.55	11.21	120.85	107.33, 136.09

Ref=reference

Note: Molecular weights used in the conversion to micromoles are as follows: RIF: 847.02 g/mole; 25-*O*-desacetylrifabutin: 804.97 g/mole.

8. Results

EVG

- Co-administration of EVG/co with ROS did not significantly alter the mean pharmacokinetic parameters (AUC_{tau}, C_{max}, and C_{tau}) of EVG (all changes were less than 10 %) as compared with when EVG/co was administered alone.
- Co-administration of EVG/co with RIF (150 mg every other day) decreased the mean AUC_{tau}, C_{max}, and C_{tau} by ~20 %, 9 %, and 67 %, respectively as compared with when EVG/co was administered alone.

ROS

- Co-administration of EVG/co with ROS increased the mean AUC_{tau}, C_{max}, and C_{tau}) of ROS by ~38 %, 90 %, and 43 % as compared with when ROS was given alone.

RIF and 25-*O*-desacetylrifabutin

- Co-administration of EVG/co with RIF (150 mg every other day) did not significantly alter the pharmacokinetic parameters (AUC_{tau}, C_{max}, and C_{tau}) of RIF when RIF (150 mg every other day) was co-administered with EVG/co as compared with when RIF (300 mg once daily) was administered alone.
- Co-administration of EVG/co with RIF (150 mg every other day) increased the mean AUC_{tau}, C_{max}, and C_{tau} of 25-O-desacetylrifabutin by 525 %, 384 %, and 394 % when RIF (150 mg every other day) was co-administered with EVG/co as compared with when RIF (300 mg once daily) was administered alone.

9. Conclusion

ROS:

- When rosuvastatin is administered in combination with QUAD, titrate rosuvastatin dose carefully and use the lowest necessary dose while monitoring for safety.

RIF:

- EVG/co and Rifabutin should not be co-administered due to the potential for significant reduction in EVG C_{tau}.

The dosing recommendations based on this trial conducted with EVG/Cobi can be extended to the QUAD tablet (EVG/Cobi/Emtricitabine/Tenofovir) due to the similarity in exposures of EVG and Cobi when given either as EVG/Cobi or the QUAD tablet.

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GS-US-216-0124

1. Title

A Phase 1, Open Label, Parallel Design, Multiple Dose Study to Evaluate the Pharmacokinetics of Cobicistat (Cobi)-Boosted Elvitegravir (EVG) in Subjects with Severe Renal Impairment

2. Objectives

The primary objective of the study was to evaluate whether the pharmacokinetics of EVG and/or cobicistat is altered in patients with severe renal impairment (and not on dialysis) compared with subjects with normal renal function. The secondary objectives of the study were to evaluate the safety of EVG/co in subjects with severe renal impairment and to evaluate renal function in subjects with severe renal impairment prior to, during, and after dosing with EVG/co.

Reviewer's Note:

The draft renal guidance (March 2010) version suggests that for drugs that are primarily metabolized or secreted in the bile, the effect of impaired renal function on the pharmacokinetics of a drug can be determined by using a "reduced PK study" design. The "reduced PK study" design compares the pharmacokinetics of the drug in subjects at the extremes of renal function (i.e. subjects with normal renal function and subjects with End Stage Renal Disease (ESRD) not yet on dialysis).

Based on the results of the mass balance trial (GS-US-183-0126) conducted with EVG/ritonavir, 6.7 % (primarily as glucuronidated metabolites; no unchanged drug) of EVG dose was excreted in the urine. Similarly, the results of the mass balance trial conducted with GS-9350 (cobicistat) showed that cobicistat is minimally excreted (~6.3 %; 5.4 % as unchanged drug) in the urine. Hence the "abbreviated study design" to assess the impact of altered renal function on the pharmacokinetics of EVG and Cobi is acceptable.

3. Trial Design

Multicenter, open label, parallel group, multiple dose (7 days) study. 24 male and non-pregnant female subjects were to be enrolled in the study; 12 subjects with severe renal impairment (eGFR < 30 mL/min) not requiring or anticipated to require dialysis within 90 days of study entry and 12 matched controls with normal renal function (eGFR ≥ 90 mL/min). The eGFR was estimated using the Cockroft-Gault equation using actual body weight. Each subject in the control group was to be matched for age (± 10 years), gender, and body mass index (BMI; ± 15 %, 18 ≤ BMI ≤ 34 kg/m^2).

Following screening procedures, eligible subjects in both groups received EVG 150 mg (1 X 150 mg tablet) + Cobi (1 X 150 mg tablet) once daily in the morning, immediately following a standard meal. The doses of EVG and Cobi selected for this trial are the doses of EVG and Cobi in the QUAD (EVG/Cobi/Emtricitabine/Tenofovir) tablet.

On day 7, the standardized meal was given following an overnight fast for at least 8 hours and plasma samples for pharmacokinetic (PK) analysis of EVG and COBI were collected at the following time points relative to EVG/co dosing: 0 (pre-dose), and 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 16, and 24 hours post-dose. At 4 hours post dose, an additional blood sample was collected for the measurement of EVG and COBI plasma protein binding.

On the morning of Day 7, subjects completely voided their bladders prior to administration of the standardized breakfast before initiation of the 24-hour urine collection. Urine was subsequently collected at pre-dose void and over intervals of 0-4, 4-8, 8-12, and 12-24 hours. The volume of urine collected over each interval was measured, and a well-mixed aliquot from each collection was taken for the PK analysis of EVG and Cobi.

Reviewer's Note:

Per the applicant, urine samples were not analyzed for determining the urinary concentrations of EVG and Cobi because there were no clinically relevant changes in the pharmacokinetics of EVG and Cobi observed in subjects with severe renal impairment as compared with subjects with normal renal function. Hence, renal clearance of EVG and Cobi, a protocol defined PK parameter, was not determined.

On Days -1 through 7, and on Day 14, a single blood sample was collected for serum creatinine analysis. On Day -1, the sample was collected at a time equivalent to approximately 4 hours after the scheduled dosing time on Days 1 through 7. On Days 1 through 7 the sample was collected 4 hours post-dose. On Day 14 the sample was collected at approximately the same time as on Days -1 and 7 (i.e., equivalent to 4 hours post-dose).

5. Drugs Used in the Trial

The trial was conducted between January 2011 (first subject screened) and April 2011 (last subject observation) using the following drugs:

EVG: 150 mg tablets (Lot # AJ0802D1 and Expiration Date April 2012).
Cobi: 150 mg tablets (Lot # BB1004B1 and Expiration Date September 2012)

6. Pharmacokinetic & Pharmacodynamic Assessments and Statistical Analysis

Pharmacokinetic Assessments

Pharmacokinetic parameters of EVG and Cobi were computed using non-compartmental pharmacokinetic analysis. To evaluate the effect of renal impairment on EVG and Cobi pharmacokinetics, AUC_{tau}, C_{max} and C_{tau} were compared between the two renal function groups after natural log transformation.

Pharmacodynamic Assessments

The exploratory pharmacodynamic end points were:

1) $eGFR_{CG}$:

$$\text{Male:} \quad \frac{(140 - \text{age in years}) \times (\text{weight in kg})}{72 \times (\text{serum creatinine in mg/dL})} = eGFR_{CG}\ (\text{mL/min})$$

$$\text{Female:} \quad \frac{(140 - \text{age in years}) \times (\text{weight in kg}) \times 0.85}{72 \times (\text{serum creatinine in mg/dL})} = eGFR_{CG}\ (\text{mL/min})$$

where serum creatinine was measured at 4 hours post dose (or equivalent time on non-dosing days), age was calculated at each visit, and weight was measured at each visit.

2) $eGFR_{MDRD}$:

$$186 \times S_{Cr}^{-1.154} \times \text{age}^{-0.203} \times 1.21\ (\text{if black}) \times 0.742\ (\text{if female}) = eGFR_{MDRD}\ (\text{mL/min/1.73 m}^2)$$

where S_{cr} is serum creatinine concentration (in mg/dL) measured at 4 hours post dose (or equivalent time on non-dosing days), age was calculated at each visit, and weight was measured at each visit.

For both $eGFR_{CG}$ and $eGFR_{MDRD}$, pre-dose values, post dose values, and changes and percent changes from baseline were summarized by renal function group for each scheduled visit (days 1 through 7 and day 14). Within each renal function group, a paired t-test was used to evaluate if changes from baseline in $eGFR_{CG}$ and $eGFR_{MDRD}$ were significantly different from 0, based on a 2 sided test with an alpha level of 0.05.

To evaluate whether $eGFR_{CG}$ and $eGFR_{MDRD}$ on days -1, 7, and 14 were similar within each renal function group, geometric mean ratios for each comparison of interest (i.e.,day 7 vs day -1 and day 14 vs day -1) and their associated 90 % CI were computed using an ANOVA model.

Statistical Analysis

90 % confidence intervals were constructed for the ratio of geometric means for each specified parameter for each analyte.

7. Results

Subject Disposition and Demographics

13 subjects with severe renal impairment (eGFR < 30 mL/min) were enrolled in the trial. 12 out of the 13 subjects received all planned doses and completed the trial; one subject withdrew consent (after having received 2 doses of the study drugs) to continued participation in the study (because of personal reasons) and dropped out of the trial. This subject was included in the safety analysis but excluded from the EVG and Cobi PK analysis.

The applicant could not find a matched healthy control subject for one of the renally impaired subjects; hence, recruitment of healthy subjects was terminated after 11 subjects with normal renal function (eGFR ≥ 90 mL/min) were enrolled. Each of the 11 healthy subjects received all planned doses of the study drugs and completed the study.

Table 1 shows the demographics (based on safety analysis datasets) of the subjects enrolled in the trial.

Table 1: Demographics (based on safety analysis datasets) of Subjects Enrolled in the Trial

	Renal Function Group	
	Normal Renal Function $eGFR_{CG} \geq 90$ mL/min (N=11)	Severe Renal Impairment $eGFR_{CG} < 30$ mL/min (N=13)
Characteristics		
Age at Day -1 (Years)		
Mean (SD)	63 (8.8)	66 (9.1)
Median	65	70
Min, Max	42, 75	44, 76
Sex, n (%)		
Male	5 (45.5%)	6 (46.2%)
Female	6 (54.5%)	7 (53.8%)
Race, n (%)		
Asian	1 (9.1%)	1 (7.7%)
Black or African Heritage	0	1 (7.7%)
White	10 (90.9%)	11 (84.6%)
Ethnicity, n (%)		
Hispanic/Latino	2 (18.2%)	3 (23.1%)
Non-Hispanic/Latino	9 (81.8%)	10 (76.9%)
Weight (kg)		
Mean (SD)	79.4 (10.58)	70.8 (16.13)
Median	82.3	70.4
Min, Max	62.5, 94.3	40.9, 99.0
BMI (kg/m^2)		
Mean (SD)	27.8 (2.81)	26.3 (4.01)
Median	28.0	27.8
Min, Max	21.9, 31.6	19.3, 31.5
$eGFR_{CG}$ (mL/min)		
Mean (SD)	97.2 (15.23)	23.5 (5.23)
Median	94.8	24.8
Min, Max	82.1, 136.1	14.2, 31.0
$eGFR_{MDRD}$ (mL/min/1.73m^2)		
Mean (SD)	90.4 (17.27)	22.3 (7.80)
Median	94.4	22.4
Min, Max	71.6, 131.4	10.2, 33.6

Baseline $eGFR_{CG}$ and $eGFR_{MDRD}$ were calculated using serum creatinine concentrations at a time on Day -1 equivalent to 0 hours (predose) on subsequent dosing days.

Bioanalysis

Plasma concentrations of EVG and Cobi were determined using validated LC/MS/MS methods. All samples were analyzed in the time frame supported by frozen stability storage data.

Table 2 shows the performance characteristics of the assay used to quantify the concentrations of EVG and Cobi.

Table 2 : Performance characteristics of the assay used to quantify the concentrations of EVG and Cobi

Parameter	Plasma	
	EVG	**COBI**
Linear range (ng/mL)	20–10,000	5–2,500
LLQ[a] (ng/mL)	20	5
Interassay precision range (%CV)[a]	2.8–8.1	3.9–8.3
Interassay accuracy range (%RE)[a]	-8.0 to 5.7	-0.3 to 9.7
Stability in frozen matrix (days)	585 days at -70°C	121 days at -10°C to -30°C 365 days at -60°C to -80°C

a   LLQ, lower limit of quantitation; %CV, percentage coefficient of variation; %RE, percent relative error

Pharmacokinetics

EVG

Fig 1 shows the mean steady state pharmacokinetic profile of EVG in subjects with severe renal impairment and subjects with normal renal function.

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Fig 1: Mean steady state pharmacokinetic profile of EVG in subjects with severe renal impairment and subjects with normal renal function

Semi-Logarithmic Scale

Normal Renal Function (n=): 11 11 11 11 11 11 11 11 11
Severe Renal Impairment (n=): 12 12 12 12 12 12 12 12 12

Table 3 shows the mean steady state pharmacokinetic parameters of EVG in subjects with severe renal impairment and subjects with normal renal function

Table 3:   Mean steady state pharmacokinetic parameters of EVG in subjects with severe renal impairment and subjects with normal renal function

	Renal Function Group	
EVG PK Parameter	**Normal** $eGFR_{CG} \geq 90$ mL/min N = 11	**Severe Renal Impairment** $eGFR_{CG} < 30$ mL/min N = 12
AUC_{tau} (ng·h/mL)	34597.3 (26.9)	26044.6 (24.3)
C_{max} (ng/mL)	3348.6 (33.9)	2224.1 (26.7)
C_{tau} (ng/mL)	761.1 (38.9)	531.2 (42.3)
T_{max} (h)	4.50 (4.00, 4.50)	4.25 (3.75, 4.52)
T_{last} (h)	24.00 (24.00, 24.05)	24.00 (24.00, 24.07)
$t_{\frac{1}{2}}$ (h)	13.97 (9.43, 19.15)	10.66 (8.40, 12.29)
V_z/F (mL)	110303.4 (46.7)	92867.3 (27.6)
CL/F (mL/min)	76.8 (25.2)	101.7 (25.6)

Data are presented as mean (%CV) except T_{max}, T_{last}, and $t_{\frac{1}{2}}$, which are presented as median (Q1, Q3)

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Reviewer's Note:

The mean steady state systemic exposure (AUC$_{tau}$) of EVG in subjects with normal renal function observed in this trial were significantly higher than the mean steady state systemic exposure of EVG observed in other trials in which EVG was co-administered with Cobi. The mean systemic exposure of EVG in some of the other clinical trials in which EVG was co-administered with Cobi are as follows:

GS-US-216-0116: AUC$_{tau}$ of EVG (when co-administered with GS-9350 formulation 2): 22,246 ng*hr/mL
GS-US-216-0123: AUC$_{tau}$ of EVG: 17,347 and 22, 325 ng*hr/mL
GS-US-183-0133: AUC$_{tau}$ of EVG: 21,278.5 ng*hr/mL

Since there were differences in systemic exposures of EVG in subjects with normal renal function in this trial and other trials, the "decrease" in EVG exposures in subjects with severe renal impairment relative to subjects with normal renal function should be interpreted with caution.

Table 4 shows the statistical comparison of the mean pharmacokinetic parameters of EVG in subjects with severe renal impairment and subjects with normal renal function.

Table 4: Statistical comparison of the mean pharmacokinetic parameters of EVG in subjects with severe renal impairment and subjects with normal renal function

EVG PK Parameter	Geometric Least-Squares Mean		Geometric Least-Squares Means Ratio (%) (90% CI)
	Test Severe Renal Impairment eGFR$_{CG}$ < 30 mL/min (N = 12)	Reference Normal Renal Function eGFR$_{CG}$ ≥ 90 mL/min (N = 11)	
AUC$_{tau}$ (ng·h/mL)	25316.69	33530.63	75.50 (62.82, 90.75)
C$_{max}$ (ng/mL)	2154.03	3200.46	67.30 (54.78, 82.68)
C$_{tau}$ (ng/mL)	491.26	711.29	69.07 (51.82, 92.06)

<u>Cobi</u>

Table 5 shows the mean pharmacokinetic parameters of Cobi in subjects with severe renal impairment and subjects with normal renal function

Table 5: Mean pharmacokinetic parameters of Cobi in subjects with severe renal impairment and subjects with normal renal function

COBI PK Parameter	Renal Function Group	
	Normal $eGFR_{CG} \geq 90$ mL/min N = 11	Severe Renal Impairment $eGFR_{CG} < 30$ mL/min N = 12
AUC_{tau} (ng•h/mL)	14212.4 (24.4)	18552.9 (36.8)
C_{max} (ng/mL)	1709.3 (22.4)	2149.4 (35.0)
C_{tau} (ng/mL)	97.0 (61.0)	165.7 (128.2)
T_{max} (h)	2.50 (2.50, 3.00)	3.04 (2.50, 4.50)
T_{last} (h)	24.00 (24.00, 24.05)	24.00 (24.00, 24.07)
$t_{1/2}$ (h)	5.14 (3.65, 6.58)	4.07 (3.36, 6.54)
V_z/F (mL)	85228.2 (34.5)	73493.8 (60.6)
CL/F (mL/min)	187.2 (28.2)	156.0 (43.2)

Data are presented as mean (%CV) except T_{max}, T_{last}, and $t_{1/2}$, which are presented as median (Q1, Q3)

Reviewer's Note:

In intensive PK sub-studies within Phase 2 and Phase 3 studies of the QUAD tablet, the mean (% CV) of Cobi systemic exposure (AUC_{tau}) was 8300 (46) ng*hr/mL. Therefore, the mean systemic exposures of Cobi observed in this trial (in subjects with normal renal function) were higher than the mean systemic exposures of Cobi observed in Phase 2 and Phase 3 trials. Therefore, the "true" magnitude of increase in Cobi systemic exposures in subjects with severe renal impairment is potentially higher than shown in this trial.

Table 6 shows the statistical comparison of the mean pharmacokinetic parameters of Cobi in subjects with severe renal impairment and subjects with normal renal function.

Table 6: Statistical comparison of the mean pharmacokinetic parameters of Cobi in subjects with severe renal impairment and subjects with normal renal function

COBI PK Parameter	Geometric Least-Squares Mean		Geometric Least-Squares Means Ratio (%) (90% CI)
	Test Severe Renal Impairment $eGFR_{CG} < 30$ mL/min (N = 12)	Reference Normal Renal Function $eGFR_{CG} \geq 90$ mL/min (N = 11)	
AUC_{tau} (ng·h/mL)	17313.3	13797.50	125.48 (98.57, 159.73)
C_{max} (ng/mL)	2041.71	1667.84	122.42 (99.82, 150.13)
C_{tau} (ng/mL)	89.02	78.88	112.85 (56.75, 224.40)

Plasma Protein Binding

The mean (SD) (%) free fraction (plasma unbound concentration) was determined on day 7 in subjects with severe renal impairment and matched control subjects with normal renal function. The free fraction of EVG was 1.42 (0.17) in renally impaired subjects and 1.16 (0.16) in the matched control subjects. In the case of Cobi, the free fraction was 2.47 (0.62) in the renally impaired subjects and 2.49 (0.29) in the matched control subjects.

Pharmacodynamics

Mean (SD) values for $eGFR_{CG}$ and $eGFR_{MDRD}$ were calculated at days 0, 7, and 14. Further, mean changes in these values at day 7 and day 14, relative to day -1 (baseline) were determined.

Table 7 shows the $eGFR_{CG}$ and change from baseline at day 7 and day 14 (safety analysis datasets)

Table 7: $eGFR_{CG}$ and change from baseline at day 7 and day 14 (safety analysis datasets)

	Estimated GFR_{CG} (mL/min; 4 h Postdose) Mean (SD)		
	Day -1	Day 7	Day 14
Normal Renal Function ($eGFR_{CG} \geq 90$ mL/min) (n = 11)	102.0 (15.87)	93.0 (17.41)	100.6 (21.57)
Change from Day -1 (mL/min)	——	-9.0 (12.47) (p = 0.037)	-1.4 (13.54) (p = 0.74)
Severe Renal Impairment ($eGFR_{CG} < 30$ mL/min) (n = 12)[a]	23.6 (5.71)	20.6 (4.89)	22.5 (5.96)
Change from Day -1 (mL/min)	——	-2.9 (4.84) (p = 0.062)	-1.0 (2.40) (p = 0.18)

a   At Day -1, for subjects with severe renal impairment, n = 13

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A decrease in eGFR$_{CG}$ was observed in both group of subjects at day 7 relative to day -1; among subjects with severe renal impairment, eGFR$_{CG}$ was 10.5 % lower on day 7 as compared with day -1 and among subjects with normal renal function, eGFR$_{CG}$ was 8.4 % lower on day 7 as compared with day -1. The observed decreases were reversible with values returning to baseline levels by day 14.

Reviewer's Note:

Based on the mean estimates shown in table 7, among subjects with severe renal impairment, eGFR$_{CG}$ was 12.7 % lower on day 7 as compared with day -1 and among subjects with normal renal function, eGFR$_{CG}$ was 8.8 % lower on day 7 as compared with day -1.

Table 8 shows the eGFR$_{MDRD}$ and change from baseline at day 7 and day 14 (safety analysis datasets)

Table 8: eGFR$_{MDRD}$ and change from baseline at day 7 and day 14 (safety analysis datasets)

	Estimated GFR$_{MDRD}$ (mL/min; 4 h Postdose) Mean (SD)		
	Day -1	Day 7	Day 14
Normal Renal Function (eGFR$_{CG}$ ≥ 90 mL/min) (n = 11)	95.7 (18.40)	86.4 (16.55)	94.2 (19.30)
Change from Day -1 (mL/min)	——	-9.3 (14.54) (p = 0.059)	-1.5 (13.50) (p = 0.72)
Severe Renal Impairment (eGFR$_{CG}$ < 30 mL/min) (n = 12)[a]	22.3 (8.10)	20.2 (9.35)	22.0 (9.71)
Change from Day -1 (mL/min)	——	-2.4 (6.06) (p = 0.20)	-0.6 (3.57) (p = 0.56)

a   At Day -1, for subjects with severe renal impairment, n = 13.

A decrease in eGFR$_{MDRD}$ was observed in both group of subjects at day 7 relative to day -1; among subjects with severe renal impairment, eGFR$_{MDRD}$ was 10.8 % lower on day 7 as compared with day -1 and among subjects with normal renal function, eGFR$_{MDRD}$ was 8.9 % lower on day 7 as compared with day -1. The observed decreases were reversible with values returning to baseline levels by day 14.

Reviewer's Note:

Based on the mean estimates shown in table 8, among subjects with severe renal impairment, eGFR$_{MDRD}$ was 9.4 % lower on day 7 as compared with day -1 and among subjects with normal renal function, eGFR$_{MDRD}$ was 9.7 % lower on day 7 as compared with day -1.

The applicant hypothesizes that the decrease in eGFR were due to the inhibition of proximal tubular secretion of creatinine by Cobi.

8. Results

Compared with subjects with normal renal function:

- Mean AUC, C_{max} and C_{tau} of EVG decreased by 24 %, 33 %, and 31 %, respectively, in subjects with severe renal impairment as compared with subjects with normal renal function.

- Mean AUC, C_{max} and C_{tau} of Cobi increased by 25 %, 22 %, and 13 %, respectively, in subjects with severe renal impairment as compared with subjects with normal renal function.

9. Discussion and Conclusion

The results of this trial showed that the pharmacokinetics of EVG and Cobi was not significantly altered in subjects with severe renal impairment (eGFR < 30 mL/min) as compared with subjects with normal renal function. As previously mentioned, the mean systemic exposures of EVG and Cobi in subjects with normal renal function in this trial were higher than the mean systemic exposures of EVG and Cobi in other trials, therefore, the "true" effect of renal impairment on the pharmacokinetics of EVG and Cobi may not have been fully characterized.

Considering that elimination of EVG and Cobi through the renal route is minimal (as shown in the mass balance trials of EVG and Cobi) and the QUAD tablet will only be used in subjects with CrCL ≥ 70 mL/min, renal impairment is not expected to significantly alter the pharmacokinetics of either EVG and Cobi.

The dosing recommendations based on the renal impairment trial conducted with EVG/Cobi can be extended to the QUAD tablet (EVG/Cobi/Emtricitabine/Tenofovir) based on the following considerations:

- Similarity in exposures of EVG and Cobi when given either as EVG/Cobi or the QUAD tablet.
- No significant differences in systemic exposures (and hence no dose adjustment) of either emtricitabine or tenofovir between subjects with normal renal function (CrCL≥ 90 mL/min)   and subjects with CrCl between 70-90 mL/min

QUAD tablet can be given to subjects with eGFR ≥ 70 mL/min without any dose adjustments.

In Vivo Trials Using Fixed Dose Combination Tablet

S. No.	Study Number	Description of the Trial	Page Number
1	GS-US-236-0105	A Phase 1 Study to Determine the Effect of Food on the Pharmacokinetics of Elvitegravir, Emtricitabine, Tenofovir DF, plus pharmacoenhancer (GS-9350; Cobicistat) Fixed Dose Combination Tablet	335
2	GS-US-236-0106	A Phase 1, Open Label, Drug Interaction Study Evaluating the Effect of Elvitegravir/Emtricitabine/Tenofovir DF/GS-9350 Fixed Dose Combination Tablet on the Pharmacokinetics of a Representative Hormonal Contraceptive Medication, Norgestimate/Ethinyl Estradiol	347
3	GS-US-236-0110	A Phase 1 Multiple Dose Study to Evaluate the Relative Bioavailability of Two Elvitegravir/Emtricitabine/Tenofovir DF/GS-9350 Fixed-Dose Combination Tablet Formulations	359

GS-US-236-0105

1. Title

A Phase 1 Study to Determine the Effect of Food on the Pharmacokinetics of Elvitegravir, Emtricitabine, Tenofovir DF, plus pharmacoenhancer (GS-9350; Cobicistat) Fixed Dose Combination Tablet

2. Objectives

The study was designed to evaluate the effect of food (high-calorie/high-fat meal or light meal) on the individual components of the fixed dose combination tablet (EVG/Cobi/FTC/TDF).

Reviewer's Note:

The terms "fixed dose combination tablet" and "QUAD" are used interchangeably in this review.

3. Trial Design

Phase I, randomized, open-label, single-dose, three treatment, three-period, six-sequence crossover study evaluating the pharmacokinetics of the individual components of the QUAD tablet under fasted conditions and two different fed conditions (high calorie/high-fat meal and light meal).

Following screening and baseline assessments, subjects were randomized (1:1:1:1:1:1) to one of the six treatment sequences as shown in table 1.

Table 1: Treatment Sequences in the Trial

Treatment Sequence	Day 1	Day 8	Day 15
I	A	B	C
II	A	C	B
III	B	A	C
IV	B	C	A
V	C	A	B
VI	C	B	A

The study treatments were as follows:

Treatment A: Single oral FDC tablet administered in the morning under **fasted conditions**.

Treatment B: Single oral FDC tablet administered in the morning under fed conditions **(light meal; 373 kcal, 20 % fat)**.

Treatment C: Single oral FDC tablet administered in the morning under fed conditions **(high calorie, high fat meal; 800 kcal, 50 % fat)**.

On day 1, 8, and 15, following an overnight fast (no food or liquids, except water, for at least 10 hours), subjects randomized to treatments B and C were to complete 100 % of the standardized breakfast within 30 minutes. Subjects assigned to treatment A were to maintain the fast. FDC was administered within 5 minutes of meal completion for subjects receiving treatment B and treatment C. All subjects were restricted from water consumption 1 hour before and 2 hours after dosing, except for the 240 mL of water given with the study drug.

After study drug administration on days 1, 8, and 15, all subjects were required to fast until the 4-hour post dose blood draw when a meal was provided. Subsequent meals and/or snacks were standardized to contain similar calorie and fat content and were taken at approximately the same time each day. Food and beverages containing caffeine/xanthine or alcohol were restricted within 48 hours of study drug dosing and while subjects were confined at the study facility.

4. Dose Selection

The dose of the individual components of the FDC tablet used in this trial is identical to the doses of the individual components in the to-be-marketed FDC tablets. Page 21 of the "Clinical Overview Section" indicates that the formulation administered in this trial was the Phase 3 (and proposed commercial) formulation.

5. Drugs Used in the Trial

The trial was conducted between Jan 8, 2009 (first subject screened) and February 17, 2009 (last subject observation) using the FDC tablet. The lot number and expiration date of the FDC tablets are BK0803B1 and June 30, 2010, respectively.

6. Plasma sampling and PK/Statistical Analysis

Serial blood samples to measure plasma concentrations of EVG, Cobi, FTC, and TFV (tenofovir) were collected up to 48 hours post-dose on days 1, 8, and 15. The plasma concentration-time data was used to compute the standard non-compartmental pharmacokinetic parameters. Plasma concentration of EVG at 0.5, 1.5 and 2.5 hours was not determined.

90 % confidence intervals (90 % CI) were constructed about the ratio of the geometric means for each pharmacokinetic parameter. A food effect on a

pharmacokinetic parameter was concluded if the 90 % CI of the geometric mean ratio of test treatment to reference treatment was outside the bioequivalence boundary of 80% to 125 %.

Reviewer's Note Regarding Changes to Planned Analysis

The applicant made the following two changes to the pharmacokinetic analysis:

- *In addition to assessing C_{max} and AUC_{inf}, AUC_{last} was also determined.*
- *In addition to comparing AUC_{last}, AUC_{inf}, and C_{max} of the two test conditions (high-calorie/high fat meal or light meal) with the reference condition (fasted) for each analyte, the aforementioned pharmacokinetic parameters were also compared between the two test conditions (high-calorie/high-fat meal versus light meal) for each analyte.*

Bioanalysis

Plasma concentrations of EVG and Cobi were determined using validated LC/MS/MS methods.

EVG

The calibration samples for EVG ranged from 20 ng/mL to 10,000 ng/mL. The LLOQ was 20 ng/mL. The quality control samples were prepared at three concentration levels (50, 750, and 7500 ng/mL).

The % CV of the QCs ranged from 4.4 % to 6.4 % and the % RE (relative error) ranged from -5.9 % to 0.5 %.

Cobi

The calibration samples for Cobi ranged from 5 ng/mL to 2500 ng/mL. The LLOQ was 5 ng/mL. The quality control samples were prepared at three concentration levels (15, 500, and 2000 ng/mL).

The % CV of the QCs ranged from 8 % to 9.2 % and the % RE ranged from -2.6 % to 2 %.

FTC

The calibration samples for FTC ranged from 5 ng/mL to 3059.5 ng/mL. The LLOQ was 5 ng/mL. The quality control samples were prepared at three concentration levels (15.5, 510, and 2550 ng/mL).

The % CV of the QCs ranged from 4.5 % to 5.4 % and the % RE ranged from -3.9 % to 1.5 %.

Reference ID: 3153809

<u>TFV</u>

The calibration samples ranged from 10 to 1007 ng/mL. The LLOQ was 10 ng/mL.   The quality control samples were prepared at three concentration levels (25, 252, and 755 ng/mL).

The % CV of the QCs ranged from 2.7 % to 4.8 % and the % RE ranged from 0 to 2.4 %.

7. Results

Subject Disposition and Demographics

46 subjects were screened, 22 were screening failures, and the remaining 24 eligible subjects were randomized equally to one of six treatment sequences. All 24 subjects completed the trial and were included in the pharmacokinetic analysis set for each analyte.

Of the 24 subjects enrolled in the trial, there was an equal distribution of male and female subjects. 15 (62.5 %) subjects were white and 9 (37.5 %) subjects were black or of African heritage. The median (min,max) age was 36 (21,45) years, weight was 70.8 (60.6, 91) kg, BMI was 26.6 (20.9-29.9) kg/m^2 and creatinine clearance was 114.6 (90.2-148.8) mL/min.

Pharmacokinetics

EVG

Fig 1 shows the mean concentration-time profile of EVG after single dose administration of the QUAD tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal.

Fig 1: Mean concentration-time profile of EVG after single dose administration of the QUAD tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal
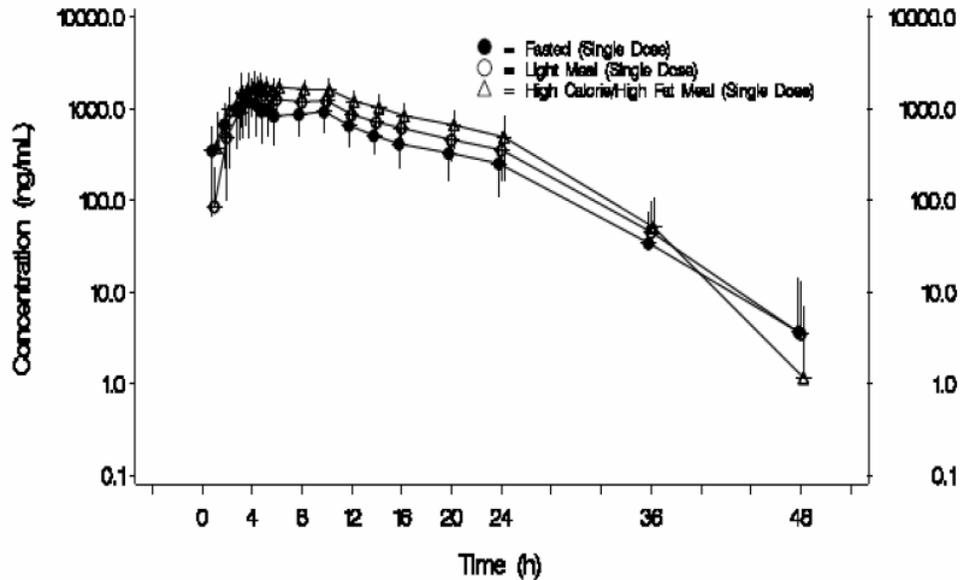

Table 2 shows the mean pharmacokinetic parameters of EVG after single dose administration of the QUAD tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal.

Table 2: Mean pharmacokinetic parameters of EVG after single dose administration of the QUAD tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal

EVG PK Parameter N=24	EVG/FTC/TDF/ GS-9350 HC/HF Meal	EVG/FTC/TDF/GS-9350 Light Meal	EVG/FTC/TDF/GS-9350 Fasted
C_{max} (ng/mL) Mean (% CV)	2227.1 (27.1)	1762.1 (31.5)	1492.8 (40.3)
T_{max} (h) Median (Q1, Q3)	4.50 (3.50, 7.00)	4.50 (4.00, 5.00)	4.00 (3.50, 5.50)
C_{last} (ng/mL) Mean (% CV)	95.1 (74.8)	82.0 (115.2)	79.3 (95.0)
T_{last} (h) Median (Q1,Q3)	36.00 (24.00, 36.00)	36.00 (24.00, 36.00)	36.00 (24.00, 36.00)
AUC_{last} (ng•h/mL) Mean (% CV)	28019.9 (22.6)	20351.6 (28.0)	15618.5 (40.2)
AUC_{exp} (%) Mean (% CV)	2.9 (89.9)	3.7 (120.4)	5.1 (102.6)
AUC_{inf} (ng•h/mL) Mean (% CV)	28796.7 (21.6)	21123.3 (27.5)	16433.1 (38.6)
$T_{\frac{1}{2}}$ (h) Median (Q1, Q3)	5.03 (4.24, 5.94)	5.31 (4.74, 6.66)	5.86 (4.97, 7.57)

CV: coefficient of variation, HC: high calorie, HF: high fat, Q1: first quartile, Q3: third quartile

Table 3 shows the statistical comparison of the mean pharmacokinetic parameters of EVG after single dose administration of the QUAD tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal.

Table 3: Statistical comparison of the mean pharmacokinetic parameters of EVG after single dose administration of the QUAD tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal

Treatment Condition N=24	EVG PK Parameters		
	C_{max} (ng/mL)	AUC_{inf} (ng•h/mL)	AUC_{last} (ng•h/mL)
HC/HF Meal GLSM	2151.74	28159.64	27338.54
Light Meal GLSM	1678.32	20269.50	19505.47
Fasted GLSM	1376.72	15095.22	14298.38
HC/HF Meal vs. Fasted GLSM ratio (90% CI), %	156.29 (138.44, 176.45)	186.55 (165.56, 210.20)	191.20 (169.56, 215.61)
Light Meal vs. Fasted GLSM ratio (90% CI), %	121.91 (107.98, 137.63)	134.28 (119.17, 151.30)	136.42 (120.97, 153.83)
HC/HF Meal vs. Light Meal GLSM ratio (90% CI), %	128.21 (113.56, 144.74)	138.93 (123.29, 156.54)	140.16 (124.29, 158.05)

CI=confidence interval, GLSM=geometric least-squares mean, HC=high calorie, HF=high fat
GLSMs were obtained using a mixed-effects model.

Cobi

Table 4 shows the mean pharmacokinetic parameters of Cobi after single dose administration of the QUAD tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal

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Table 4: Mean pharmacokinetic parameters of Cobi after single dose administration of the QUAD tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal

GS-9350 PK Parameter, N= 24	EVG/FTC/TDF/GS-9350 HC/HF Meal	EVG/FTC/TDF/GS-9350 Light Meal	EVG/FTC/TDF/GS-9350 Fasted
C_{max} (ng/mL) Mean (% CV)	944.1 (43.9)	1243.1 (35.5)	1189.1 (34.5)
T_{max} (h) Median (Q1, Q3)	4.50 (3.00, 5.00)	4.00 (3.00, 4.50)	3.00 (2.00, 4.00)
C_{last} (ng/mL) Mean (% CV)	21.1 (80.4)	16.4 (70.3)	15.5 (96.4)
T_{last} (h) Median (Q1,Q3)	24.00 (22.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
AUC_{last} (ng•h/mL) Mean (% CV)	6570.2 (49.1)	8012.3 (44.4)	8290.9 (49.5)
AUC_{exp} (%) Mean (% CV)	1.5 (80.4)	1.0 (61.6)	1.0 (92.7)
AUC_{inf} (ng•h/mL) Mean (% CV)	6678.9 (49.5)	8092.3 (44.5)	8368.9 (49.7)
$T_{½}$ (h) Median (Q1, Q3)	3.00 (2.67, 3.53)	3.04 (2.72, 3.70)	2.97 (2.87, 3.70)

CV: coefficient of variation, HC: high calorie, HF: high fat, Q1: first quartile, Q3: third quartile

Table 5 shows the statistical comparison of the mean pharmacokinetic parameters of Cobi after single dose administration of the QUAD tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal.

Table 5: Statistical comparison of the mean pharmacokinetic parameters of Cobi after single dose administration of the QUAD tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal

Treatment Condition N=24	GS-9350 PK Parameters[a]		
	C_{max} (ng/mL)	AUC_{inf} (ng•h/mL)	AUC_{last} (ng•h/mL)
HC/HF Meal GLSM	845.51	5816.23	5727.63
Light Meal GLSM	1156.80	7207.17	7138.74
Fasted GLSM	1117.47	7020.03	6951.03
HC/HF Meal vs. Fasted GLSM ratio (90% CI), %	75.66 (68.44, 83.64)	82.85 (72.52, 94.65)	82.40 (71.92, 94.40)
Light Meal vs. Fasted GLSM ratio (90% CI), %	103.52 (93.64, 114.44)	102.67 (89.87, 117.29)	102.70 (89.64, 117.66)
HC/HF Meal vs. Light Meal GLSM ratio (90% CI), %	73.09 (66.12, 80.80)	80.70 (70.64, 92.19)	80.23 (70.03, 91.92)

CI=confidence interval, GLSM=geometric least squares mean, HC=high calorie, HF=high fat
GLSMs were obtained using a mixed-effects model.

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Emtricitabine (FTC)

Table 6 shows the mean pharmacokinetic parameters of FTC after single dose administration of the QUAD tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal.

Table 6: Mean pharmacokinetic parameters of FTC after single dose administration of the QUAD tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal

FTC PK Parameter N=24	EVG /FTC /TDF/GS-9350 HC/HF Meal	EVG/FTC/TDF/GS-9350 Light Meal	EVG/FTC/TDF/GS-9350 Fasted
C_{max} (ng/mL) Mean (% CV)	1823.5 (26.5)	1811.5 (28.8)	1908.7 (29.1)
T_{max} (h) Median (Q1, Q3)	2.25 (2.00, 3.50)	2.75 (2.00, 3.00)	2.00 (1.50, 3.00)
C_{last} (ng/mL) Mean (% CV)	16.8 (34.7)	17.9 (43.2)	16.4 (39.0)
T_{last} (h) Median (Q1,Q3)	48.00 (48.00, 48.00)	48.00 (48.00, 48.00)	48.00 (48.00, 48.00)
AUC_{last} (ng•h/mL) Mean (% CV)	10417.9 (19.1)	10293.6 (19.6)	10987.6 (21.8)
AUC_{exp} (%) Mean (% CV)	3.5 (59.7)	4.3 (84.7)	3.3 (64.5)
AUC_{inf} (ng•h/mL) Mean (% CV)	10785.2 (18.8)	10738.6 (18.6)	11348.6 (21.0)
$T_{½}$ (h) Median (Q1, Q3)	13.98 (11.67, 15.03)	13.85 (11.84, 18.55)	14.31 (12.51, 15.89)

CV: coefficient of variation, HC: high calorie, HF: high fat, Q1: first quartile, Q3: third quartile

Table 7 shows the statistical comparison of the mean pharmacokinetic parameters of FTC after single dose administration of the QUAD tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal.

Table 7: Statistical comparison of the mean pharmacokinetic parameters of FTC after single dose administration of the QUAD tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal

Treatment Condition N=24	FTC PK Parameters		
	C_{max} (ng/mL)	AUC_{inf} (ng•h/mL)	AUC_{last} (ng•h/mL)
HC/HF Meal GLSM	1756.19	10608.74	10240.55
Light Meal GLSM	1740.91	10567.28	10108.96
Fasted GLSM	1825.06	11088.13	10716.68
HC/HF Meal vs. Fasted GLSM ratio (90% CI), %	96.23 (87.22, 106.16)	95.68 (91.58, 99.95)	95.56 (91.45, 99.85)
Light Meal vs. Fasted GLSM ratio (90% CI), %	95.39 (86.46, 105.24)	95.30 (91.23, 99.56)	94.33 (90.28, 98.56)
HC/HF Meal vs. Light Meal GLSM ratio (90% CI), %	100.88 (91.44, 111.29)	100.39 (96.10, 104.88)	101.30 (96.95, 105.85)

CI: confidence interval, GLSM: geometric least squares mean, HC: high calorie, HF: high fat
GLSMs were obtained using a mixed-effects model.

Tenofovir (TFV)

Table 8 shows the mean pharmacokinetic parameters of tenofovir after single dose administration of the QUAD tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal

Table 8: Mean pharmacokinetic parameters of tenofovir after single dose administration of the QUAD tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal

TFV PK Parameter N=24	EVG/FTC/TDF/GS-9350 HC/HF Meal	EVG/FTC/TDF/GS-9350 Light Meal	EVG/FTC/TDF/GS-9350 Fasted
C_{max} (ng/mL) Mean (% CV)	355.7 (45.7)	386.3 (29.2)	326.2 (33.4)
T_{max} (h) Median (Q1, Q3)	2.00 (1.50, 3.25)	2.00 (1.50, 2.50)	1.25 (1.00, 2.00)
C_{last} (ng/mL) Mean (% CV)	15.1 (16.8)	15.4 (19.5)	13.9 (23.9)
T_{last} (h) Median (Q1,Q3)	48.00 (48.00, 48.00)	48.00 (48.00, 48.00)	48.00 (42.00, 48.00)
AUC_{last} (ng•h/mL) Mean (% CV)	2782.0 (19.7)	2765.2 (17.1)	2243.2 (24.4)
AUC_{exp} (%) Mean (% CV)	11.6 (27.2)	11.9 (20.9)	13.0 (31.2)
AUC_{inf} (ng•h/mL) Mean (% CV)	3143.1 (18.9)	3139.0 (17.2)	2579.9 (24.2)
$T_{\frac{1}{2}}$ (h) Median (Q1, Q3)	15.64 (14.39, 18.37)	16.57 (15.01, 17.25)	15.86 (14.24, 17.65)

CV: coefficient of variation, HC: high calorie, HF: high fat, Q1: first quartile, Q3: third quartile

Table 9 shows the statistical comparison of the mean pharmacokinetic parameters of tenofovir after single dose administration of the QUAD tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal.

Table 9: Statistical comparison of the mean pharmacokinetic parameters of tenofovir after single dose administration of the QUAD tablet under fasted conditions, with a light meal, and with a high calorie/high fat meal

Treatment Condition N=24	TFV PK Parameters[a]		
	C_{max} (ng/mL)	AUC_{inf} (ng•h/mL)	AUC_{last} (ng•h/mL)
HC/HF Meal GLSM	319.39	3083.60	2724.93
Light Meal GLSM	370.76	3094.48	2726.58
Fasted GLSM	308.90	2505.63	2178.74
HC/HF Meal vs. Fasted GLSM ratio (90% CI), %	103.39 (89.38, 119.60)	123.07 (117.08, 129.36)	125.07 (119.09, 131.35)
Light Meal vs. Fasted GLSM ratio (90% CI), %	120.02 (103.76, 138.84)	123.50 (117.50, 129.81)	125.14 (119.16, 131.42)
HC/HF Meal vs. Light Meal GLSM ratio (90% CI), %	86.14 (74.47, 99.65)	99.65 (94.80, 104.74)	99.94 (95.16, 104.95)

CI: confidence interval, GLSM: geometric least squares mean, HC: high calorie, HF: high fat

GLSMs were obtained using a mixed-effects model.

Reviewer's Note:

Per the approved package insert of TRUVADA (Emtricitabine/Tenofovir 200/300 mg), administration of TRUVADA with a high fat meal (784kcal; 49gms fat) or a light meal (373kcal; 8gms fat) resulted in a mean increase in tenofovir AUC and C_{max} by 35 % and 15 %, respectively (the clinical pharmacology review of TRUVADA indicates that light meal and high fat meal cause similar increase in the pharmacokinetic parameters of tenofovir). Emtricitabine systemic exposures were unaffected when TRUVADA was administered either a high fat meal or a light meal.

It should be noted that although the "kcal" of the light meal and high fat meal used in the two trials (QUAD food effect trial and TRUVADA food effect trial) are similar, the "gms" of fat cannot be directly compared because the food effect trial conducted with the QUAD tablet did not provide information pertaining to the gms of fat used (only % fat content was provided).

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8. Results

Single dose administration of QUAD tablet with a <u>light meal vs fasted</u> conditions:

- The mean C_{max} and AUC_{inf} of EVG increased by 22 % and 34 %, respectively.
- There was no significant change (all changes < 10 %) in either the C_{max} or AUC_{inf} of Cobi.
- There was no significant change (all changes < 10 %) in either the C_{max} or AUC_{inf} of FTC.
- The mean C_{max} and AUC_{inf} of tenofovir increased by 20 % and 24 %, respectively.

Single dose administration of QUAD tablet with a <u>high fat meal vs fasted</u> conditions:

- The mean C_{max} and AUC_{inf} of EVG increased by 56 % and 87 %, respectively.
- The mean C_{max} and AUC_{inf} of Cobi decreased by 24 % and 17 %, respectively.
- There was no significant change (all changes < 10 %) in either the C_{max} or AUC_{inf} of FTC.
- The mean C_{max} of tenofovir was not significantly changed (< 10 %); mean AUC_{inf} of tenofovir increased by 23 %, respectively.

Comparison of the mean PK parameters of individual components of the QUAD tablet with <u>high calorie-high fat meal vs light meal</u>:

- The mean C_{max} and AUC_{inf} of EVG increased by 28 % and 39 %, respectively.
- The mean C_{max} and AUC_{inf} of Cobi were decreased by 27 % and 19 %, respectively.
- There was no significant change in either the C_{max} or AUC_{inf} of FTC.
- The mean C_{max} of tenofovir was decreased by 14 %; no significant change in the AUC_{inf} of tenofovir.

9. Discussion and Conclusion

The results of this trial suggest:

- *Similar magnitude of change (23-24 % in the QUAD trial vs 35 % in the TRUVADA trial) in the pharmacokinetic parameters of tenofovir (after administration of the QUAD tablet) under light meal and high fat meal conditions when compared with the pharmacokinetic parameters of*

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tenofovir (after administration of TRUVADA) under light meal and high fat meal conditions .

- *No significant change in the pharmacokinetic parameters of emtricitabine (after administration of the QUAD tablet) under light meal and high fat meal conditions when compared with the pharmacokinetic parameters of emtricitabine (after administration of TRUVADA) under light meal and high fat meal conditions .*

Recommendation: QUAD tablets should be taken with food due to the increase in systemic exposure of EVG. The increase in systemic exposure (AUC_{inf}) of EVG and decrease in systemic exposure of Cobi when QUAD was administered with a high fat meal (800kcal) as compared with when QUAD was administered with a light meal (373 kcal) is not expected to be clinically relevant.

Of note, the QUAD tablet was given with food in pivotal Phase III trials in which the safety and efficacy of the individual components of the QUAD tablets were determined.

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1. Title

A Phase 1, Open Label, Drug Interaction Study Evaluating the Effect of Elvitegravir/Emtricitabine/Tenofovir DF/GS-9350 Fixed Dose Combination Tablet on the Pharmacokinetics of a Representative Hormonal Contraceptive Medication, Norgestimate/Ethinyl Estradiol

2. Objectives

The primary objective of the study was to determine the effect of EVG/GS-9350 (cobicistat)/FTC/TDF [QUAD; FDC tablet; EVG/COBI/FTC/TDF] on the pharmacokinetics of individual components (norgestimate [NGM] and ethinyl estradiol [EE]) of a representative hormonal contraceptive medication, Ortho Tri-Cyclen® Lo.

3. Trial Design

Phase I, single-center, open-label, fixed sequence, multiple dose study.

The study treatment period consisted of two periods, Part A and Part B. Subjects who had not taken Ortho Tri-Cyclen® Lo or generic equivalent for one full menstrual cycle were required to enroll into Part A of the study. Part A was a lead- in period and consisted of dosing with Ortho Tri-Cyclen® Lo for one full menstrual cycle. Subjects who had taken Ortho Tri-Cyclen® Lo or generic equivalent for at least 1 full menstrual cycle prior to day 1 were eligible to enroll directly into Part B. The first study visit for a subject (Part A or Part B) was required to be within 21 days after the screening visit.

In Part A, subjects returned to the clinic at Lead-in Baseline on Day 28 of their current menstrual cycle. Subjects were confined overnight and began dosing with Ortho Tri-Cyclen® Lo on Day 1 of their menstrual cycle (Day 1 of Lead-in period [L1]). Subjects were discharged on Day L1 and completed the 1-month period self-administering Ortho Tri-Cyclen® Lo at home.

Part B consisted of two 28-day menstrual cycles. The treatment scheme in Part B was as follows:

- Part B Cycle 1: Ortho Tri-Cyclen® Lo administered from Days 1 to 28
- Part B Cycle 2: Ortho Tri-Cyclen® Lo administered from Days 29 to 56, with EVG/COBI/FTC/TDF co-administered on days 40 to 49.

On Days 21 (of part B, cycle 1) and 49 (of part B, cycle 2), following an overnight fast (no food or liquids, except water, for at least 8 hours), study drugs (Ortho Tri-Cyclen® Lo in part B cycle 1 and Ortho Tri-Cyclen® Lo + EVG/COBI/FTC/TDF in

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part B, cycle 2) were administered in the morning within 5 minutes of completing a standardized breakfast (400 calories; 13g of fat). On these days, subjects were restricted from food intake until after collection of the blood draw 4 hours after dosing. Subjects were restricted from water consumption from 1 hour before until 2 hours after dosing, except for the 240 mL of water given with the study drug.

4. Dose Selection

The dose of the individual components of the Fixed Dose Combination (FDC) tablet (EVG/COBI/FTC/TDF) used in this trial is identical to the doses of the individual components in the to-be-marketed fixed dose combination tablets.

Ortho Tri-Cyclen® Lo is a commercially available product containing norgestimate 0.180 mg/0.215 mg/0.250 mg/ethinyl estradiol 0.025 mg.

5. Drugs Used in the Trial

The trial was conducted between August 2009 (first subject screened) and February 2010 (last subject observation) using the following drugs:

FDC Tablet: Lot # BK0803B1 and Expiration Date June 2010
Norgestimate/Ethinyl Estradiol (Ortho Tri-Cyclen® Lo): Lot # 9DM591 and Expiration Date Feb 2011.

6. Plasma sampling and Pharmacokinetic/Pharmacodynamic/Statistical Analysis

Pharmacokinetic Analysis

Serial blood samples were collected up to 24 hours on day 21 and 49 to determine plasma concentrations of norgestromin (NGMN; the primary and pharmacologically active metabolite of norgestimate, also known as 17-desacetyl norgestimate), EE, EVG, and COBI. The plasma concentration-time data was used to compute the standard non-compartmental pharmacokinetic parameters.

The null hypothesis was that the geometric mean ratio of the test treatment (NGM/EE + ECG/COBI/FTC/TDF) to the reference treatment (NGM/EE) for each of the two pharmacokinetic parameters (AUC_{tau} and C_{max}) for NGMN and EE was outside the 80% -125 % limits. The alternative hypothesis was that the each of those two ratios was within the boundary of 80 % and 125 %.

The pharmacokinetic analysis set for the study was to include at least 12 subjects who had evaluable NGMN and EE PK profiles for both the reference and test treatments.

Pharmacodynamic Analysis

Progesterone, Follicle stimulating hormone (FSH), and Luteinizing hormone (LH) were measured in serum samples obtained on days 0 (baseline), 21, 28, and 49 as measures of pharmacodynamic activity of the study treatments.

8. Results

Subject Disposition and Demographics

21 subjects were enrolled and received the study drug (19 subjects enrolled in Part A and 2 subjects enrolled directly into part B). Of the 19 subjects enrolled in part A, 3 subjects (16 %) discontinued and did not continue on to part B (1 subject was lost to follow up after the first day of dosing in Part A; one subject withdrew consent after completing part A and one subject was discontinued after completing part A because part B was fully enrolled). The remaining 16 subjects continued on to part B.

Of the 18 subjects (16 subjects who continued from part A + 2 subjects who enrolled directly into part B) who were administered the reference treatment, Ortho Tri-Cyclen® Lo, in the first menstrual cycle of Part B, 2 discontinued the study early (consent withdrawn after dosing on Days 15 and 17 of the reference treatment). The remaining 16 subjects continued and were treated with the test treatment, Ortho Tri-Cyclen Lo + EVG/COBI/FTC/TDF, in the second menstrual cycle in Part B. Of the 16 subjects who received the test treatment, 15 completed the study, and 1 discontinued the study early (consent withdrawn after dosing on Day 1 of the test treatment).

The median (min-max) age of the 21 enrolled female subjects was 30 years (19-45 years). The median (min-max) BMI was 23.5 kg/m^2 (19.1 to 27.9 kg/m^2) and the median (min-max) CL_{cr} was 109.7 mL/min (87.2 to 164.4 mL/min).

Bioanalysis

Plasma concentrations of NGMN, EE, EVG, and Cobi were determined using validated LC/MS/MS methods.

<u>NGNM</u>

The calibration samples for NGNM ranged from 20 ng/mL to 10,000 ng/mL. The quality control samples were prepared at four concentration levels (60, 500, 3000, and 8000 ng/mL).

The % CV of the QCs ranged from 2.5 % - 8.3 % and the % RE (relative error) ranged from -0.7 % to 2.1 %.

<u>EE</u>

The calibration samples for EE ranged from 2.5 ng/mL to 500 ng/mL. The quality control samples were prepared at three concentration levels (7.5, 80, and 400 ng/mL).

The % CV of the QCs ranged from 4.1 % - 5.9 % and the % RE ranged from -2.8 % to 1.6 %.

<u>EVG</u>

The calibration samples for EVG ranged from 20 ng/mL to 10,000 ng/mL. The quality control samples were prepared at three concentration levels (60, 800, and 8000 ng/mL).

The % CV of the QCs ranged from 2.7 % - 4.6 % and the % RE ranged from -0.3 % to 8 %.

<u>COBI</u>

The calibration samples for EVG ranged from 5 ng/mL to 2500 ng/mL. The quality control samples were prepared at three concentration levels (15, 200, and 2000 ng/mL).

The % CV of the QCs ranged from 5.3 % -6.9 % and the % RE ranged from -2.5 % to 5.3 %.

Pharmacokinetics

<u>NGMN</u>

Fig 1 shows the mean concentration time profile of NGMN following administration of NGM/EE alone and in combination with EVG/COBI/FTC/TDF.

Fig 1: Mean concentration time profile of NGMN following administration of NGM/EE alone and in combination with EVG/COBI/FTC/TDF
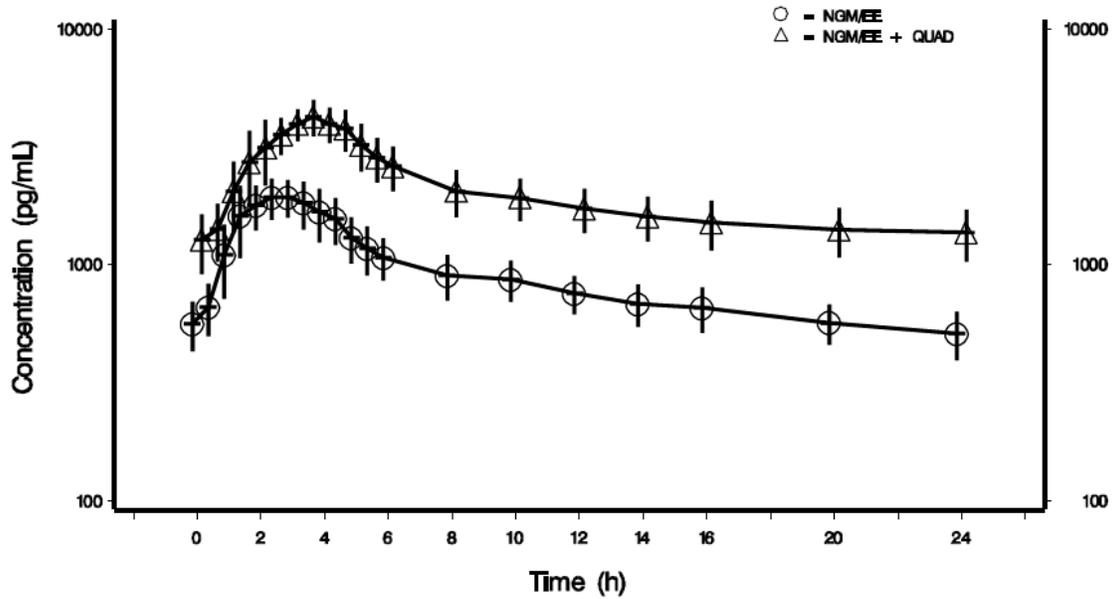
NGM/EE: Norgestimate/Ethinyl Estradiol. QUAD: EVG/FTC/TDF/GS-9350.

Table 1 shows the mean pharmacokinetic parameters of NGMN after administration of NGM/EE and NGM/EE + EVG/COBI/FTC/TDF.

Table 1: Mean pharmacokinetic parameters of NGMN after administration of NGM/EE and NGM/EE + EVG/COBI/FTC/TDF

	Treatment	
NGMN PK Parameter	**NGM/EE** **Part B, First Menstrual Cycle** **Reference** **(N=15)**	**NGM/EE + EVG/COBI/FTC/TDF** **Part B, Second Menstrual Cycle** **Test** **(N=15)**
C_{max} (pg/mL) Mean (%CV)	2147.0 (15.4)	4461.1 (14.5)
T_{max} (h) Median (Q1, Q3)	2.50 (2.00, 3.00)	3.50 (2.50, 3.50)
C_{tau} (pg/mL) Mean (%CV)	510.3 (23.1)	1368.2 (24.7)
T_{last} (h) Median (Q1, Q3)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
AUC_{tau} (h·pg/mL) Mean (%CV)	21367.27 (17.1)	48339.22 (17.7)
$T_{\frac{1}{2}}$ (h) Median (Q1, Q3)	23.70 (19.25, 28.30)	36.42 (31.53, 39.31)[a]
Cl/F (mL/h) Mean (%CV)	12012.0 (16.4)	5323.9 (17.6)

NGM/EE = Ortho Tri-Cyclen Lo

NGM/EE + EVG/COBI/FTC/TDF = Ortho Tri-Cyclen Lo plus a FDC tablet containing EVG 150 mg, COBI 150 mg, FTC 200 mg, and TDF 300 mg.

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Table 2 shows the statistical comparison of the mean pharmacokinetic parameters of NGMN after administration of NGM/EE and NGM/EE + EVG/COBI/FTC/TDF.

Table 2: Statistical comparison of the mean pharmacokinetic parameters of NGMN after administration of NGM/EE and NGM/EE + EVG/COBI/FTC/TDF

| NGMN PK Parameter	Geometric Least-squares Means		Geometric Least-squares Means Ratios (Test/Reference) (%)	90% Confidence Intervals
	NGM/EE Reference (N=15)	NGM/EE + EVG/COBI/FTC/TDF Test (N=15)		
AUC_{tau} (h·pg/mL)	21084.51	47642.85	225.96	(215.13, 237.34)
C_{tau} (pg/mL)	497.64	1326.57	266.57	(243.06, 292.35)
C_{max} (pg/mL)	2125.16	4420.00	207.98	(199.74, 216.57)

NGM/EE = Ortho Tri-Cyclen Lo

NGM/EE + EVG/COBI/FTC/TDF = Ortho Tri-Cyclen Lo plus a fixed-dose combination tablet containing EVG 150 mg, COBI 150 mg, FTC 200 mg, and TDF 300 mg.

EE

Fig 2 shows the mean concentration time profile of EE following administration of NGM/EE alone and in combination with EVG/COBI/FTC/TDF.

Fig 2: Mean concentration time profile of EE following administration of NGM/EE alone and in combination with EVG/COBI/FTC/TDF

NGM/EE: Norgestimate/Ethinyl Estradiol. QUAD: EVG/FTC/TDF/GS – 9350.

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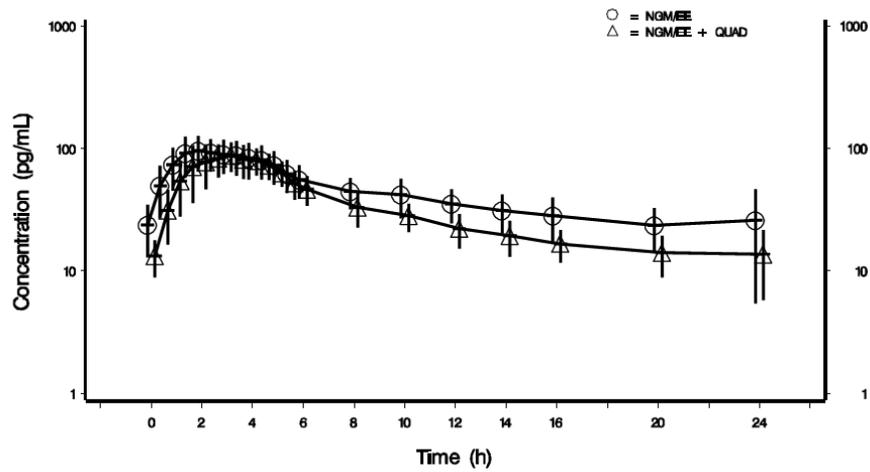
Table 3 shows the mean pharmacokinetic parameters of EE after administration of NGM/EE and NGM/EE + EVG/COBI/FTC/TDF.

Table 3: Mean pharmacokinetic parameters of EE after administration of NGM/EE and NGM/EE + EVG/COBI/FTC/TDF

EE PK Parameter	Treatment	
	NGM/EE Part B, First Menstrual Cycle Reference (N=15)	NGM/EE + EVG/COBI/FTC/TDF Part B, Second Menstrual Cycle Test (N=15)
C_{max} (pg/mL) Mean (%CV)	105.7 (30.7)	98.6 (27.8)
T_{max} (h) Median (Q1, Q3)	2.00 (1.50, 3.00)	3.00 (2.50, 3.50)
C_{tau} (pg/mL) Mean (%CV)	25.8 (78.9)	13.7 (57.8)
T_{last} (h) Median (Q1, Q3)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
AUC_{tau} (h·pg/mL) Mean (%CV)	1050.562 (32.1)	775.036 (26.1)
$T_{1/2}$ (h) Median (Q1, Q3)	16.97 (13.93, 18.81) [a]	13.94 (11.26, 16.48) [a]
Cl/F (mL/h) Mean (%CV)	26104.0 (30.6)	34273.2 (24.6)

NGM/EE = Ortho Tri-Cyclen Lo

NGM/EE + EVG/COBI/FTC/TDF = Ortho Tri-Cyclen Lo plus a FDC tablet containing EVG 150 mg, COBI 150 mg, FTC 200 mg, and TDF 300 mg.

Table 4 shows the statistical comparison of the mean pharmacokinetic parameters of EE after administration of NGM/EE and NGM/EE + EVG/COBI/FTC/TDF.

Table 4: Statistical comparison of the mean pharmacokinetic parameters of EE after administration of NGM/EE and NGM/EE + EVG/COBI/FTC/TDF

EE PK Parameter	Geometric Least-squares Means		Geometric Least-squares Means Ratios (Test/Reference) (%)	90% Confidence Intervals
	NGM/EE Reference (N=15)	NGM/EE + EVG/COBI/FTC/TDF Test (N=15)		
AUC_{tau} (h·pg/mL)	1002.29	751.44	74.97	(69.41, 80.98)
C_{tau} (pg/mL)	21.72	12.27	56.48	(51.88, 61.49)
C_{max} (pg/mL)	101.24	95.25	94.09	(85.54, 103.50)

NGM/EE = Ortho Tri-Cyclen Lo

NGM/EE + EVG/COBI/FTC/TDF = Ortho Tri-Cyclen Lo plus a FDC tablet containing EVG 150 mg, COBI 150 mg, FTC 200 mg, and TDF 300 mg.

Reviewer's Comment

There was a significant increase in the concentrations of NGNM after co-administration of NGM/EE and EVG/COBI/FTC/TDF as compare with when NGM/EE was given alone. Increase in the concentrations of NGNM, albeit of a lower magnitude, has been previously observed with HIV-1 protease inhibitors such as atazanavir/ritonavir (NGNM mean C_{max} and AUC_{tau} increased by 68 % and 85 % in the presence of atazanavir/ritonavir). Further, a decrease in the mean C_{max} (by 16 %) and mean AUC_{tau} (by 19 %) of EE was observed when atazanavir/ritonavir and NGM/EE are co-administered. The mean AUC_{tau} of EE was decreased by a similar magnitude (~25 %) when NGM/EE was co-administered with EVG/COBI/FTC/TDF as compared with when NGM was administered alone.

The package insert for atazanavir indicates that when atazanavir/ritonavir and NGM/EE are co-administered, caution should be exercised and the dose of EE should be increased to 35 μg. However, a similar recommendation cannot be provided for co-administration of EVG/COBI/FTC/TDF and NGM/EE due to the following considerations:

- *The magnitude of increase in NGNM concentrations observed in the presence of EVG/COBI/FTC/TDF was higher (126 % vs 85 %) as compared with the magnitude of increase in NGNM concentrations observed in the presence of ATV/rtv*
- *Ortho Tri-Cyclen® Lo is a combination tablet of NGM and EE. Hence, in order to increase the dose of EE (to compensate for the decrease in EE concentrations in the presence of QUAD tablet), a higher strength NGM/EE tablet will need to be used (which will thereby increase the amount of NGM administered and the probability of increasing NGNM related adverse events).*

The clinical significance of the increased NGMN exposures and the benefit-vs-risk assessment of using QUAD with oral contraceptives in general was discussed with the Division of Reproductive and Urology Products (DRUP). The response to the formal consult request and follow up discussions indicate that there is a potential risk of rare thromboembolic events which may occur due to higher NGMN exposures; however, the rarity of these thromboembolic events makes it challenging to quantify the risk. On the other hand, Ortho Tricyclen Lo is a widely used oral contraceptive and is a convenient option for contraception. Further, the results of the trial indicate that the efficacy of Ortho Tricyclen Lo is not expected to decrease when given with QUAD because the progestin component is considered more important (than the estrogen component) for contraceptive efficacy. Based on the aforementioned considerations, it was determined that a) describing the PK changes of NGMN and EE in the package insert, b) indicating that DDIs between QUAD and oral contraceptives containing other progestins has not been conducted and cannot be predicted based on the

available data, c) not recommending an increase in the dose of EE to 35 µg [(b) (4)] and d) suggesting healthcare professionals may consult with experts in the area of pregnancy and women's health concerns before co-administration of QUAD and Ortho Tricyclen Lo will convey sufficient benefit-to-risk information regarding use of QUAD and Ortho Tri Cyclen Lo.

<u>EVG and COBI:</u>

QUAD tablet was not administered alone in the trial; hence, the pharmacokinetic parameters of the individual components of the QUAD tablet (when administered alone) could not be determined. A comparison of the mean pharmacokinetic parameters of EVG [AUC_{tau} and C_{tau} were 26,917.65 ng*hr/mL and 414.8 ng/mL, respectively] and Cobi [AUC_{tau} and C_{tau} were 10,715.73 ng*hr/mL and 25.6 ng/mL respectively] were within the range of AUC and C_{tau} of EVG and Cobi observed in other healthy volunteer trials. The similarity between the mean pharmacokinetic parameters of EVG and Cobi in this trial and the mean pharmacokinetic parameters of EVG and Cobi in other trials suggests that NGM/EE did not have a clinically significant impact on the pharmacokinetics of EVG and Cobi.

Pharmacodynamics:

<u>Progesterone:</u>

Table 5 shows the median progesterone concentration on day 0 and day 21 in the two treatment arms.

Table 5: Median progesterone concentration on day 0 and day 21 in the two treatment arms

Progesterone Concentration (ng/mL)	Treatment	
	NGM/EE Part B, Menstrual Cycle 1 (N=14)	NGM/EE + EVG/COBI/FTC/TDF Part B, Menstrual Cycle 2 (N=14)
Day 0 Median Q1, Q3	0.5 0.3, 0.5	0.4 0.3, 0.5
Day 21 Median Q1, Q3	0.5 0.3, 0.6	0.5 0.3, 0.5
Change from Day 0 to Day 21 Median Q1, Q3	0.0 0.0, 0.1	0.0 -0.1, 0.2

NGM/EE = Ortho Tri-Cyclen Lo

NGM/EE + EVG/COBI/FTC/TDF = Ortho Tri-Cyclen Lo plus a FDC tablet containing EVG 150 mg, COBI 150 mg, FTC 200 mg, and TDF 300 mg.

Cobicistat (COBI) is the new generic name for GS-9350.

Progesterone values reported as < 0.2 ng/mL were treated as 0.1 ng/mL in the summary table.

Progesterone PD analysis set included subjects with progesterone values for both Day 0 and Day 21 in a treatment (NGM/EE or NGM/EE + EVG/COBI/FTC/TDF).

NGM/EE treatment: Subjects 4949-1006, 4949-1013, 4949-1014, and 4949-1016 were excluded due to missing values on Day 0 (4949-1006, 4949-1014) or on Day 21 (4949-1013, 4949-1016).

NGM/EE + EVG/COBI/FTC/TDF treatment: Subjects 4949-1001 and 4949-1008 were excluded due to missing values on Day 0 (4949-1008) or Day 21 (4949-1001).

There were no changes in median progesterone concentrations (day 0 to day 21) following administration of NGM/EE alone or in combination with EVG/COBI/FTC/TDF.

Luteinizing Hormone

Table 6 shows the median luteinizing hormone concentrations at day 0 and day 21 in the two treatment arms.

Table 6:   Median luteinizing hormone concentrations at day 0 and day 21 in the two treatment arms

LH Concentration (mIU/mL)	Treatment	
	NGM/EE Part B, Menstrual Cycle 1 (N=16)	NGM/EE + EVG/COBI/FTC/TDF Part B, Menstrual Cycle 2 (N=13)
Day 0 Median Q1, Q3	3.37 2.54, 5.22	4.76 4.02, 7.98
Day 21 Median Q1, Q3	1.68 0.94, 3.39	1.02 0.78, 1.69
Change from Day 0 to Day 21 Median Q1, Q3	−1.56 −2.70, −0.39	−4.11 −5.73, −2.05

NGM/EE = Ortho Tri-Cyclen Lo

NGM/EE + EVG/COBI/FTC/TDF = Ortho Tri-Cyclen Lo plus a FDC tablet containing EVG 150 mg, COBI 150 mg, FTC 200 mg, and TDF 300 mg.

Cobicistat (COBI) is the new generic name for GS-9350.

LH PD analysis set included subjects with LH values for both Day 0 and Day 21 in a treatment (NGM/EE or NGM/EE + EVG/COBI/FTC/TDF)

NGM/EE treatment: Subjects 4949-1013 and 4949-1016 were excluded due to missing values on Day 0 (4949-1013) and/or on Day 21 (4949-1013, 4949-1016).

NGM/EE + EVG/COBI/FTC/TDF treatment: Subjects 4949-1001, 4949-1006, and 4949-1008 were excluded due to missing values on Day 0 (4949-1006, 4949-1008) or Day 21 (4949-1001).

Follicle-stimulating Hormone

Table 7 shows the median follicle-stimulating hormone concentrations at day 0 and day 21 in the two treatment arms.

Table 7: Median follicle-stimulating hormone concentrations at day 0 and day 21 in the two treatment arms

FSH Concentration (mIU/mL)	Treatment	
	NGM/EE Part B, Menstrual Cycle 1 (N=16)	NGM/EE + EVG/COBI/FTC/TDF Part B, Menstrual Cycle 2 (N=13)
Day 0 Median Q1, Q3	5.96 5.32, 6.98	5.28 4.74, 6.15
Day 21 Median Q1, Q3	1.83 1.42, 2.47	1.59 1.19, 1.99
Change from Day 0 to Day 21 Median Q1, Q3	−4.62 −4.97, −3.04	−4.06 −4.88, −2.67

NGM/EE = Ortho Tri-Cyclen Lo

NGM/EE + EVG/COBI/FTC/TDF = Ortho Tri-Cyclen Lo plus a FDC tablet containing EVG 150 mg, COBI 150 mg, FTC 200 mg, and TDF 300 mg.

Cobicistat (COBI) is the new generic name for GS-9350.

FSH PD analysis set included subjects with FSH values for both Day 0 and Day 21 in a treatment (NGM/EE or NGM/EE + EVG/COBI/FTC/TDF).

NGM/EE treatment: Subjects 4949-1013 and 4949-1016 were excluded due to missing values on Day 0 (4949-1013) and/or on Day 21 (4949-1013, 4949-1016).

NGM/EE + EVG/COBI/FTC/TDF treatment: Subjects 4949-1001, 4949-1006, and 4949-1008 were excluded due to missing values on Day 0 (4949-1006, 4949-1008) or Day 21 (4949-1001).

9. Results

Pharmacokinetics:

- The mean C_{max}, C_{tau}, and AUC_{tau} of NGMN increased by 108 %, 167 %, and 126 % after co-administration of NGM/EE with EVG/COBI/FTC/TDF as compared with when NGM/EE was administered alone.
- The mean C_{tau}, and AUC_{tau} of EE decreased by 43% and 25 % after co-administration of NGM/EE with EVG/COBI/FTC/TDF as compared with when NGM/EE was administered alone. There was no significant change (< 10 %) in the C_{max} of EE.
- Comparison of the mean pharmacokinetic parameters of EVG and Cobi observed in this trial with the mean pharmacokinetic parameters of EVG and Cobi observed in previous trials suggest that NGM/EE did not alter the pharmacokinetics of either EVG or Cobi.

10. Conclusion

Coadministration of QUAD and a norgestimate/ethinyl estradiol containing hormonal oral contraceptive resulted in decreased plasma concentrations of ethinyl estradiol and increased concentrations of the progestational component. The clinical significance of the increased exposures of the progestational component is unknown; therefore, experts in the area of pregnancy and women's health may be consulted before coadministration of QUAD and norgestimate/ethinylestradiol. Alternatively, use of non-hormonal methods of contraception can be considered.

The effect of [TRADENAME] on other progestins is unknown.

GS-US-236-0110

1. Title

A Phase 1 Multiple Dose Study to Evaluate the Relative Bioavailability of Two Elvitegravir/Emtricitabine/Tenofovir DF/GS-9350 Fixed-Dose Combination Tablet Formulations

2. Objectives

The primary objective of the study was to evaluate the relative bioavailability of two formulations of a fixed dose combination (FDC) tablet containing EVG/COBI/FTC/TDF.

3. Rationale for Conducting the Trial:

The FDC formulation 2 tablet is _____ (b) (4) than the FDC formulation 1 tablet and the process used in manufacturing GS-9350 (cobicistat) for FDC formulation 2 tablet _____ (b) (4) relative to that for manufacturing FDC formulation 1 tablet. Formulation 1 was used in trial GS-US-236-0101 in which the pharmacokinetics of EVG from QUAD (with Cobi 100 mg and 150 mg) vs EVG/rtv was determined. Therefore, the goal of the trial was to enable selection of a fixed dose formulation for subsequent evaluation in Phase III trials. Fixed dose formulation 2 was compared to both FDC formulation 1 (EVG and secondarily GS-9350 exposures) and to commercially available FTC capsules + TDF stand alone tablets.

4. Trial Design

Randomized, open-label, multiple-dose, six sequence, three period, cross-over study evaluating the relative bioavailability of two tablet formulations of EVG/COBI/FTC/TDF. A total of 42 subjects were to be enrolled in the trial to obtain 36 evaluable subjects.

Eligible subjects were randomized to one of six treatment sequences (ABC, ACB, BAC, BCA, CAB, CBA). On the first day of each period (days 1, 11, and 21), subjects were administered 1 of the following 3 drug treatments to be taken orally as a single daily dose for 10 days.

Treatment A (FDC Formulation 2): 1 fixed dose combination tablet (150 mg EVG/150 mg COBI/200 mg FTC/300 mg TDF).
Treatment B (FDC Formulation 1): 1 fixed dose combination tablet (150 mg EVG/150 mg COBI/200 mg FTC/300 mg TDF).
Treatment C (FTC + TDF): 1 X 200 mg FTC capsule + 1 X 300 mg TDF tablet.

All study drug doses were administered at the study center in the morning with 240 mL of water and within 5 minutes of consuming a standardized meal. On days 10, 20, and 30 (all treatments) and on days 6, 16, and 26 (treatments A and B only), study drug doses were administered after an overnight fast (no food or liquids, except water, for at least 8 hours). As the pharmacokinetic profiles of the individual components of the FDC has been established in other trials, a 10-day regimen was determined (by the sponsor) to be sufficient to conduct assessment under clinically relevant steady-state conditions.

5. Drugs Used in the Trial

The trial was conducted between August 4, 2009 and October 29, 2009 using the following formulations:

FDC Formulation 1: Lot # BK0902B1 and expiration date June 2011
FDC Formulation 2: Lot # BK0901B1 and expiration date June 2011
Emtricitabine capsules: Lot # 67038AF21 and expiration date October 2011
Tenofovir tablets: Lot # A160721 and expiration date Feb 2010

6. Plasma sampling and PK/Statistical Analysis

Serial blood samples were collected up to 24 hours following dosing of study drugs on days 10, 20, and 30. Plasma concentrations of FTC and TFV were determined and pharmacokinetic parameters estimated at all time points (treatment A and treatment C only). Plasma concentrations of EVG and cobicistat were determined and pharmacokinetic parameters estimated at all time points for treatment A and treatment B.

Additional trough samples were collected for steady state assessment of EVG on days 7, 17, and 27 of treatments A and B only.

Bioanalysis

Plasma concentrations of EVG and Cobi were determined using validated LC/MS/MS methods.

EVG

The calibration samples for EVG ranged from 20 ng/mL to 10,000 ng/mL. The LLOQ was 20ng/mL. The quality control samples were prepared at three concentration levels (60, 800, and 8000 ng/mL).

The % CV of the QCs ranged from 3.8 % to 5.8 % and the % RE (relative error) ranged from -2.8 % to 4.4 %.

<u>Cobi</u>

The calibration samples for Cobi ranged from 5 ng/mL to 2500 ng/mL. The LLOQ was 5 ng/mL. The quality control samples were prepared at three concentration levels (15, 200, and 2000 ng/mL).

The % CV of the QCs ranged from 5.8 % - 9.7 % and the % RE ranged from -2.4 % to 3.8 %.

<u>FTC</u>

The calibration samples for FTC ranged from 5 ng/mL to 3000 ng/mL. The quality control samples were prepared at three concentration levels (15, 150, 600 and 2400 ng/mL).

The % CV of the QCs ranged from 3 % - 6.2 % and the % RE ranged from -3.1 % to 2.1 %.

<u>TFV</u>

The calibration samples for TFV ranged from 5 ng/mL to 3000 ng/mL. The quality control samples were prepared at three concentration levels (15, 150, 600 and 2400 ng/mL).

The % CV of the QCs ranged from 4.1 % - 8.5 % and the % RE ranged from -1.8 % - 2.7 %.

7. Results

Subject Disposition and Demographics

Of the 42 subjects randomized and treated, 39 subjects received FDC formulation 2 as a single tablet (treatment A), 40 subjects received FDC formulation 1 as a single tablet (treatment B) and 39 subjects received concurrent FTC capsule + TDF tablet. 36 subjects completed all study treatments and 6 subjects discontinued study drug prematurely (4 discontinued due to AEs and 2 discontinued at investigator's discretion).

Of the 42 subjects enrolled in the trial, there was an equal distribution of male and female subjects. 26 (~62 %) subjects were white and 12 (28.6 %) subjects were black. The median (min,max) age was 29 (20,45) years, median (min,max) BMI for males and females was 24.5 (19.1-29.3) and 26 (21.9-29.9) kg/m^2

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respectively. The median (min,max) creatinine clearance was 124 (84.7-182.5) mL/min.

Pharmacokinetics

GS-9137 (EVG)

Fig 1 shows the mean plasma concentration-time profile of EVG after oral administration of multiple doses of FDC formulation 2 and FDC formulation 1

Fig 1: Mean plasma concentration-time profile of EVG after oral administration of multiple doses of FDC formulation 2 and FDC formulation 1
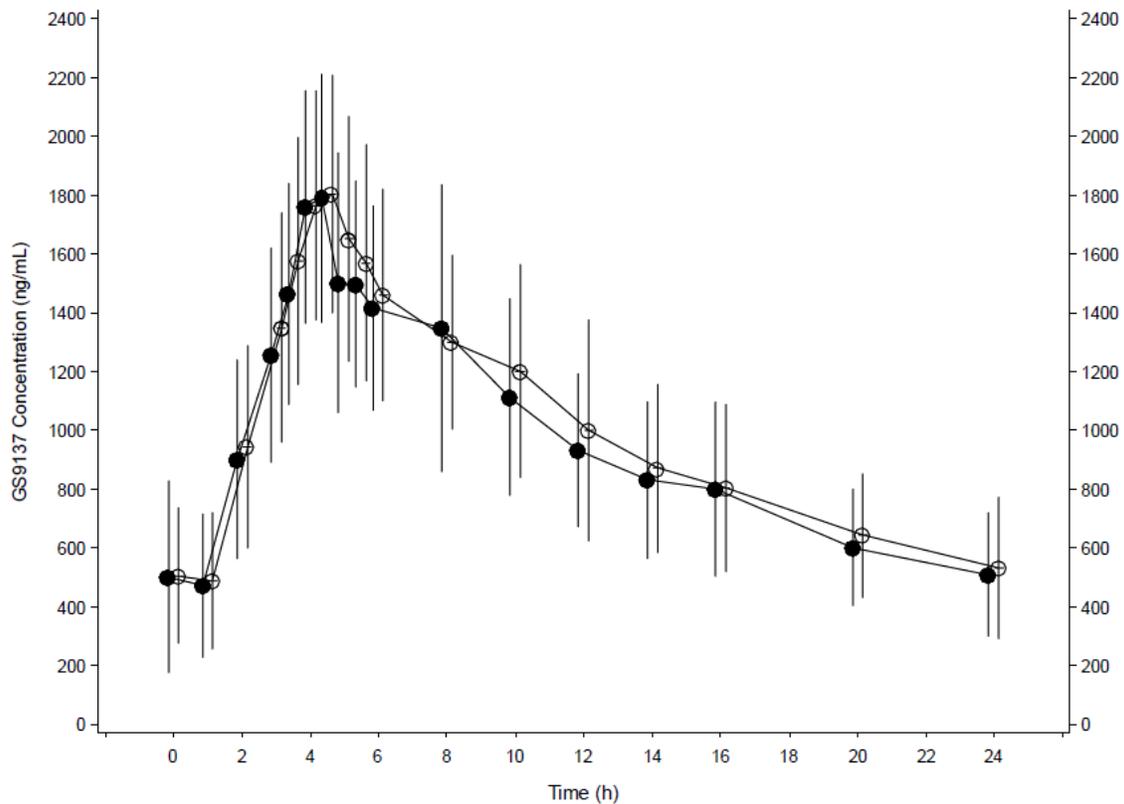

Table 1 shows the mean pharmacokinetic parameters of EVG after oral administration of multiple doses of FDC formulation 2 and formulation 1.

Table 1: Mean pharmacokinetic parameters of EVG after oral administration of multiple doses of FDC formulation 2 and formulation 1

Elvitegravir PK Parameter	FDC Formulation 2 (Treatment A) (N = 36)	FDC Formulation 1 (Treatment B) (N = 36)
AUC_{tau} (ng•h/mL), Mean (% CV)	22,485.9 (26.7)	23,415.7 (24.2)
C_{max} (ng/mL), Mean (% CV)	1919.3 (24.3)	1977.0 (23.4)
C_{tau} (ng/mL), Mean (% CV)	508.2 (41.3)	531.0 (45.0)
$T_{1/2}$ (h), Median (Q1, Q3)	12.89 (8.61, 14.34)	12.64 (9.16, 14.61)
T_{max} (h), Median (Q1, Q3)	4.50 (4.00, 4.53)	4.06 (4.00, 4.50)

Note: Subjects 2003, 2018, 2024, 2030, 2038, and 2042 did not have PK data for a treatment pair of interest and were excluded from summaries.

Table 2 shows the statistical comparison of the mean pharmacokinetic parameters of EVG after oral administration of multiple doses of FDC formulation 2 and formulation 1.

Table 2: Statistical comparison of the mean pharmacokinetic parameters of EVG after oral administration of multiple doses of FDC formulation 2 and formulation 1

Elvitegravir PK Parameter	Geometric Least Squares Means		Geometric Least Squares Mean Ratio (%)	90% Confidence Interval
	FDC Formulation 2 (Treatment A)	FDC Formulation 1 (Treatment B)		
Without Carry-over Effect in the Model				
n	36	36		
AUC_{tau} (ng•h/mL)	21,716.05	22,801.19	95.24	91.08, 99.60
C_{max} (ng/mL)	1857.26	1923.66	96.55	90.87, 102.58
C_{tau} (ng/mL)	471.39	491.30	95.95	88.08, 104.51
With Carry-over Effect in the Model				
AUC_{tau} (ng•h/mL)	—	—	91.35	85.03, 98.14

Note: The statistical model for comparisons without carry-over effect included treatment, sequence, and period as fixed effects and subject within sequence as a random effect. The full statistical model with carry-over effect included treatment, sequence, period and carry-over as fixed effects and subject within sequence as a random effect. This model was only performed for PK parameters with significantly unbalanced carry-over effect.

<u>Cobi</u>

Table 3 shows the mean pharmacokinetic parameters of Cobi after oral administration of multiple doses of FDC formulation 2 and formulation 1.

Table 3: Mean pharmacokinetic parameters of Cobi after oral administration of multiple doses of FDC formulation 2 and formulation 1

GS-9350 PK Parameter	FDC Formulation 2 (Treatment A) (N = 36)	FDC Formulation 1 (Treatment B) (N = 36)
AUC_{tau} (ng•h/mL), Mean (% CV)	11,288.2 (28.5)	11,371.1 (29.7)
C_{max} (ng/mL), Mean (% CV)	1528.0 (25.2)	1593.4 (25.4)
C_{tau} (ng/mL), Mean (% CV)	45.5 (88.1)	48.0 (109.5)
$T_{1/2}$ (h), Median (Q1, Q3)	3.54 (3.13, 4.11)	3.63 (3.18, 4.27)
T_{max} (h), Median (Q1, Q3)	4.00 (3.02, 4.50)	3.75 (3.00, 4.50)

Note: Subjects 2003, 2018, 2024, 2030, 2038, and 2042 did not have PK data for a treatment pair of interest and were excluded from summaries.

Table 4 shows the statistical comparison of the mean pharmacokinetic parameters of Cobi after oral administration of multiple doses of FDC formulation 2 and formulation 1.

Table 4: Statistical comparison of the mean pharmacokinetic parameters of Cobi after oral administration of multiple doses of FDC formulation 2 and formulation 1

GS-9350 PK Parameter	Geometric Least Squares Means		Geometric Least Squares Mean Ratio (%)	90% Confidence Interval
	FDC Formulation 2 (Treatment A)	FDC Formulation 1 (Treatment B)		
n	36	36		
AUC_{tau} (ng•h/mL)	10,826.85	10,904.83	99.28	96.39, 102.27
C_{max} (ng/mL)	1480.85	1545.35	95.83	93.44, 98.27
C_{tau} (ng/mL)	33.14	34.53	95.98	86.50, 106.49

Note: The statistical model included treatment, sequence, and period as fixed effects and subject within sequence as a random effect.

FTC

Table 5 shows the statistical comparison of the mean pharmacokinetic parameters of FTC after oral administration of multiple doses of FDC formulation 2 and Emtricitabine + Tenofovir (treatment C)

Table 5: Statistical comparison of the mean pharmacokinetic parameters of FTC after oral administration of multiple doses of FDC formulation 2 and Emtricitabine + Tenofovir (treatment C)

Emtricitabine PK Parameter	Geometric Least Squares Means		Geometric Least Squares Mean Ratio (%)	90% Confidence Interval
	FDC Formulation 2 (Treatment A)	Emtricitabine + Tenofovir DF (Treatment C)		
n	37	37		
AUC_{tau} (ng•h/mL)	12,269.10	10,128.93	121.13	118.51, 123.81
C_{max} (ng/mL)	1905.56	1646.47	115.74	111.94, 119.66
C_{tau} (ng/mL)	112.67	95.75	117.67	112.53, 123.04

Note: The statistical model included treatment, sequence, and period as fixed effects and subject within sequence as a random effect.

TFV

Table 6 shows the statistical comparison of the mean pharmacokinetic parameters of TFV after oral administration of multiple doses of FDC formulation 2 and Emtricitabine + Tenofovir (treatment C)

Table 6: Statistical comparison of the mean pharmacokinetic parameters of TFV after oral administration of multiple doses of FDC formulation 2 and Emtricitabine + Tenofovir (treatment C)

Tenofovir PK Parameter	Geometric Least Squares Means		Geometric Least Squares Mean Ratio (%)	90% Confidence Interval
	FDC Formulation 2 (Treatment A)	Emtricitabine + Tenofovir DF (Treatment C)		
n	37	37		
AUC_{tau} (ng•h/mL)	3952.08	3136.81	125.99	122.61, 129.46
C_{max} (ng/mL)	464.70	308.95	150.41	142.90, 158.32
C_{tau} (ng/mL)	81.13	63.32	128.13	122.68, 133.81

Note: The statistical model included treatment, sequence, and period as fixed effects and subject within sequence as a random effect.

Reviewer's Note:

The mean pharmacokinetic parameters of TFV were higher after administration of QUAD formulation 2 as compared with QUAD formulation 1. However, these increases in TFV pharmacokinetic parameters are not expected to be clinically relevant because similar increases in mean TFV exposure have been previously observed in the presence of other boosted PIs (for example when tenofovir is co-administered [as tenofovir disoproxil fumarate; TDF] with darunavir/ritonavir, the mean C_{max}, AUC_{tau}, and C_{min} of tenofovir are increased by 24 %, 22 %, and 37 %, respectively, as compared with when tenofovir is administered [as tenofovir disoproxil fumarate; TDF] alone). No dose adjustment of tenofovir is recommended when TDF is co-administered with darunavir/ritonavir.

8. Results

- There were no significant differences between the pharmacokinetic parameters (C_{max}, C_{tau}, AUC_{tau}) of EVG and Cobi after administration of formulation 2 and formulation 1.
- The mean C_{max}, C_{tau}, AUC_{tau} of FTC increased by 16 %, 18 %, and 21 %, respectively after administration of FDC formulation 2 as compared to administration of FTC capsules + TDF tablet.
- The mean C_{max}, C_{tau}, AUC_{tau} of TFV 50 %, 28 %, and 26 %, respectively after administration of FDC formulation 2 as compared to administration of FTC capsules + TDF tablet.

9. Conclusion

QUAD formulation 2 provided similar exposures of EVG and Cobi as QUAD formulation 1. The increase in the systemic exposure of FTC and TDF is not expected to be clinically relevant.

Of note, QUAD formulation 2 was used in Phase III trials (GS-US-236-0102 and GS-US-236-0103) and is the proposed commercial formulation.

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Review of In Vitro Studies

In Vitro Studies of Elvitegravir

In vitro studies pertinent to distribution in blood and plasma

Studies
Study JTK-303-AD-014
Study JTK-303-AD-013

Summary
These two studies evaluated the plasma protein binding and the distribution into blood cells of radiolabeled JTK-303. Plasma and blood specimens from rats, dogs, monkeys, and humans were used. JTK-303 was determined to be stable in protein and blood and to be highly protein-bound (>99%, primarily to α_1 acidic glycoprotein) in human plasma and moderately distributed into human blood cells (ranging from 20.8-24.0% over a range of 0.1-10 ug/mL JTK-303).

Brief reviews of the individual study reports are appended below.

Study JTK303-AD-014 – Protein Binding of JTK-303 *In Vitro*
Study Report Date: 26 Mar 2004

Introduction
In this study, the *in vitro* extent of protein binding of JTK-303 was investigated in plasma from rats, dogs, monkeys, and humans. The stability of JTK-303 in plasma was also calculated.

Materials and methods
Reagents and biospecimens
The reference standard JTK-303 was manufactured by Japan Tobacco Inc. JTK-303 was radiolabeled with ^{14}C (specific radioactivity: 4.99 MBq/mg) (b) (4)
  Human serum albumin (HSA) and human α_1 acidic glycoprotein were obtained from (b) (4) . Whole blood was collected from the vena cava of euthanized male Sprague-Dawley rats (n=14, (b) (4)) and the cephalic vein of the foreleg of male beagle dogs (n=6, (b) (4)). Whole blood from male cynomolgus monkeys was purchased (n=6, (b) (4)). Whole blood was also collected from healthy adult male volunteers (b) (4) Whole blood was centrifuged (3000 rpm, 4°C, 15 min) to obtain plasma, which was refrigerated until use.

Stability of high concentration ^{14}C-JTK-303 solution in plasma
Plasma samples were pooled by species and pre-incubated at 37°C for 5 minutes. Radiolabeled JTK-303 (4 ul of 1.0 mg/mL solution) was added to 400 uL plasma and 100 uL aliquots were incubated at 37°C for 0 and 8 hours. The reaction was terminated upon addition of 200 uL acetonitrile solution containing 0.1% acetic acid and the samples were centrifuged (10000 rpm at 4°C for 5 minutes). Radioactivity was subsequently measured in the supernatant (100 uL). ^{14}C-JTK-303 was quantified in two reaction samples per species.

Stability of low concentration ^{14}C-JTK-303 solution in plasma
Plasma samples were pooled by species and pre-incubated at 37°C for 5 minutes. Radiolabeled JTK-303 (10 ul of 0.01 mg/mL solution) was added to 1 mL plasma and 100 uL aliquots were incubated at 37°C for 0 and 8 hours. The reaction was terminated upon addition of 800 uL acetonitrile solution containing 0.1% acetic acid to 400 uL samples and the samples were centrifuged (10000 rpm at 4°C for 5 minutes). Radioactivity was subsequently measured in the supernatant (100 uL). ^{14}C-JTK-303 was quantified in two reaction samples per species.

Rate of ^{14}C-JTK-303 protein binding
Rat, dog, and monkey plasma (0.1, 1, and 10 ug/mL), HSA (5%), and α_1 acidic glycoprotein/HSA mixed solution were pre-incubated at 37°C for 5 minutes. Drug solution (0.01, 0.1, or 1 mg/mL) was added to the plasma and solution samples to a final concentration of 1%. After 10 minutes of incubation at 37°C, 0.7 mL of

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reaction sample was injected into the central cell of the dialysis unit, with phosphate-buffered isotonic saline (PBIS) in the right and left cells. The equilibrium dialysis times for both human plasma and the calibration standard (0.07% α_1 acidic glycoprotein solution) were determined to be 6 hours (detailed methods were described in the study report). Samples were incubated at 37°C with gentle shaking for 6 hours. Post-incubation, plasma and solution samples, as well as the PBIS solution in the left and right cells of the dialysis unit, were collected for ^{14}C-JTK-303 quantification. Three samples or solutions (pooled for rat plasma and the plasma solutions, individual for dog and monkey plasma) were used at each concentration.

Measurement of radioactivity and calculation of results
ULTIMA GOLD was added to the experimental samples and radioactivity was measured once for 5 minutes using a liquid scintillation analyzer. JTK-303 stability in plasma was calculated from the ratio of the JTK-303 peak area to the total peak area. The fraction of drug bound in plasma was calculated by the following equation:

$$\% \text{ protein binding} = 100 - \left[\frac{\text{Radioactivity in PBIS sample after dialysis / Sample weight}}{\text{Radioactivity in plasma sample after dialysis / Sample weight}} \times 100 \right]$$

Results
The results of the investigation of ^{14}C-JTK-303 stability in plasma from rats, dogs, monkeys, and humans are shown in Table 1. The residual ratio was greater than 99.1% in plasma from all species tested, indicating that ^{14}C-JTK-303 is stable in plasma from these species.

Table 1: Stability of ^{14}C-JTK-303 in Pooled Plasma from Rats, Dogs, Monkeys, and Humans (source: Study Report Table 14-2)

Animal species	Concentration (µg/mL)	Reaction time (hr)	Residual ratio (%) JTK-303
Rats	0.1	0	100.0
		8	100.0[1]
	10	0	99.1
		8	99.2
Dogs	0.1	0	100.0
		8	100.0
	10	0	99.7
		8	99.5
Monkeys	0.1	0	100.0
		8	100.0
	10	0	99.2
		8	100.0
Humans	0.1	0	100.0
		8	100.0
	10	0	99.4
		8	100.0

Residual ratio: The mean of two cases
1): Value from one case
Rats: Pooled plasma from seven male Sprague-Dawley rats
Dogs: Pooled plasma from six male beagle dogs
Monkeys: Pooled plasma from three male Cynomolgus monkeys
Humans: Pooled plasma from three healthy adult men

[14]C-JTK-303 was very highly plasma protein bound in a species- and concentration-independent manner, with the percent of drug bound to protein ranging from 99.89-99.93 in rat plasma, 99.19-99.23 in dog plasma, 98.80-98.83 in monkey plasma, and 99.31-99.35 in human plasma in concentrations ranging from 0.1 and 10 ug/mL (see Table 2). ^{14}C-JTK-303 protein binding was similar in human plasma and human serum albumin (HSA) solution (range: 99.38-99.4%), but the extent of protein binding was lower in human α1 acidic glycoprotein solution (range: 39.05-40.68%), indicating that JTK-303 is primarily bound to albumin in human plasma.

Table 2: Extent of ^{14}C-JTK-303 Protein Binding in Plasma from Rats, Dogs, Monkeys, and Humans, and Human Plasma Protein Solutions (mean ± SD, n=3 [individual dog, monkey, and human plasma samples; pooled plasma from 7 rats; pooled samples for human plasma protein solutions]; source: Study Report Table 14-7)

Animal species	Sample	Concentration (μg/mL)	Protein binding rate (%)
Rat	Plasma	0.1	99.89 ± 0.01
		1	99.93 ± 0.01
		10	99.93 ± 0.00
Dog	Plasma	0.1	99.23 ± 0.17
		1	99.22 ± 0.15
		10	99.19 ± 0.16
Monkey	Plasma	0.1	98.83 ± 0.11
		1	98.81 ± 0.09
		10	98.80 ± 0.09
Human	Plasma	0.1	99.35 ± 0.05
		1	99.34 ± 0.07
		10	99.31 ± 0.04
	5% HSA	0.1	99.40 ± 0.02
		1	99.39 ± 0.01
		10	99.38 ± 0.01
	0.07% α_1 acidic glycoprotein	0.1	39.25 ± 1.04
		1	39.05 ± 0.93
		10	40.68 ± 1.99
	0.05% α_1 acidic glycoprotein /HSA	0.1	99.45 ± 0.01
		1	99.39 ± 0.01
		10	99.36 ± 0.03
	0.1% α_1 acidic glycoprotein /HSA	0.1	99.06 ± 0.63
		1	99.33[1]
		10	99.44 ± 0.01
	0.2% α_1 acidic glycoprotein /HSA	0.1	99.44 ± 0.02
		1	99.43 ± 0.01
		10	99.41 ± 0.01

Mean ± SD
[n = 3 (individual dog, monkey and human plasma, pooled plasma from seven rats, and pooled samples for 5% HSA, 0.07% α_1 acidic glycoprotein, and α_1 acidic glycoprotein/HSA)]
1): The mean of two cases
Rat plasma: Male Sprague-Dawley rats, Dog plasma: Male beagle dogs,
Monkey plasma: Male cynomolgus monkeys, Human plasma: Healthy adult men

Conclusion

The extent of ^{14}C-JTK-303 protein binding in human plasma ranged from 99.89 to 99.93%, primarily due to binding with albumin (99.38 to 99.40% protein-bound in 5% HSA solution). Drug was stable and highly protein-bound in plasma, irrespective of species or drug concentration.

Reference ID: 3153809

Study JTK303-AD-013 – Distribution of JTK-303 into Blood Cells *In Vitro*
Study Report Date: 23 Mar 2004

Introduction
In this study, the degrees of distribution of JTK-303 into blood cells from rats, dogs, monkeys, and humans were evaluated *in vitro*. The stability of JTK-303 in rat, dog, monkey, and human blood was also calculated.

Materials and methods
Reagents and biospecimens
The reference standard JTK-303 was manufactured by Japan Tobacco Inc. JTK-303 was radiolabeled with ^{14}C (specific radioactivity: 4.99 MBq/mg) (b) (4)
Whole blood was collected from the vena cava of euthanized male Sprague-Dawley rats (n=12, (b) (4) .) and the cephalic vein of the foreleg of male beagle dogs (n=3, (b) (4) .). Whole blood from male cynomolgus monkeys was purchased (n=6, (b) (4)). Whole blood was also collected from healthy adult male volunteers (b) (4)

Rate of ^{14}C-JTK-303 distribution into blood cells
Drug solution (10 uL of 0.01, 0.1, or 1.0 mg/mL) was added to 1 mL pre-warmed (37˚C for 5 min) blood samples from rat, dog, monkey, and human and the reaction solution was incubated at 37˚C for 0.5 h (detailed methods for determining the reaction time are described in the study report). An aliquot of blood (100 uL) was taken for radioactivity measurements, and the remaining blood was centrifuged (10000 rpm for 5 min). Radioactivity was measured in 100 uL of the resulting plasma from three reaction samples per species.

Stability of high concentration ^{14}C-JTK-303 solution in blood
Drug solution (1.0 mg/mL) was added at a ratio of 1% to pre-warmed (37˚C for 5 min) blood samples from rat, dog, monkey, and human (4 mL for human, 1 mL for other species), which were subsequently aliquoted into 100 uL reaction samples. The reaction samples were incubated at 37˚C for 0 and 2 hours. The reaction was terminated by the addition of 200 uL acetonitrile solution containing 0.1% acetic acid to 100 uL aliquots of the reaction samples. The samples were centrifuged (10000 rpm at 4˚C for 5 min) and radioactivity was measured in 100 uL of the resulting supernatant from three reaction samples per species.

Stability of low concentration ^{14}C-JTK-303 solution in blood
Drug solution (0.01 mg/mL) was added at a ratio of 1% to pre-warmed (37˚C for 5 min) blood samples from rat, dog, monkey, and human (4 mL for human, 1.5 mL for other species), which were subsequently aliquoted into 100 uL reaction samples. The reaction samples were incubated at 37˚C for 0 and 2 hours. The reaction was terminated by the addition of 800 uL acetonitrile solution containing 0.1% acetic acid to 400 uL of the reaction samples. The samples were

centrifuged (10000 rpm at 4°C for 5 minutes) and radioactivity was measured in 100 uL of the resulting supernatant from three reaction samples per species.

Measurement of radioactivity and calculation of results
ULTIMA GOLD was added to the experimental samples and radioactivity was measured once for 5 minutes using a liquid scintillation analyzer. JTK-303 stability in plasma was calculated from the ratio of the JTK-303 peak area to the total peak area. Drug concentrations in blood were calculated by the following equation:

$$\text{ug/mL in blood} = \frac{\text{Radioactivity in blood sample / Sample volume}}{\text{Specific radioactivity}} / 1000 \times \text{Dilution ratio}$$

Results

The percentage of ^{14}C-JTK-303 that was distributed into blood cells is shown in Table 1. Within a drug concentration range of 0.1-10 ug/mL, the percentage of drug in blood cells was 2.2-3.2% in rats, 25.6-32.4% in dogs, 26.1-28.6% in monkeys, and 20.8-24.0% in humans. These results indicate that there is a moderate degree of ^{14}C-JTK-303 distribution into rat, dog, and monkey blood cells but low distribution into blood cells from rats.

Table 1: Distribution of ^{14}C-JTK-303 into Rat, Dog, Monkey, and Human Blood Cells (source: Study Report Table 14-3)

Animal species	Concentration (µg/mL)	Distribution into blood cells (%)
		Mean ± SD
Rats	0.1	2.2 ± 1.9
	1	3.0 ± 2.0
	10	3.2 ± 2.8
Dogs	0.1	32.4 ± 1.9
	1	30.8 ± 3.0
	10	25.6 ± 3.4
Monkeys	0.1	26.4 ± 4.9
	1	28.6 ± 3.4
	10	26.1 ± 8.0
Humans	0.1	24.0 ± 5.4
	1	21.9 ± 6.4
	10	20.8 ± 2.0

Mean ± SD (n = 3)
Rat blood: Male Sprague-Dawley strain, Dog blood: Male beagle dogs
Monkey blood: Male Cynomolgus, Human blood: Healthy adult men

The results of the investigation of ^{14}C-JTK-303 stability in blood from rats, dogs, monkeys, and humans are shown in Table 2. The residual ratio was at least 98.8% in blood from all species tested, indicating that ^{14}C-JTK-303 is stable in blood from these species.

Table 2: Stability of ^{14}C-JTK-303 into Rat, Dog, Monkey, and Human Blood Cells (source: Study Report Table 14-4)

Animal species	Concentration (µg/mL)	Reaction time (hr)	Residual ratio (%) JTK-303
Rats	0.1	0	100.0
		2	100.0
	10	0	98.8
		2	99.4
Dogs	0.1	0	100.0
		2	100.0
	10	0	99.5
		2	99.6
Monkeys	0.1	0	100.0
		2	100.0
	10	0	99.6
		2	99.6
Humans	0.1	0	100.0
		2	100.0
	10	0	100.0
		2	100.0

Residual ratio: The mean of three cases
Rats: Male Sprague-Dawley strain, Dogs: Male beagle dogs
Monkeys: Male Cynomolgus, Humans: Healthy adult men

Conclusion
The extent of ^{14}C-JTK-303 distribution into human blood cells ranged from 20.8 to 24.0% and did not appear to be concentration-dependent. Distribution into rat blood cells was substantially lower than other species tested, ranging from 2.2 to 3.2%. JTK-303 was stable in blood, irrespective of species or drug concentration.

In vitro studies pertinent to hepatic metabolism

Studies
JTK-303-AD-015
JTK-303-AD-016
JTK-303-AD-017
JTK-303-AD-018
JTK-303-AD-024

Summary
These five studies evaluated the metabolism of ^{14}C-JTK-303 in liver microsomes from rats, dogs, monkeys, and/or humans. In liver microsomes from all species, the primary oxidative metabolite was M1, a chlorofluorophenyl group hydroxide of JTK-303, and the primary glucuronide conjugate was M4, an acyl-glucuronide conjugate of JTK-303. In human liver microsomes, oxidative metabolism of JTK-303 was more extensive (rate of metabolism: 129.4 pmol/min/mg protein) than glucuronide conjugation of JTK-303 (rate of metabolism: 0.9 pmol/min/mg protein).

CYP3A4 was responsible for the majority of JTK-303 metabolism in human liver microsomes, although metabolism via CYP3A5 and CYP1A1 was also detected. In the presence of the CYP3A4 inhibitor ketoconazole, metabolism of JTK-303 in human liver microsomes was markedly reduced (69.8 and 97.5% inhibition by 0.2 and 2 uM ketoconazole, respectively), but the CYP2C9 inhibitor sulfaphenazole and the CYP2D6 inhibitor quinidine did not substantially influence JTK-303 metabolism. The K_m and V_{max} of JTK-303 metabolic activity (as determined in human liver microsomes) were 21.46 uM and 1265 pmol/min/mg protein, respectively.

Brief reviews of the individual study reports are appended below.

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Study JTK303-AD-015 – In Vitro Metabolism of ^{14}C-JTK-303 (Oxidative Reaction in Liver Microsomes)
Study Report Date: 23 Mar 2004

Introduction
In this study, the rate of metabolism of JTK-303 and the rate of formation of JTK-303 oxidative metabolites were investigated with ^{14}C-JTK-303 in the presence of NADPH in liver microsomes from rats, dogs, monkeys, and humans.

Materials and methods
Reagents and biospecimens
The reference standard JTK-303 was manufactured by Japan Tobacco Inc. JTK-303 was radiolabeled with ^{14}C (specific radioactivity: 4.99 MBq/mg)   (b) (4)
                                 Frozen pooled liver microsomes from human, dog (beagle), rat, and cynomolgus monkey were purchased at concentrations of 20 mg/mL from   (b) (4) (Lots 0310156, 0310058, 0310113, and 0210403, respectively).

Reaction procedure
Radiolabeled JTK-303 (final concentration: 1.0 ug/mL), liver microsomes (final concentration: 1.0 ug/mL), and potassium phosphate buffer (pH 7.4) were combined (total volume: 160 uL) and incubated at 37˚C for 5 min. The reaction was initiated by the addition of 40 uL of NADPH generating system (0.4 U/mL G-6-PDH solution, 1.3 mM NADP$^+$, 3.3 mM G-6-P, and 3.3 mM MgCl$_2$) followed by a 10 min incubation at 37˚C. Potassium phosphate buffer was added to control samples in place of the NADPH generating system. After incubation, a representative aliquot was taken for radioactivity counting (the reaction sample), and 0.1% acetic acid in acetonitrile was added to the remainder to terminate the reaction. The reaction mixture was vortexed and centrifuged (10000 rpm, 4˚C, 5 min) and an aliquot of the supernatant was taken for radioactivity counting (the extraction sample); the remaining supernatant was dried under N$_2$, dissolved in acetonitrile and ammonium formate buffer and its composition was analyzed by HPLC. The pellet was sonicated in water for 30 min and dissolved (50˚C, 1 h) and radioactivity was counted. All metabolic reactions were performed in duplicate.

Measurement of radioactivity and calculation of results
ULTIMA GOLD or HIONIC FLUOR was added to experimental samples and radioactivity was counted (once for 5 min) in triplicate for each sample using a liquid scintillation analyzer. A specific radioactivity of 299.4 dpm/ng was used to calculate radioactivity in experimental samples. The quantifiable limit of radioactivity was set as 2x the radioactivity of the background samples. The lowest detection limit for the peak heights using radio-HPLC was set as 40 cpm.

The rate of JTK-303 metabolism was calculated by the following equation, where A is the mean percentage of JTK-303 radioactivity at time 0, and B is that percentage at 10 min:

$$\text{Rate of metab (pmol/min/mg protein)} = \frac{A-B}{100} \times [\text{substrate}] \ (uM) \times 1000 \times \frac{1}{\text{rxn time (min)}} \times \frac{1}{[\text{protein}] \ (mg/mL)}$$

The rate of formation of JTK-303 metabolites was calculated by the following equation, where A is the mean percentage of JTK-303 radioactivity at time 0, and B is that percentage at 10 min:

$$\text{Rate of metab formation (pmol/min/mg protein)} = \frac{B-A}{100} \times [\text{substrate}] \ (uM) \times 1000 \times \frac{1}{\text{rxn time (min)}} \times \frac{1}{[\text{protein}] \ (mg/mL)}$$

Results

Oxidative metabolism of JTK-303 was more extensive in liver microsomes from monkey (rate of metabolism: 193.8 pmol/min/mg protein) compared to liver microsomes from rat, human, or dog (rates of metabolism: 130.8, 129.4, and 15.4 pmol/min/mg protein, respectively). The primary metabolite in all species was a chlorofluorophenyl group hydroxide of JTK-303 (M1). Metabolites M5 (human, dog, monkey) and M8 (human, rat, monkey) were also detected, but their structures were not determined. Representative radio-HPLC chromatograms of ^{14}C-JTK-303 and metabolites are shown in Figure 1.

Figure 1: Typical Radio-HPLC Chromatograms of the Parent Drug and its Metabolites after Reaction with Liver Microsomes in the Presence of NADPH (source: Study Report Figure 15-5)
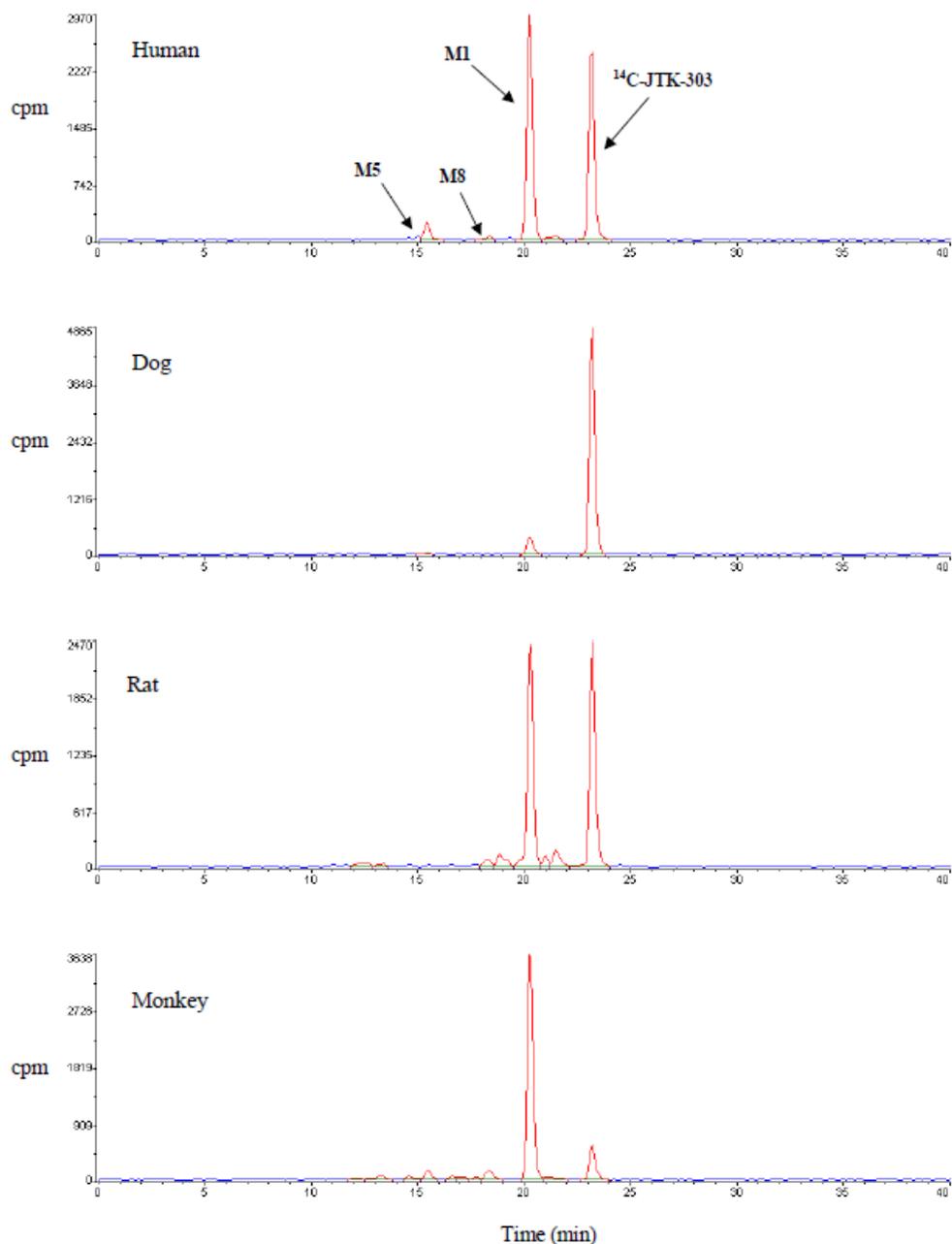
Conclusion

[14]C-JTK-303 was metabolized the presence of NADPH more extensively in liver microsomes from monkey compared to liver microsomes from rat, human, and dog. ^{14}C-JTK-303 was primarily metabolized to M1, a chlorofluorophenyl group hydroxide of JTK-303, in all species. Minor metabolites M5 (human, dog, and monkey) and M8 (human, rat, and monkey) were also detected. These oxidative reactions did not proceed in the absence of the reducing agent NADPH.

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Study JTK303-AD-016 – In Vitro Metabolism of ^{14}C-JTK-303 (Glucuronide Conjugation in Liver Microsomes)

Study Report Date: 23 Mar 2004

Introduction

In this study, the rate of metabolism of JTK-303 and the rate of formation of JTK-303 glucuronide metabolites were investigated with ^{14}C-JTK-303 in the presence of uridine diphosphoglucuronic acid (UDPGA) in liver microsomes from rats, dogs, monkeys, and humans.

Materials and methods

Reagents and biospecimens

The reference standard JTK-303 was manufactured by Japan Tobacco Inc. JTK-303 was radiolabeled with ^{14}C (specific radioactivity: 4.99 MBq/mg) (b) (4) Frozen pooled liver microsomes from human, dog (beagle), rat, and cynomolgus monkey were purchased at concentrations of 20 mg/mL from (b) (4) (Lots 0310156, 0310058, 0310113, and 0210403, respectively).

Reaction procedure

Radiolabeled JTK-303 (final concentration: 1.0 ug/mL), liver microsomes (final concentration: 1.0 ug/mL), alamethicin (final concentration: 25 ug/mL), D-saccharic acid 1,4-lactone (final concentration: 5 mM), and Tris-HCl buffer (pH 7.5) were combined (total volume: 160 uL) and incubated at 37°C for 5 min. The reaction was initiated by the addition of 40 uL of a mixture of UDPGA and MgCl$_2$ (5 mM UDPGA and 10 mM MgCl$_2$) followed by a 60 min incubation at 37°C. After incubation, a representative aliquot was taken for radioactivity counting (the reaction sample), and 0.1% acetic acid in acetonitrile was added to the remainder to terminate the reaction. The reaction mixture was vortexed and centrifuged (10000 rpm, 4°C, 5 min) and an aliquot of the supernatant was taken for radioactivity counting (the extraction sample); the remaining supernatant was dried under N$_2$, dissolved in acetonitrile and ammonium formate buffer and its composition was analyzed by HPLC. The pellet was sonicated in water for 30 min and dissolved (50°C, 1 h) and radioactivity was counted. All metabolic reactions were performed in duplicate.

Measurement of radioactivity and calculation of results

ULTIMA GOLD or HIONIC FLUOR was added to experimental samples and radioactivity was counted (once for 5 min) in triplicate for each sample using a liquid scintillation analyzer. A specific radioactivity of 299.4 dpm/ng was used to calculate radioactivity in experimental samples. The quantifiable limit of radioactivity was set as 2x the radioactivity of the background samples. The lowest detection limit for the peak heights using radio-HPLC was set as 40 cpm.

The rate of JTK-303 metabolism was calculated by the following equation, where A is the mean percentage of JTK-303 radioactivity at time 0, and B is that percentage at 10 min:

$$\text{Rate of metab (pmol/min/mg protein)} = \frac{A\text{-}B}{100} \times [\text{substrate}]\ (uM) \times 1000 \times \frac{1}{\text{rxn time (min)}} \times \frac{1}{[\text{protein}]\ (mg/mL)}$$

The rate of formation of JTK-303 metabolites was calculated by the following equation, where A is the mean percentage of JTK-303 radioactivity at time 0, and B is that percentage at 10 min:

$$\text{Rate of metab formation (pmol/min/mg protein)} = \frac{B\text{-}A}{100} \times [\text{substrate}]\ (uM) \times 1000 \times \frac{1}{\text{rxn time (min)}} \times \frac{1}{[\text{protein}]\ (mg/mL)}$$

Results

Glucuronide conjugation of JTK-303 was more extensive in liver microsomes from rats (rate of metabolism: 17.9 pmol/min/mg protein) followed by monkeys, dogs, and humans (rates of metabolism: 7.1, 3.7, and 0.9 pmol/min/mg protein, respectively). The primary metabolite in all species was an acyl-glucuronide conjugate of JTK-303 (M4). An ether-glucuronide (M3) was also formed in rat and dog. Representative radio-HPLC chromatograms of ^{14}C-JTK-303 and its glucuronide conjugates are shown in Figure 1.

Figure 1: Typical Radio-HPLC Chromatograms of the Parent Drug and its Glucuronide Conjugates after Reaction with Liver Microsomes in the Presence of UDPGA (source: Study Report Figure 15-5)
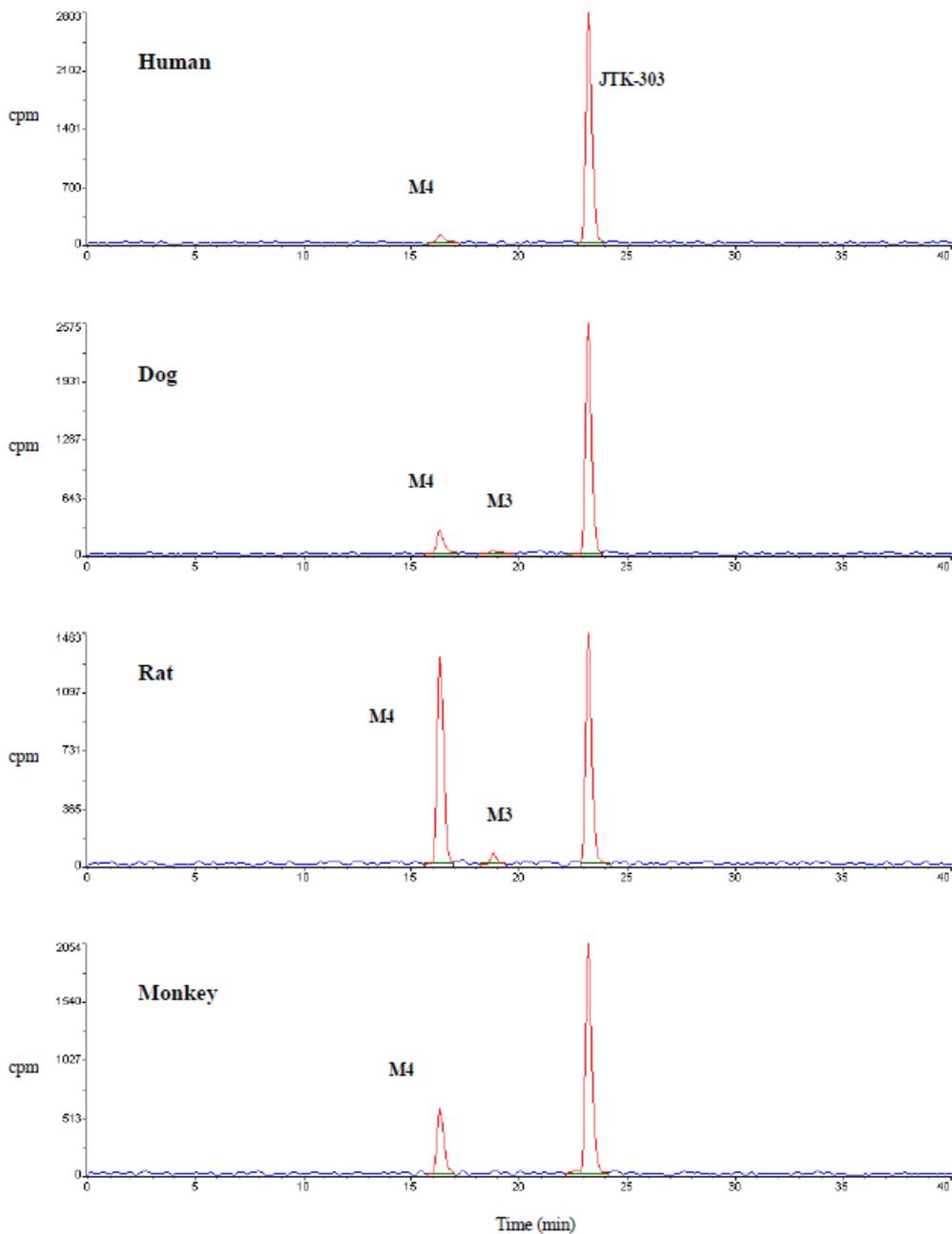
Conclusion

Glucuronide conjugates of ^{14}C-JTK-303 were formed in the presence of UDPGA more extensively in liver microsomes from rat compared to liver microsomes from monkey, dog, and human. ^{14}C-JTK-303 was primarily metabolized to M4, an acyl-glucuronide of JTK-303, in all species. The ether-glucuronide M3 was also formed in rat and dog.

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Study JTK303-AD-017 – Metabolism of JTK-303 by Recombinant Human CYP Isoforms

Study Report Date: 17 Dec 2004

Introduction

In this study, the rate of metabolism of JTK-303 and the rate of formation of JTK-303 oxidative metabolites were investigated when ^{14}C-JTK-303 was incubated with recombinant CYP isoforms.

Materials and methods

Reagents and biospecimens

The reference standard JTK-303 was manufactured by Japan Tobacco Inc. JTK-303 was radiolabeled with ^{14}C (specific radioactivity: 4.99 MBq/mg) (b) (4) Microsomes expressing human CYPs (CYP1A1: Lot 19; CYP1A2: Lot 27; CYP2A6: Lot 11; CYP2B6: Lot 10; CYP2C8: Lot 16; CYP2C9*1: Lot 26; CYP2C19: Lot 18; CYP2D6: Lot 41; CYP2E1: Lot 15; CYP3A4: Lot 58; CYP3A5: Lot 18) were purchased frozen from (b) (4).
A microsome solution (final microsomal protein concentration: 1 mg/mL) was made by combining CYP microsomes, control microsomes (P450 reductase control microsomes for CYP1A1, CYP1A2, CYP2D6, CYP3A5; P450 reductase + cytochrome b5 control microsomes for CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2E1, CYP3A4), and potassium phosphate buffer (pH 7.4) or Tris-HCl buffer (pH 7.5).

Reaction procedure

Radiolabeled JTK-303 (final concentration: 10 ug/mL), liver microsome solution (final P450 concentration: 50 pmol/mL), and potassium phosphate buffer (pH 7.4) or Tris-HCL buffer (pH 7.5) were combined (total volume: 160 uL) and incubated at 37˚C for 5 min. The reaction was initiated by the addition of 40 uL of NADPH generating system (0.4 U/mL G-6-PDH solution, 1.3 mM NADP$^+$, 3.3 mM G-6-P, and 3.3 mM MgCl$_2$) followed by a 30 min incubation at 37˚C. After incubation, a representative aliquot was taken for radioactivity counting (the reaction sample), and 0.1% acetic acid in acetonitrile was added to the remainder to terminate the reaction. The reaction mixture was vortexed and centrifuged (10000 rpm, 4˚C, 5 min) and an aliquot of the supernatant was taken for radioactivity counting (the extraction sample); the remaining supernatant was dried under N$_2$, dissolved in acetonitrile and ammonium formate buffer and its composition was analyzed by HPLC. The pellet was sonicated in water for 30 min and dissolved (50˚C, 1 h) and radioactivity was counted. All metabolic reactions were performed in duplicate.

Measurement of radioactivity and calculation of results

ULTIMA GOLD or HIONIC FLUOR was added to experimental samples and radioactivity was counted (once for 5 min) in triplicate for each sample using a liquid scintillation analyzer. A specific radioactivity of 299.4 dpm/ng was used to calculate radioactivity in experimental samples. The quantifiable limit of

radioactivity was set as 2x the radioactivity of the background samples. The lowest detection limit for the peak heights using radio-HPLC was set as 40 cpm.

The rate of JTK-303 metabolism was calculated by the following equation, where A is the percentage of JTK-303 radioactivity of control microsomes at time 0, and B is the percentage of JTK-303 radioactivity of control or P450-expressing microsomes at 30 min:

$$\text{Rate of metab (pmol/min/mg protein)} = \frac{A-B}{100} \times [\text{substrate}] \, (uM) \times 1000 \times \frac{1}{\text{rxn time (min)}} \times \frac{1}{[\text{P450}] \, (\text{pmol/mL})}$$

The rate of formation of JTK-303 metabolites was calculated by the following equation, where A is the percentage of JTK-303 radioactivity of control microsomes at time 0, and B is the percentage of JTK-303 radioactivity of control or P450-expressing microsomes at 30 min:

$$\text{Rate of metab formation (pmol/min/mg protein)} = \frac{B-A}{100} \times [\text{substrate}] \, (uM) \times 1000 \times \frac{1}{\text{rxn time (min)}} \times \frac{1}{[\text{protein}] \, (\text{mg/mL})}$$

Results
JTK-303 was metabolized by CYP1A1, CYP3A4, and CYP3A5 (rates of metabolism: 0.9, 9.4, and 0.4 pmol/min/mg protein, respectively); no other CYP isoforms were involved in JTK-303 metabolism. M1, the hydroxide at the chlorofluorophenyl group of JTK-303, was the primary metabolite formed by CYP3A4 and CYP3A5 (rates of formation: 7.5 and 0.4 pmol/min/pmol P450, respectively); this was also the main metabolite formed in human liver microsomes. M2, the hydroxide at the benzyl group of JTK-303, was formed by CYP1A1 (rate of metabolism: 0.9 pmol/min/pmol P450). Small amounts of the minor metabolites M5 and M8 were also formed by CYP3A4.

Conclusion
CYP3A4, CYP1A1, and CYP3A5 mediate the metabolism of ^{14}C-JTK-303 in humans (rates of metabolism: 9.4, 0.9, and 0.4 pmol/min/pmol P450, respectively). M1 was formed by all of these CYPs; the rate of M1 formation by CYP3A4 was 7.5 pmol/min/pmol P450, which was higher than the other isoforms. CYP1A1 was also involved in the formation of the minor metabolite M2, while CYP3A4 and CYP3A5 were involved in the formation of the minor metabolites M5 and M8. Qualitatively, JTK-303 metabolism using recombinant human CYP isoforms was similar to that of human liver microsomes.

Study JTK303-AD-018 – Effects of CYP Inhibitors on the Metabolism of JTK-303 in Human Liver Microsomes

Study Report Date: 25 Mar 2004

Introduction

In this study, CYP inhibitors were used to identify the CYP isoforms involved in ^{14}C-JTK-303 metabolism in human liver microsomes.

Materials and methods

Reagents and biospecimens

The reference standard JTK-303 was manufactured by Japan Tobacco Inc. JTK-303 was radiolabeled with ^{14}C (specific radioactivity: 4.99 MBq/mg) (b) (4) Frozen pooled human liver microsomes were purchased at a concentration of 20 mg/mL from (b) (4) (Lot 0310156). The selective inhibitors sulfaphenazole (CYP2C8/9), quinidine (CYP2D6), and ketoconazole (CYP3A4) were purchased from (b) (4).

Reaction procedure

CYP inhibitor solution was added to a 2 mL Eppendorf tube and evaporated to dryness under a nitrogen stream. Radiolabeled JTK-303 (final concentration: 1.0 ug/mL), liver microsomes (final concentration: 1 mg protein/mL), and potassium phosphate buffer (pH 7.4) were combined in the Eppendorf tube (total volume: 160 uL) and incubated at 37°C for 5 min. The reaction was initiated by the addition of 40 uL of NADPH generating system (0.4 U/mL G-6-PDH solution, 1.3 mM NADP$^+$, 3.3 mM G-6-P, and 3.3 mM MgCl$_2$) followed by a 10 min incubation at 37°C. After incubation, a representative aliquot was taken for radioactivity counting (the reaction sample), and 0.1% acetic acid in acetonitrile was added to the remainder to terminate the reaction. The reaction mixture was vortexed and centrifuged (10000 rpm, 4°C, 5 min) and an aliquot of the supernatant was taken for radioactivity counting (the extraction sample); the remaining supernatant was dried under N$_2$, dissolved in acetonitrile and ammonium formate buffer and its composition was analyzed by HPLC. The pellet was sonicated in water for 30 min and dissolved (50°C, 1 h) and radioactivity was counted. All metabolic reactions were performed in duplicate.

Measurement of radioactivity and calculation of results

ULTIMA GOLD or HIONIC FLUOR was added to experimental samples and radioactivity was counted (once for 5 min) in triplicate for each sample using a liquid scintillation analyzer. A specific radioactivity of 299.4 dpm/ng was used to calculate radioactivity in experimental samples. The quantifiable limit of radioactivity was set as 2x the radioactivity of the background samples. The lowest detection limit for the peak heights using radio-HPLC was set as 40 cpm.

The rate of JTK-303 metabolism was calculated by the following equation, where A is the mean percentage of JTK-303 radioactivity at time 0, and B is that percentage at 10 min:

$$\text{Rate of metab (pmol/min/mg protein)} = \frac{A-B}{100} \times [\text{substrate}] \, (uM) \times 1000 \times \frac{1}{\text{rxn time (min)}} \times \frac{1}{[\text{protein}] \, (mg/mL)}$$

The rate of formation of JTK-303 metabolites was calculated by the following equation, where A is the mean percentage of JTK-303 radioactivity at time 0, and B is that percentage at 10 min:

$$\text{Rate of metab formation (pmol/min/mg protein)} = \frac{B-A}{100} \times [\text{substrate}] \, (uM) \times 1000 \times \frac{1}{\text{rxn time (min)}} \times \frac{1}{[\text{protein}] \, (mg/mL)}$$

The percentage of inhibition of JTK-303 metabolism was calculated by the following equation:

$$\text{Percentage of inhibition} = 100 \times 1 - \frac{\text{Rate of metabolism in the presence of an inhibitor}}{\text{Rate of metabolism in the absence of an inhibitor}}$$

Results
JTK-303 oxidative metabolism in human liver microsomes was inhibited 69.8 and 97.5% by 0.2 and 2 uM ketoconzole (a CYP3A4 inhibitor), respectively. The CYP2C9 inhibitor sulfaphenazole inhibited JTK-303 metabolism by 8.0 and 14.6% (0.2 and 2 uM, respectively), and the CYP2D6 inhibitor quinidine inhibited JTK-303 metabolism by 4.1 and 5.1% (0.2 and 2 uM, respectively). The formation of M1, the metabolite formed by CYP3A, was inhibited by 68.0 and 96.8% by 0.2 and 2 uM ketoconzole, respectively.

Conclusion
Ketoconazole markedly reduced formation of the JTK-303 metabolite M1, indicating that CYP3A4 is primarily responsible for JTK-303 metabolism in human liver microsomes. In contrast, the CYP2C9 inhibitor sulfaphenazole and the CYP2D6 inhibitor quinidine did not substantially inhibit JTK-303 metabolism, suggesting that these CYP isoforms are minor pathways of JTK-3030 metabolism.

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Study JTK303-AD-024 – In Vitro Study of JTK-303 [II]: Determination of K_m and V_{max} Using Human Liver Microsomes

Study Report Date: 28 Apr 2005

Introduction
In this study, the Michaelis constant (K_m) and maximal velocity (V_{max}) of JTK-303 were determined from the metabolic activity of ^{14}C-JTK-303 and M1 in human liver microsomes.

Materials and methods
Reagents and biospecimens

The reference standard JTK-303 was manufactured by Japan Tobacco Inc. JTK-303 was radiolabeled with [14]C (specific radioactivity: 4.99 MBq/mg) (b) (4) Frozen pooled human liver microsomes were purchased at a concentration of 20 mg/mL from (b) (4) (Lot 0410042).

Reaction procedure

Radiolabeled JTK-303 (final concentration: 2, 4, 10, 20, 40, 60, or 120 uM), liver microsomes (final concentration: 1 mg protein/mL), and potassium phosphate buffer (pH 7.4) were combined (total volume: 330 uL) and incubated at 37°C for 5 min. The reaction was initiated by the addition of 170 uL of NADPH generating system (0.4 U/mL G-6-PDH solution, 1.3 mM $NADP^+$, 3.3 mM G-6-P, and 3.3 mM $MgCl_2$) followed by incubation at 37°C. After 10 min incubation at 37°C, a representative aliquot was taken for radioactivity counting (the reaction sample), and 0.1% acetic acid in acetonitrile was added to the remainder to terminate the reaction. The reaction mixture was vortexed and centrifuged (10000 rpm, 4°C, 5 min) and an aliquot of the supernatant was taken for radioactivity counting (the extraction sample); the remaining supernatant was dried under N_2, dissolved in acetonitrile and ammonium formate buffer and its composition was analyzed by HPLC. The pellet was sonicated in water for 30 min and dissolved (50°C, 1 h) and radioactivity was counted. All metabolic reactions were performed in duplicate.

Measurement of radioactivity and calculation of results

ULTIMA GOLD or HIONIC FLUOR was added to experimental samples and radioactivity was counted (once for 5 min) in triplicate for each sample using a liquid scintillation analyzer. A specific radioactivity of 299.4 dpm/ng was used to calculate radioactivity in experimental samples. The quantifiable limit of radioactivity was set as 2x the radioactivity of the background samples. The lowest detection limit for the peak heights using radio-HPLC was set as 40 cpm.

The metabolic activity of ^{14}C-JTK-303 (pmol/min/mg protein) was calculated by the following equation, where A is the relative amount of ^{14}C-JTK-303 in the incubation mixture, B is the relative amount of ^{14}C-JTK-303 in the control mixture (not containing the NADPH generating system), C is the initial concentration, D is the incubation time, and E is the microsomal concentration:

$$\text{Metabolic activity of }^{14}\text{C-JTK-303} = \frac{(B - A) \times C}{D \times E}$$

The metabolic activity of M1 (pmol/min/mg protein) was calculated by the following equation, where A is the relative amount of M1 in the incubation mixture, B is the relative amount of M1 in the control mixture (not containing the NADPH generating system), and C, D, and E are as above:

$$\text{Metabolic activity of M1} = \frac{(B - A) \times C}{D \times E}$$

K_m and V_{max} were calculated from the metabolic activity of JTK-303 or M1 and the initial concentration of JTK-303 by fitting the data to a Michaelis-Menten equation using WinNonlin (version 2.1, Pharsight Corp.).

Results

[14]C-JTK-303 metabolic activity and M1 formation activity in human liver microsomes were used to determine K_m and V_{max}. Based on JTK-303 metabolic activity, the K_m and V_{max} were 21.46 uM and 1265 pmol/min/mg protein, respectively. Based on the M1 formation activity, the K_m and V_{max} were 20.36 uM and 1083 pmol/min/mg protein, respectively. For comparison, the V_{max} determined from the metabolic activity of CYP3A4 (based on the probe substrate midazolam) in human liver microsomes was 704.5 pmol/min/mg protein.

Conclusion

K_m and V_{max} were determined using JTK-303 metabolic activity (21.46 uM and 1265 pmol/min/mg protein, respectively) and M1 formation activity (20.36 uM and 1083 pmol/min/mg protein, respectively).

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In vitro studies pertaining to potential drug interactions

Studies
JTK-303-AD-025
JTK-303-AD-027
JTK-303-AD-023
AD-183-2028
AD-183-2034
JTK-303-AD-026
AD-183-2030

Summary
These seven studies evaluated the interactions between JTK-303 (elvitegravir) and CYP450 enzymes, the transporters P-glycoprotein, OATP1B1, and OATP1B3, and UDP-glucuronosyl transferases (UGTs) using human liver microsomes, primary cultured human hepatocytes, or transfected cell culture assays.

JTK-303 metabolism was influenced by a panel of potentially concomitant medications, all of which interact with CYP3A (JTK303-AD-025); however, the inhibition observed in vitro is unlikely to be clinically relevant in the presence of low-dose ritonavir or cobicistat. JTK-303 was a weak inhibitor of CYP3A4 (IC_{50} value of 63 uM) but did not inhibit any other CYP isoforms (JTK303-AD-027). JTK-303 was also a weak inducer of CYP2C9 and CYP3A4 (JTK303-AD-023), although the latter is unlikely to be clinically relevant in the presence of low-dose ritonavir or cobicistat.

In the presence of ritonavir (with which elvitegravir will be coadministered clinically), glucuronidation becomes the primary route of elvitegravir metabolism. Elvitegravir glucuronidation was determined to be primarily mediated by UGT1A1 and UGT1A3 (AD-183-2034). An in vitro study indicated that at pharmacological concentrations, ketoconazole and atazanavir inhibited JTK-303 glucuronidation (AD-183-2028). Clinical drug-drug interaction studies were subsequently performed to inform dosing recommendations when elvitegravir is coadministered with ketoconazole, lopinavir, or atazanavir.

Studies conducted in cell culture systems demonstrated that JTK-303 is a substrate and a weak inhibitor of P-glycoprotein (JTK-303-AD-026) as well as a weak inhibitor of OATP1B1 and a more potent inhibitor of OATP1B3 (AD-183-2030). Clinical drug-drug interaction studies were performed in which ritonavir-boosted elvitegravir was coadministered with the P-gp inducer tipranavir or the OATP1B1/3 substrate rosuvastatin; no clinically significant pharmacokinetic alterations were observed.

Brief reviews of the individual study reports are appended below.

Study JTK303-AD-025 – In Vitro Study of JTK-303 [III]: Interaction Study of JTK-303 with Coadministered Drugs
Study Report Date: 28 Apr 2005

Introduction
In this study, the potential of ten drugs that may potentially be administered concomitantly with JTK-303 (amprenavir, efavirenz, indinavir sulfate, ketoconazole, lopinavir, nelfinavir, nevirapine, ritonavir, saquinavir, and zidovudine) to inhibit the metabolism of ^{14}C-JTK-303 was tested in human liver microsomes.

Materials and methods
Reagents and biospecimens
Amprenavir and lopinavir were purchased from   (b) (4); efavirenz, indinavir sulfate, nevirapine, ritonavir, and saquinavir from   (b) (4)   ; and ketoconazole and zidovudine from   (b) (4). Nelfinavir and the reference standard JTK-303 were supplied by Japan Tobacco Inc. JTK-303 was radiolabeled with ^{14}C (specific radioactivity: 4.99 MBq/mg)   (b) (4)   Frozen pooled human liver microsomes were purchased at a concentration of 20 mg/mL from   (b) (4) (Lot 0410042). All of the coadministered antiretrovirals except for zidovudine and ritonavir were tested at concentrations of 0.1, 0.5, 1, 5, 10, and 50 uM. Zidovudine was tested at concentrations of 1, 10, and 100 uM. Ritonavir and ketoconazole were tested at 0.003, 0.01, 0.03, 0.1, 0.3, and 1 uM. The CYP3A4 probe substrate midazolam was used at 10 uM.

Reaction procedure
Radiolabeled JTK-303 (final concentration: 2 uM), liver microsomes (final concentration: 1.0 mg/mL), and potassium phosphate buffer (pH 7.4) were combined (total volume: 330 uL) and incubated at 37°C for 5 min. The reaction was initiated by the addition of 170 uL of NADPH generating system (0.4 U/mL G-6-PDH solution, 1.3 mM NADP$^+$, 3.3 mM G-6-P, and 3.3 mM MgCl$_2$) followed by a 10 min incubation at 37°C. After incubation, a representative aliquot was taken for radioactivity counting (the reaction sample), and 0.1% acetic acid in acetonitrile was added to the remainder to terminate the reaction. The reaction mixture was ultrasonicated and centrifuged (3000 rpm, 4°C, 5 min) and an aliquot of the supernatant was taken for radioactivity counting (the extraction sample). The residue was dissolved in the tissue solubilizer Soluene-350 and the remaining supernatant was dried under N$_2$ and dissolved in acetonitrile and ammonium formate buffer; the radioactivity of both samples was counted. The pellet was sonicated in water for 30 min and dissolved (50°C, 1 h) and radioactivity was counted.

Calculation of results
The metabolic activity of ^{14}C-JTK-303 (pmol/min/mg protein) was calculated by the following equation, where A is the relative amount of ^{14}C-JTK-303 in the

incubation mixture, B is the relative amount of ^{14}C-JTK-303 in the control mixture (not containing the NADPH generating system), C is the initial concentration, D is the incubation time, and E is the microsomal concentration:

$$\text{Metabolic activity of } ^{14}\text{C-JTK-303} = \frac{(B - A) \times C}{D \times E}$$

The metabolic activity of M1 (pmol/min/mg protein) was calculated by the following equation, where A is the relative amount of M1 in the incubation mixture, B is the relative amount of M1 in the control mixture (not containing the NADPH generating system), and C, D, and E are as above:

$$\text{Metabolic activity of M1} = \frac{(B - A) \times C}{D \times E}$$

The degree of inhibition of metabolic activity was calculated by the following equation, where A is the metabolic activity of ^{14}C-JTK-303 and M1 in the sample with the coadministered drug and B is the metabolic activity of ^{14}C-JTK-303 and M1 in the control sample:

$$\text{Degree of inhibition (\% of inhibition)} = 100 - 100 \times \frac{A}{B}$$

The IC$_{50}$ was calculated based on the relationship between the coadministrated drug concentration and the percent of control using Excel. The percent of control was calculated by the following equation, where C is the final concentration of the coadminstered drug:

$$\text{Percent of control} = \frac{\text{IC}_{50} \text{ value}}{\text{IC}_{50} \text{ value} + C} \times 100$$

Results

The amounts of JTK-303 and M1 were determined from radiochromatograms (percent of peak) and the metabolic activity was determined. These values were used to calculate percent of control and degree of inhibition, and subsequently IC$_{50}$ values were determined (Table 1).

Table 1: IC$_{50}$ values for drugs coadministered with JTK-303

Drug	IC$_{50}$ (uM)
Amprenavir	1.1
Indinavir sulfate	0.51
Ketoconazole	0.099
Lopinavir	3.1
Nelfinavir	1.1
Ritonavir	0.079
Saquinavir	4.5
Efavirenz	Inhibition ratio <50%
Nevirapine	Inhibition ratio <50%
Zidovudine	Inhibition ratio <50%

Conclusion

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The IC_{50} values for amprenavir, indinavir sulfate, lopinavir, nelfinavir, ritonavir, and ketoconazole were less than 5 uM, suggesting that these drugs may inhibit JTK-303 metabolism at clinically relevant plasma concentrations. In contrast, zidovudine, nevirapine, and efavirenz did not significantly influence JTK-303 metabolism, even at supratherapeutic concentrations.

(b) (4)

Therefore, the drugs that affected JTK-303 metabolism primarily via CYP3A inhibition (i.e. amprenavir, indinavir sulfate, lopinavir, nelfinavir, and ketoconazole) will likely not influence elvitegravir exposures to a clinically significant degree in the presence of the potent CYP3A inhibitors ritonavir or cobicistat.

Study JTK303-AD-027 – In Vitro Metabolism of ^{14}C-JTK-303 [I]: Enzyme Inhibition Study Using Human Liver Microsomes – Determination of IC$_{50}$
Study Report Date: 11 May 2005

Introduction
In this study, the inhibitory effect of JTK-303 on human CYP450 isoforms (CYP1A, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) was investigated using human liver microsomes.

Materials and methods
Reagents and biospecimens
The reference standard JTK-303 was manufactured by Japan Tobacco Inc. Frozen pooled human liver microsomes were purchased at a concentration of 20 mg/mL from ▨▨▨▨ (b) (4) (Lot H0610). The model substrates and typical inhibitors for the CYP450 isoforms were selected based on reports in the literature and are listed in Tables 1 and 2, respectively.

Table 1: Model substrates, internal standards, and concentrations (source: Study Report Table 4.2.5)

Isoform	Model substrate	Final concentration	Incubation time (min)	Internal standard (concentration)
CYP1A	Ethoxyresorufin[1]	0.5 μmol/L	10	Nothing
CYP2A6	Coumarin[1]	2 μmol/L	10	Nothing
CYP2C9	Tolbutamide[1]	400 μmol/L	30	Chlorpropamide (10 μg/mL)
CYP2C19	S-(+)-Mephenytoin[2]	60 μmol/L	60	Phenacetin (10 ng/mL)
CYP2D6	(±)-Bufuralol[3]	10 μmol/L	15	Alprenolol (5 μg/mL)
CYP2E1	Chlorzoxazone[1]	100 μmol/L	15	Phenacetin (10 μg/mL)
CYP3A4	Testosterone[1]	120 μmol/L	30	Phenacetin (10 μg/mL)
CYP3A4	Midazolam[4]	10 μmol/L	10	Phenacetin (1 μg/mL)

Concentrations of substrates were decided based on the following references:
1) *Xenobio. Metabol. and Dispos.*, vol. 16 (2), 115–126, 2001
2) *Xenobiotica*, vol. 28 (12), 1203–1253, 1998
3) *Drug Metabolism and Disposition*, vol. 23 (11), 1231–1241, 1995
4) *Drug Metabolism and Disposition*, vol. 24 (9), 940–947, 1996

Table 2: Typical inhibitors and concentrations for P450 isoforms (source: Study Report Table 4.2.6)

Isoform	Typical inhibitor	Final concentration[*] (μmol/L)
CYP1A	α-Naphthoflavone	1
CYP2A6	Methoxsalen	5
CYP2C9	Sulfaphenazole	3
CYP2C19	Tranylcypromine	20
CYP2D6	Quinidine	4
CYP2E1	Diethyldithiocarbamate	100
CYP3A4	Ketoconazole	1

<References>
1) *Drug Metabolism and Disposition*, vol. **23** (1), 154–158, 1995
2) *Xenobiotica*, vol. **26** (7), 681–693, 1996
3) *Drug Metabolism and Disposition*, vol. **29** (5), 748–753, 2001
4) *Drug Metabolism and Disposition*, vol. **24** (9), 948–954, 1996
[*] Concentrations of inhibitors were decided based on the above references.

Reaction procedure
JTK-303 (final concentrations: 0, 0.1, 0.3, 1, 3, 10, and 30 ug/mL, equivalent to 0, 0.2, 0.7, 2.2, 6.7, 22.3, and 67.0 uM), liver microsomes (final concentrations of CYP1A, 2C9, 2C19, and 2E1: 0.5 mg protein/mL; CYP2A6: 0.2 mg protein/mL; CYP2D6: 1.0 mg protein/mL; CYP3A4: 0.1 mg protein/mL), model substrate, typical inhibitor (if applicable), and phosphate buffer (pH 7.4) were combined (total volume: 0.5 mL) and incubated at 37°C for 5 min. The reaction was initiated by the addition of NADPH generating system (0.4 U/mL G-6-PDH solution, 1.3 mM $NADP^+$, 3.3 mM G-6-P, and 3.3 mM $MgCl_2$) followed by incubation at 37°C for the time specified in Table 1. The reaction was subsequently terminated and the reaction mixture was vortexed and centrifuged. After extraction or filtration (if necessary) the supernatant was subjected to HPLC or spectrofluorophotometric analysis for substrate quantitation. All metabolic reactions were performed in duplicate.

Calculation of results
The metabolic activity (pmol/min/mg protein) was calculated by dividing the concentration of the model substrate metabolites in the incubation mixtures (determined by regression from a calibration curve) by incubation time and microsomal protein concentration in the incubation mixture. The remaining activity and degree of inhibition were subsequently calculated from the metabolic activity values in the presence and absence of the typical inhibitor:

$$\text{Remaining activity (\% of control)} = \frac{\text{Metabolic activity in the presence of test article or typical inhibitor}}{\text{Metabolic activity in the absence of test article or typical inhibitor}}$$

$$\text{Degree of inhibition (\% of inhibition)} = 100 - \text{Remaining activity}$$

The IC_{50} value was calculated for CYP3A4, which was the only isoform that was inhibited by greater than 50% by JTK-303, using the equation below:

$$\% \text{ of control} = IC_{50} / (IC_{50} + I) \times 100$$

The IC_{50} values for the other CYP isoforms are expressed as greater than the highest inhibitor concentration used.

Results

Over a concentration range of 0.1 to 30 ug/mL (0.2 to 67 uM), maximal JTK-303 inhibition was 52.8% of the CYP3A4 isoform, using the model substrate testosterone. CYP3A4 was the only isoform for which an IC50 value was able to be determined over the range of JTK-303 concentrations tested. The inhibitory effects of JTK-303 on all of the isoforms studied are displayed in Table 3 and the IC50 values are listed in Table 4.

Table 3: Inhibitory effect of JTK-303 on CYP450 activity in human liver microsomes (percent of control; source: Study Report Table 12-1)

	% of control							
Cytochrome P450	Concentration of JTK-303 [μg/mL (μmol/L)]							Positive control
	0 (0)	0.1 (0.2)	0.3 (0.7)	1 (2.2)	3 (6.7)	10 (22.3)	30 (67.0)	
CYP1A	100.0	99.7	98.8	100.2	102.1	105.0	107.4	5.6
CYP2A6	100.0	102.3	95.3	91.2	95.5	90.8	70.1	<10.2
CYP2C9	100.0	92.3	97.3	96.1	81.5	71.4	56.5	42.5
CYP2C19	100.0	83.7	86.9	92.2	82.2	78.5	60.6	29.6
CYP2D6	100.0	99.1	93.2	95.5	95.1	90.9	84.1	<17.5
CYP2E1	100.0	110.6	106.0	124.6	134.6	146.3	157.7	48.3
CYP3A4 (Testosterone)	100.0	97.8	94.3	94.7	89.0	76.9	47.2	5.6
CYP3A4 (Midazolam)	100.0	85.6	79.8	86.0	83.0	68.1	51.5	<9.6

Concentration of positive control
CYP1A: α-Naphthoflavone (1 μmol/L)
CYP2A6:   Methoxsalen (5 μmol/L)
CYP2C9:   Sulfaphenazole (3 μmol/L)
CYP2C19:   Tranylcypromine (20 μmol/L)
CYP2D6:   Quinidine (4 μmol/L)
CYP2E1:   Diethyldithiocarbamate (100 μmol/L)
CYP3A4:   Ketoconazole (1 μmol/L)
CYP3A4:   Ketoconazole (1 μmol/L)

Table 4: IC_{50} of JTK-303 for model substrate metabolism for CYP450 isoforms in human liver microsomes (source: Study Report Table 12-3)

IC$_{50}$ [µg/mL (µmol/L)]							
CYP1A	CYP2A6	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A4 (Testosterone)	CYP3A4 (Midazolam)
>30 (>67.0)	>30 (>67.0)	>30 (>67.0)	>30 (>67.0)	>30 (>67.0)	>30 (>67.0)	28.32 (63.19)	>30 (>67.0)

Conclusion

At JTK-303 concentrations between 0.1 and 30 ug/mL (0.2 and 67 uM), CYP3A4 (using testosterone as a model substrate) was the only CYP450 isoform that was inhibited by more than 50%, with an IC$_{50}$ value of 28.32 ug/mL (63.19 uM). These results suggest that JTK-303 does not inhibit CYP1A, CYP2A6, CYP2C9, CYP2C19, CYP2D6, or CYP2E1 and is a weak inhibitor of CYP3A4, although this inhibition is unlikely to be clinically relevant (b) (4)

Study JTK303-AD-023 – Enzyme Induction Study of JTK-303 in Fresh Primary Cultured Hepatocytes
Study Report Date: 26 Apr 2005

Introduction
In this study, the induction potential of JTK-303 on human hepatic drug metabolizing enzymes (CYP1A2, CYP2C9, CYP2C19, and CYP3A4) was investigated in primary cultured human hepatocytes.

Materials and methods
Reagents and biospecimens
The reference standard JTK-303 was manufactured by Japan Tobacco Inc and was used at concentrations of 0.1, 1, and 10 ug/mL (0.2, 2.2, and 22.3 uM). Fresh primary cultured human hepatocytes (two individuals; Lots 66 and 68). The probe substrate for CYP1A2 was phenacetin (final concentration: 10 uM); for CYP2C9, tolbutamide (400 uM); for CYP2C19, (S)-mephenytoin (100 uM); and for CYP3A4, midazolam (10 uM). Positive controls included 20 uM -naphthoflavone for CYP1A2; 20 uM rifampicin for CYP2C9 and CYP2C19; and 10 uM rifampicin for CYP3A4. Probe substrates, positive controls, and concentrations were selected based on the literature.

Induction study procedure
Each induction treatment was performed using two wells of hepatocytes per concentration. Cells were incubated in culture medium containing inducer at 37˚C for 48 h (CYP1A2) or 72 h (CYP2C9, CYP2C19, and CYP3A4). Cells were washed and the culture medium was replaced with medium containing probe substrate. Cells were incubated at 37˚C for 1 h (CYP3A4), 2 h (CYP1A2), or 3 h (CYP2C9 and CYP2C19). After incubation, the culture medium was collected, frozen in dry ice, and stored at -80˚C until analysis by LC-MS/MS. Hepatocytes were washed with PBS and protein content was measured using the BCA assay. Cytotoxicity was measured using the lactose dehydrogenase assay.

Calculation of results
Concentrations of metabolites were calculated using a regression equation based on relative peak areas on chromatograms resulting from LC-MS/MS analysis. Enzyme activity was expressed as the amount of metabolite produced per hour per mg protein (pmol/h/mg protein).

The enzyme induction ration (IR) was calculated using the following equation:

$$IR = \frac{\text{Enzyme activity after exposure of test article or typical inhibitor}}{\text{Enzyme activity of solvent control}}$$

Results
In primary cultured human hepatocytes, JTK-303 did not induce CYP1A2 activity. The induction potential of JTK-303 on CYP2C19 could not be evaluated because concentrations of metabolite were below the lower limit of the calibration curve.

JTK-303 demonstrated some induction potency on CYP2C9 and CYP3A4, as shown in Table 1.

Table 1: Effect of JTK-303 on CYP2C9 (tolbutamide 4-hydroxylation) and CYP3A4 (midazolam 1'-hydroxylation) in fresh primary cultured human hepatocytes (source: Study Report Tables 11-3, 11-4, 11-7, and 11-8)

	Test compound	Induction Ratio (Pct of Pos Ctrl) Lot 66	Induction Ratio (Pct of Pos Ctrl) Lot 68
CYP2C9	0.1 ug/mL JTK-303	30	21
	1 ug/mL JTK-303	38	38
	10 ug/mL JTK-303	47	63
	20 uM rifampin	100	100
CYP3A4	0.1 ug/mL JTK-303	5	6
	1 ug/mL JTK-303	19	25
	10 ug/mL JTK-303	56	41
	10 uM rifampin	100	100

The results of the cytotoxicity studies indicated that there was no cytotoxicity that inhibited enzyme induction.

Conclusion

In cultured primary human hepatocytes, JTK-303 is a weak inducer of CYP2C9 and CYP3A4 at clinically relevant concentrations. Induction of CYP3A4 is likely to be counteracted by coadministration of the potent CYP3A inhibitor RTV. The potential for JTK-303 induction of CYP2C9 exists and may result in altered efficacy and/or safety profiles for CYP2C9 substrate drugs such as warfarin and phenytoin.

Study AD-183-2028 – In Vitro Assessment of Inhibition of Human Elvitegravir Glucuronidation by Ketoconazole
Study Report Date: 27 July 2009

Introduction
In this study, the potential for ketoconzole to inhibit the glucuronidation of elvitegravir (EVG) was determined in human hepatic microsomal fractions using the UGT1A1 inhibitor atazanavir (ATV) as a positive control.

Materials and methods
Reagents and biospecimens
EVG and its glucuronides GS-9200 (M4) and GS-9201 were synthesized by Japan Tobacco Inc. and Gilead Sciences. All other reagents were purchased from [redacted] (b) (4) except for ATV, which was purchased from [redacted] (b) (4) Human hepatic microsomal fraction and insect cell microsomal fraction containing baculovirus-expressed recombinant UGT1A1 (Supersomes™) were provided by [redacted] (b) (4).

Reaction procedure
Diluted microsomal fraction was incubated on ice for 15 min with alamethicin (50 ug/mg microsomal protein), 10 mM $MgCl_2$, and 5 mM D-saccharic acid 1,4 lactone. EVG and inhibitor (ketoconazole or ATV) were added and the mixture was warmed to 37°C for 5 min. The reaction was initiated by the addition of 5 mM UDP-glucuronic acid in potassium phosphate buffer and incubated at 37°C with shaking. Aliquots were removed at intervals. Reactions were terminated by the addition of ice-cold water, methanol, and acetonitrile (1:2:1 v/v/v) containing 0.1% formic acid. The mixtures were centrifuged and supernatants were analyzed by LC-MS/MS.

The formation of EVG-glucuronide was linear up to 1 mg/mL hepatic microsomal protein and incubation times up to 60 min. The EVG K_m was determined to be 21 uM using a Michaelis-Menten model; an EVG concentration of 10 uM was selected for use in inhibition studies.

For the positive control, the inhibitory effect of ATV on glucuronidation in insect cell microsomal fraction expressing recombinant UGT1A1 was determined. The protein concentration was 0.5 mg/mL and the EVG concentration was 3 uM; all other conditions were as stated above.

Data analysis and calculation of results
LC-MS/MS was used to quantify EVG and the GS-9200 glucuronide. Activities were expressed as the percent of control activity (i.e. in the absence of inhibitor) remaining. To determine IC50 values, a non-linear regression was fit to a graph of the reaction rates at each substrate concentration in the presence of inhibitor versus those in the absence of inhibitor (100% activity) using a sigmoidal inhibition model (GraphPad Prism 5.01).

Results

The IC50 values for ketoconazole and ATV inhibition of EVG glucuronidation were 9.6 and 0.4 uM, respectively. Maximum inhibition by ATV in human hepatic microsomal fraction was 83% at 100 uM, suggesting that UGT1A1 is the primary enzyme involved in EVG glucuronidation. (For comparison, 100 uM ATV inhibited UGT1A1 by 99.3% in the recombinant system.) A plot of the inhibitory effects of ketoconazole and ATV on EVG glucuronidation is shown in Figure 1.

Figure 1: Effect of ketoconazole and atazanavir on the glucuronidation of EVG by human hepatic microsomal fraction (source: Study Report Figure 2)
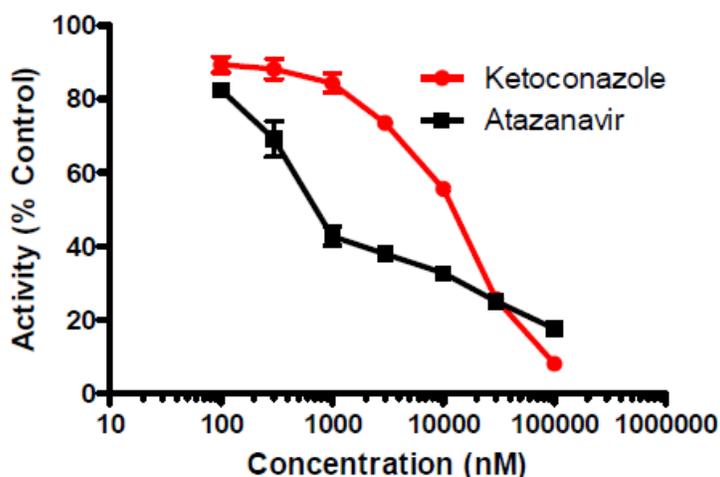
A representative C_{max} value for ketoconazole when dosed 200 mg BID is 5311 ng/mL (10 uM). The C_{max} increases to 10501 ng/mL (19.8 uM) in the presence of ritonavir 100 mg BID (in the manner that EVG will be dosed), suggesting that inhibition of EVG glucuronidation would be expected in a clinical setting.

Conclusion

Ketoconazole modestly inhibited elvitegravir glucuronidation in human hepatic microsomal fraction with an IC_{50} of 9.6 uM. In the presence of low-dose ritonavir (with which EVG will be coadministered), glucuronidation will become the primary route of EVG metabolism. Ritonavir will also substantially increase ketoconazole plasma concentrations, likely resulting in inhibition of EVG glucuronidation.

Clinical drug-drug interaction studies were performed to investigate pharmacokinetic changes after coadministration of ritonavir-boosted EVG with ketoconazole (GS-US-183-0146), atazanavir (GS-US-183-0108), and the UGT1A1 inhibitor lopinavir (GS-US-183-0116). The findings of these studies resulted in a maximum recommended ketoconazole dose (200 mg daily) when coadministered with ritonavir-boosted elvitegravir, as well as a reduced elvitegravir dose (85 mg QD) when coadministered with ritonavir and atazanavir or lopinavir.

Study AD-183-2034 – UDP-Glucuronosyl Transferase Phenotyping of Elvitegravir
Study Report Date: 8 June 2011

Introduction
In this study, human UDP glucuronosyl transferase (UGT) enzyme preparations were used to determine the rate of and UGT isoform(s) involved in formation of the elvitegravir acyl glucuronide metabolite GS-9200 (M4).

Materials and methods
Reagents and biospecimens
EVG, EVG-glucuronide (GS-9200), and the internal standard for mass spectrometry (GS-224337) were manufactured by Japan Tobacco and Gilead Sciences. Insect cell microsomal fractions containing baculovirus-expressed human UGT enzyme preparations (Supersomes) were purchased from (b) (4)   . Chemicals were purchased from   (b) (4) . or similar vendors.

Reaction procedure
Individual Supersome preparations were diluted in phosphate buffer (pH 7.4) to a final concentration of 2 mg protein/mL and preincubated with the permeabilizing agent alamethicin (final concentration 12.5 ug/mg microsomal protein). Subsequently, EVG (final concentration 20 uM) or control substrates (raloxifene, trifluoperazine, 7-hydroxy-coumarin, 4-hydroxyestradiol, or scopoletin; final concentrations 3, 3, 10, 10, and 10 uM, respectively) were added. The metabolic reactions were initiated by addition of UDP-glucuronic acid (final concentration 2 mM). Reaction samples were incubated at 37˚C. Aliquots were removed after 0, 10, 25, 45, or 65 min and the reactions were stopped by the addition of methanol:acetonitrile:water:formic acid (50:25:24.9:0.1 v/v/v/v) mixture. Proteins were removed via precipitation and samples were centrifuged. The resulting supernatants were analyzed by LC-MS/MS.

Calculation of results
The rate of GS-9200 generation from EVG was determined by linear regression of the initial (linear) portion of the GS-9200 concentration-incubation time plot. The rate of metabolism of control substrates were calculated from a log-linear regression of a plot of the decline in substrate concentration vs. time (first-order kinetics were assumed).

Results
The rates of metabolism of positive control substrates and of EVG to GS-9200 are listed in Table 1. Relatively high rates of metabolism of control substrates were observed. The UGT1A1 and 1A3 isoforms were primarily responsible for GS-9200 formation, with UGT1A9 and 2B15 playing minor roles.

Table 1: Rates of metabolism of control substrates and of generation of GS-9200 from EVG by major human UGT enzymes (source: Study Report Table 1)

Enzyme	Positive Control	Positive control T½ (min)	GS-9200 formation (pmol/mg protein/min)
None	(None)		< 0.1
UGT1A1	Raloxifene	31.5	3.75
UGT1A3	Raloxifene	89.1	16.6
UGT1A4	Trifluoperazine	176	< 0.1
UGT1A6	7-Hydroxycoumarin	< 10	< 0.1
UGT1A7	7-Hydroxycoumarin	18.0	< 0.1
UGT1A8	7-Hydroxycoumarin	193	< 0.1
UGT1A9	7-Hydroxycoumarin	< 10	0.2
UGT1A10	Raloxifene	122	< 0.1
UGT2B4	4-Hydroxyestradiol	51.1	< 0.1
UGT2B7	4-Hydroxyestradiol	< 10	< 0.1
UGT2B15	Scopoletin	< 10	0.12
UGT2B17	4-Hydroxyestradiol	58.3	< 0.1

Conclusion

Formation of the EVG acyl glucuronide metabolite GS-9200 occurred mainly via UGT1A1 and UGT1A3. UGT1A9 and UGT2B15 also formed GS-9200, albeit very slowly; no other UGT isoforms tested were involved in EVG glucuronidation. Inhibitors of UGT1A1 or UGT1A3 may influence the pharmacokinetics of EVG if CYP-mediated metabolism is inhibited.

Study JTK303-AD-026 – Involvement of MDR1 in Membrane Permeation of JTK-303 and Inhibitory Effect of JTK-303 on Digoxin Transport
Study Report Date: 26 Apr 2005

Introduction
In this study, P-glycoprotein (P-gp) mediated transport of JTK-303 was investigated using MDR1-expressing LLC-PK1 cells. The inhibitory potential of JTK-303 on digoxin transport was also studied.

Materials and methods
Reagents and biospecimens
This study was performed by   (b) (4)   The JTK-303 reference standard and ^{14}C-JTK-303 were supplied by Japan Tobacco, Inc. The P-gp probe substrate ^{3}H-digoxin was purchased from   (b) (4)   and the P-gp control inhibitor verapamil was purchased from   (b) (4) . MDR1- and control vector-expressing porcine kidney (LLC-PK1) cells were used under sublicense from   (b) (4) .

Reaction procedure
LLC-PK1 cells expressing MDR1 or control vector were seeded in Transwell® inserts and incubated in Medium 199 (with 9% FBS and 50 ug/mL gentamicin) for eight days prior to the experiment. To determine transcellular transport of JTK-303, the medium on the apical or basal side was replaced with HBSS with 1 uM ^{14}C-JTK-303 or the probe substrate ^{3}H-digoxin. After 1, 2, and 4 h incubations at 37°C, 50 uL was taken from the opposite compartment (basal or apical) and radioactivity was measured using a liquid scintillation counter. To determine the P-gp inhibitory potential of JTK-303, the medium on the apical or basal side was replaced with HBSS with 1 uL ^{3}H-digoxin and JTK-303 (0.3, 1, 3, 10, and 30 uM) or 10 uM verapamil as a positive control. After a two-hour incubation at 37°C, 50 uL was taken from the opposite compartment (basal or apical) and radioactivity was measured using a liquid scintillation counter. All incubations were performed in triplicate.

Calculation of results
The transcellular transport activity (flux) was calculated using the following equation:

$$\text{flux (uL/mg protein/h)} = [\text{transported amount}]/[\text{sampling time}]/[\text{protein amount}]/C_0$$

The flux ratio was defined using the following equation:

$$\text{flux ratio} = [\text{flux}_{\text{basal-apical}}]/[\text{flux}_{\text{apical-basal}}]$$

Results
The transcellular transport of ^{14}C-JTK-303 was almost linear across the time period studied. The JTK-303 basal-apical:apical-basal flux ratios after a four-hour incubation were 14.5 and 1.5 in MDR1- and vector-expressing cells,

respectively, indicating that JTK-303 is a P-gp substrate. In comparison, the flux ratios for the P-gp probe substrate digoxin were 10.3 and 2.2 in MDR1- and vector-expressing cells, respectively. (Transcellular transport of the permeability control mannitol was low and similar in both flux directions, indicating that the experimental system is adequate for the study performed).

The effect of JTK-303 on digoxin transcellular transport in MDR1-expressing cells is shown in Figure 1. The digoxin flux ratio in MDR1-expressing cells was 9.9. In the presence of 0.3, 1, 3, 10, and 30 uM JTK-303, the flux ratios were 10.4, 7.9, 10.7, 9.8, and 6.0, respectively. In comparison, the digoxin flux ratio in MDR1-expressing cells was 3.2 in the presence of the P-gp inhibitor verapamil.

Figure 1: Effect of JTK-303 on the digoxin transport across MDR1-expressing cell monolayers (source: Study Report Figure 9-2)
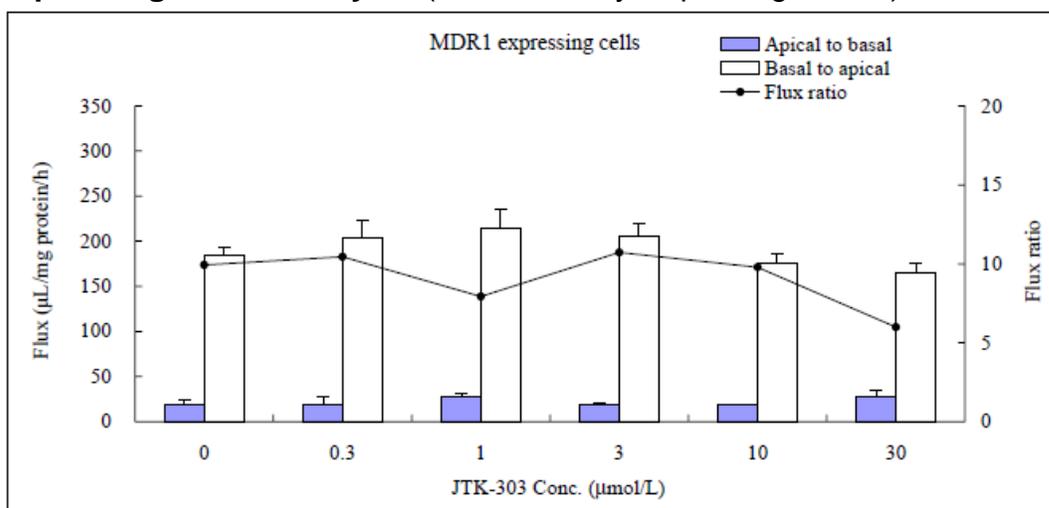
The Applicant notes that while the flux ratio at 30 uM JTK-303 suggests that JTK-303 is a P-gp inhibitor (9.9 and 6.0 in the absence and presence of 30 uM JTK-303, respectively), the basal:apical flux values (183.6 and 164.6 uL/mg protein/h in the absence and presence of 30 uM JTK-303, respectively) indicate little inhibitory effect. The IC_{50} was greater than 30 uM (the highest JTK-303 concentration tested).

The JTK-303 C_{max} values resulting from multiple dosing of elvitegravir (JTK-303) 150 mg coadministered with 100 mg ritonavir and from multiple dosing of the QUAD single tablet regimen (150 mg elvitegravir, 150 mg cobicistat, 200 mg emtricitabine, and 300 mg tenofovir disoproxil fumarate) are in the range of 2000-2500 ng/mL (4.5-5.5 uM). Therefore, the $[I]_1/IC_{50}$ is not more than approximately 0.18.

Conclusion
Based on in vitro data, JTK-303 is a substrate of P-gp with a net flux ratio of 14.5. An in vivo drug interaction study indicated that elvitegravir plasma levels were

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unaffected by coadministration with the P-gp inducer tipranavir in the presence of low-dose ritonavir (GS-US-183-0110). JTK-303 appears to be a weak inhibitor of P-gp at supratherapeutic concentrations (IC_{50} > 30 uM) and an in vivo drug interaction study with a P-gp substrate is not necessary.

Study AD-183-2030 – In Vitro Assessment of Elvitegravir Inhibition of Human OATP1B1 and OATP1B3

Study Report Date: 13 May 2011

Introduction

In this study, the inhibitory potential of elvitegravir (EVG) on OATP1B1 and OATP1B3 was investigated using the fluorescent OATP1B1/3 substrate Fluo 3 in transfected Chinese Hamster Ovary (CHO) cells.

Materials and methods

Reagents and biospecimens

EVG was synthesized at Gilead Sciences (Lot 3). All other chemicals were purchased from ⬛⬛⬛⬛⬛⬛ (b) (4).

Reaction procedure

Untransfected CHO cells and CHO cells transfected with OATP1B1 or OATP1B3 were seeded in BioCoat 96-well black cell culture plates and incubated in supplemented Dulbecco's Modification of Eagle's Medium overnight. To determine the OATP1B1/3 inhibitory potential of EVG, the medium was replaced by assay buffer containing 2 uM Fluo 3 (the approximate K_m) and EVG (over a range of 0.0027 to 2 uM) and cells were pre-incubated for 1 h. After removal of the assay buffer, cells were lysed for 15 min in 0.05% SDS in 1 mM $CaCl_2$. Fluo 3 fluorescence was measured at an excitation of 485 nm and emission of 530 nm. Three independent experiments were performed in duplicate.

Calculation of results

The percent of transport inhibition was calculated using the following equation, where $OATP_I$ represents the fluorescence in OATP1B1/3-transfected cells in the presence of EVG, $OATP_{NI}$ represents the fluorescence in OATP1B1/3-transfected cells in the absence of EVG, and WT_{NI} represents the fluorescence in untransfected cells in the absence of EVG:

$$\% \text{ inhibition} = 1 - \frac{OATP_I - WT_{NI}}{OATP_{NI} - WT_{NI}} \times 100$$

IC_{50} values were calculated using non-linear fitting of percent inhibition versus concentration to a sigmoidal curve with a variable Hill coefficient using GraphPad Prism 5 software.

Results

EVG inhibited OATP1B1-mediated Fluo 3 uptake by approximately 40% at the highest tested concentration (2 uM). The IC_{50} value for OATP1B1 was greater than 2 uM (the highest EVG concentration tested). EVG inhibited OATP1B3-mediated Fluo 3 uptake with a calculated IC_{50} value of 0.44 uM. The percent inhibition versus EVG concentration curves for OATP1B1- and OATP1B3-transfected CHO cells are shown in Figures 1 and 2, respectively.

Figure 1: Inhibition of OATP1B1-mediated Fluo 3 uptake by EVG (line represents nonlinear curve fitting to a sigmoidal dose-response model; source: Study Report Figure 2)
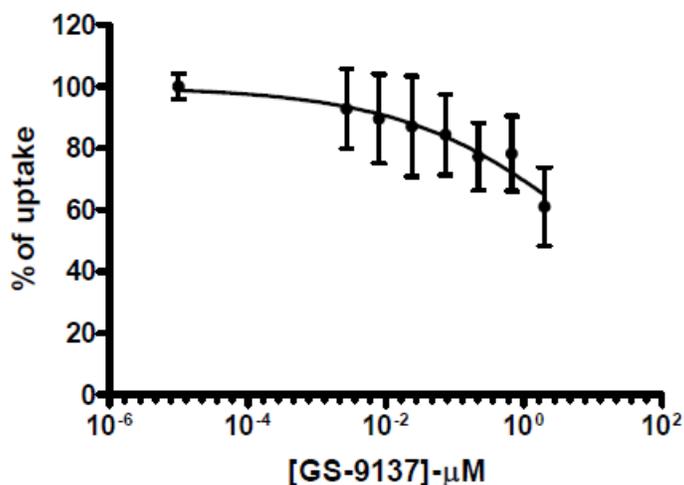
Figure 2: Inhibition of OATP1B3-mediated Fluo 3 uptake by EVG (line represents nonlinear curve fitting to a sigmoidal dose-response model; source: Study Report Figure 3)
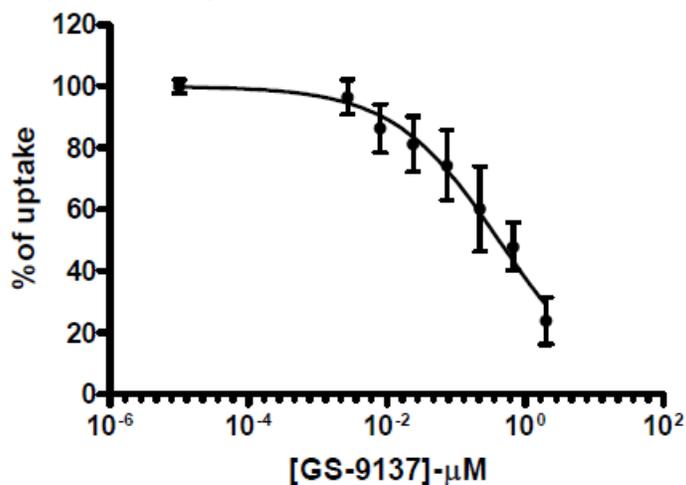
The EVG C_{max} values resulting from multiple dosing of EVG 150 mg coadministered with 100 mg ritonavir and from multiple dosing of the QUAD single tablet regimen (150 mg EVG, 150 mg cobicistat, 200 mg emtricitabine, and 300 mg tenofovir disoproxil fumarate) are in the range of 2000-2500 ng/mL (4.5-5.5 uM). Therefore, the $[I]_1/IC_{50}$ estimate for OATP1B1 is not greater than 2.75, while the $[I]_1/IC_{50}$ for OATP1B3 is 12.5.

Conclusion
Based on in vitro data, EVG inhibits both OATP1B1 and OATP1B3. At the highest EVG concentration tested (2 uM) there was 40% inhibition of OATP1B1-mediated Fluo 3 uptake. The IC_{50} for OATP1B3 was 0.44 uM. The $[I]_1/IC_{50}$

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Reference ID: 3153809

values for both transporters indicate the need for an in vivo study. A drug interaction study was conducted to investigate the effect of cobicistat-boosted EVG on the pharmacokinetics of the OATP1B1/3 substrate rosuvastatin (GS-US-216-0123) and found a clinically insignificant increase in rosuvastatin exposures (AUC increased by approximately 38%).

In vitro studies of Cobicistat

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1. Title

In Vitro Metabolism of GS-9350 in Hepatocytes and Hepatic Subcellular Fractions from Rat, Dog, Monkey, and Human

2. Objectives

The primary objective of the study was to determine the vitro metabolism rate and the predicted intrinsic clearance in the liver of cobicistat in humans, dogs, rats, and monkeys.

3. Methods

The vitro metabolism of cobicistat was evaluated in humans, beagle dogs, Sprague Dawley rats, and cynomolgus monkeys using hepatocytes and hepatic microsomes. In hepatic microsomes, the metabolism of 3 µM of cobicistat and ritonavir was evaluated at 0, 2, 5, 10, 15, 30, 45, and 60 minutes using NADPH cofactor to initiate the process. In hepatocytes, the metabolism of 2 µM of cobicistat and ritonavir was evaluated at 0, 1, 3, and 6 hours. LC/MS/MS was used to analyze the samples.

The cobicistat concentrations that were evaluated in the study covers the C_{max} concentration for cobicistat 150 mg once daily in healthy subjects: approximately 1560 ng/mL (2 µM).

Information used in deriving the predicted cobicistat clearance is displayed in Table 1. The clearance was calculated using a well stirred liver model and no adjustments were made for either plasma protein or microsomal binding. Hepatic blood flow values of 4.2, 1.8, 1.6, and 1.3 L/kg for rat, dogs, monkeys, and humans, respectively, were used. A comparison of the predicted hepatic clearance to the hepatic blood flow was used to obtain the predicted percent hepatic extraction.

Table 1- Information used in deriving the predicted cobicistat clearance

Species	Hepatocytes			Hepatic Microsomes		
	V (L)	Y (× 106/kg)	P (× 106/kg)	V (L)	Y (mg)	P (mg/kg)
Rat				0.001	0.5	45
Monkey				0.001	0.5	45
Dog				0.001	0.5	45
Human	0.00025	5000	0.25	0.001	0.5	45

V   Incubation volume (L)

Y   Microsome protein yield (mg protein/kg body weight) or hepatocyte
      yield (millions of hepatocytes/kg body weight)

P   Mass of protein in the incubation (mg) or number of hepatocytes
      $(\times 10^6)$

4. Results

The predicted cobicistat hepatic clearance and predicted percent hepatic extraction in hepatocytes (2 µM) in humans and hepatic microsomes (3 µM) in humans, dogs, rats, and monkeys are displayed in Table 2 and Table 3.

Table 2-Predicted cobicistat (2 µM) hepatic clearance and predicted percent hepatic extraction in humans using hepatocytes

Species	$t_{\frac{1}{2}}$ (h)		Predicted Hepatic Cl (L/hr/kg)		Predicted Hepatic Extraction (%)	
	GS-9350	RTV	GS-9350	RTV	GS-9350	RTV
Human	12.7	> 39.5	0.19	< 0.07	14.4	< 5.1

Table 3-Predicted cobicistat (3 µM) hepatic clearance and predicted percent hepatic extraction in humans, dogs, rats, and monkeys using hepatic microsomes

Species	$t_{\frac{1}{2}}$ (min)		Predicted Hepatic Cl (L/hr/kg)		Predicted Hepatic Extraction (%)	
	GS-9350	RTV	GS-9350	RTV	GS-9350	RTV
Dog	43.7	96.5	0.88	0.58	48.8	32.4
Rat	82.1	229.8	1.50	0.70	35.6	16.6
Monkey	8.9	13.7	1.35	1.24	84.7	77.4
Human	154.9	260.0	0.37	0.28	28.3	21.9

5. Conclusions

In hepatic microsomes, when comparing humans and various animal species, the lowest predicted cobicistat hepatic clearance and predicted percent hepatic extraction was in humans. In hepatocytes, with 2 µM of cobicistat, in humans, the predicted cobicistat hepatic clearance and the predicted percent hepatic extraction were 0.19 L/hr/kg and 14.4%. In human liver microsomes, with 3 µM of cobicistat, in humans, the predicted cobicistat hepatic clearance and the predicted percent hepatic extraction were 0.37 L/hr/kg and 28.3%. Overall, the results support classifying cobicistat as low hepatic extraction ratio (<0.3) drug in humans.

1. Title

Cytochrome P450 Phenotyping for GS-9350

2. Objectives

The primary objective of the study was to evaluate whether various cytochrome P450 enzymes metabolize cobicistat.

3. Methods

Cobicistat was incubated with five different cDNA expressing human cytochrome P450 enzymes (Bactosomes): CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 for 0, 5, 15, 30 and 45 minutes. A 5 µM concentration of cobicistat was evaluated. The cobicistat concentration that was evaluated in the study covers the C_{max} concentration for cobicistat 150 mg once daily in healthy subjects: approximately 1560 ng/mL (2 µM). The following concentrations of the cytochrome P450 enzymes were used: 100 pmol/mL (CYP1A2 and CYP2C19), 25 pmol/L (CYP2C9 and CYP3A4), and 50 pmol/mL (CYP2D6). Before the addition of NADPH to initiate the process, the CYP enzymes were pre-incubated at 37⁰C. Ritonavir and positive control compounds were also evaluated as part of the experiments. LC/MS/MS was used to analyze the samples.

4. Results

The metabolism rates of cobicistat for CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 is displayed in Table 1. For cobicistat, the highest metabolism rate was observed for CYP2D6, and secondly by CYP 3A4. For all five CYP enzymes that were evaluated, higher metabolism rates were observed for the positive control compounds compared to cobicistat.

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Table 1- Metabolism rates of cobicistat for CYP1A2, CYP2C9, 2C19, CYP2D6, and CYP3A4

Compound	Metabolism Rate (min-1 pmol-1)				
	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4
GS-9350 (% Positive Control)	0.00 (0.0%)	0.00 (0.0%)	0.00 (0.0%)	0.105 (22.5%)	0.003 (4.5%)
RTV (% Positive Control)	0.001 (0.2%)	0.001 (0.2%)	0.003 (8.6%)	0.139 (29.8%	0.004 (6.0%)
Ethoxycoumarin	0.407	—	—	—	—
Diclofenac	—	0.467	—	—	—
Diazepam*	—	—	0.035	—	—
Dextromethorphan	—	—	—	0.467	—
Testosterone	—	—	—	—	0.066

* Diazepam is a relatively poor substrate for CYP2C19.

5. Conclusions

Based on the in vitro metabolism rates of cobicistat for CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4, the metabolism rates were highest for CYP 2D6 (0.105 min^{-1} $pmol^{-1}$) and CYP 3A4 (0.003 min^{-1} $pmol^{1}$) but were lower than the metabolism rate of the respective positive control compounds. The metabolism rates of cobicistat for CYP1A2, CYP2C9, and CYP2C19 were reported as zero, indicating either no metabolism or minimal metabolism of cobicistat occurred.

AD-216-2026

1. Title

Plasma Protein Binding of GS-9350

2. Objectives

The primary objective of the study was to determine the protein binding for cobicistat in human, dog, rat, and monkey plasma.

3. Methods

Equilibrium dialysis was used to evaluate the protein binding for cobicistat in human, beagle dog, Sprague Dawley rat, and cynomolgus monkey plasma. Pooled plasma from a minimum of three male and three female donors was used. The final concentrations that were evaluated for cobicistat were 1, 10 and 30 µM and dialysis was performed over three hours at 37°C. The range of cobicistat concentrations that were evaluated in the study covers the C_{max} concentration for cobicistat 150 mg once daily in healthy subjects: approximately 1560 ng/mL (2 µM). The applicant also evaluated the plasma protein binding for ritonavir at 1 and 10 µM. LC/MS/MS was used to analyze the samples. The percent unbound (or free fraction) was calculated using the following equation: $100* (C_f/C_t)$, with C_f representing the buffer concentration after dialysis and C_t representing the plasma concentration.

4. Results

The results of the plasma protein experiments are displayed in Table 1.

Table 1-Plasma protein binding in human, beagle dog, Sprague Dawley rat, and cynomolgus monkey plasma from 1 to 30 µM

Matrix	Compound	Free Fraction (%)				Study
		1 µM	10 µM	30 µM	Mean	
Dog Plasma	GS-9350	5.68 ± 0.60	6.46 ± 0.60	6.33 ± 0.40	6.16	(b) (4) 60N−708
	RTV	1.18 ± 0.08	1.50 ± 0.05	—	1.34	60N−712
Rat Plasma	GS-9350	2.33 ± 0.06	5.34 ± 0.24	8.51 ± 0.48	5.40	60N−708
	RTV	1.37 ± 0.10	3.27 ± 0.34	—	2.32	60N−712
Monkey Plasma	GS-9350	4.31 ± 0.50	6.17 ± 0.50	9.13 ± 0.30	6.54	60N−708
	RTV	2.06 ± 0.13	3.20 ± 0.27	—	2.63	60N−712
Human Plasma	GS-9350	6.33 ± 0.80	8.92 ± 0.9	7.54 ± 0.60	7.60	60N−708
	RTV	3.04 ± 0.04	3.38 ± 0.23	—	3.21	60N−712

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5. Conclusions

Based on the vitro plasma protein binding experiments, in humans, cobicistat is greater than 90% bound to plasma proteins with minimal differences in the free fraction percentage over the range of 1 to 30 µM.

1. Title

Induction of Metabolizing Enzymes by GS-9350 In Vitro

2. Objectives

The primary objective of the in vitro study was to evaluate whether cobicistat induces the pregnane X receptor (PXR) or the aryl hydrocarbon receptor (AhR). PXR is involved in the induction of the CYP3A and CYP2C enzymes and AhR is involved in the induction of the CYP1A2 enzymes.

3. Methods

The in vitro induction experiments were conducted using Puracyp's hepatoma derived cell lines (DRE12.6 and DPX2). Table 1 below provides information on the concentrations of cobicistat, ritonavir, and the positive control compounds that were evaluated.

Table 1- Concentrations of cobicistat, ritonavir, and the positive control compounds evaluated for the in vitro PXR and AXR induction experiments

| | Concentrations Investigated (μM) | |
| | AhR Assessment | PXR Assessment |
Inducing Agent	DRE12.6 Cells	DPX2 Cells
Rifampicin	—	0.3, 1, 3, 10
Mifepristone	—	10
Androstanol	—	10
β-Naphthoflavone	0.1, 1, 5, 10	—
Omeprazole	25, 50, 100, 200	—
GS-9350	1, 3, 10	1, 3, 10
RTV	1, 3, 10 μM	1, 3, 10

The cells were placed in a 96 well plate that were stored at 37°C. The test compounds were added (three wells per compound), and the experimental conditions were maintained for 24 hours. After toxicity measurements were made, luminescence was measured using a BMG luminometer. Based on the toxicity assessment, the luminescence values were adjusted, the three values were averaged and the fold induction was determined by comparing to the concentrations of the DMSO vehicle control.

4. Results

The cobicistat concentration that was evaluated in the study covers the C_{max} concentration for cobicistat 150 mg once daily in healthy subjects: approximately 1560 ng/mL (2 µM). For PXR, the degree of cobicistat induction was lower than the degree of induction observed for ritonavir and the positive controls (see Table 2). For AhR, the degree of cobicistat induction was higher than the degree of induction observed for ritonavir but lower than the degree of induction for the positive controls (see Table 3).

Table 2-Comparison of PXR induction with cobicistat, ritonavir, and positive control compounds

Concentration	Fold Induction Over 0.1% DMSO Control				
	GS-9350	RTV	Rifampicin	Mifepristone	Androstanol
0.3 µM	—		3.15	—	—
1 µM	1.57	3.64	6.09	—	—
3 µM	1.61	7.62	9.90	—	—
10 µM	2.24	10.14	14.30	8.58	3.38

Table 3-Comparison of AhR induction with cobicistat, ritonavir, and positive control compounds

Concentration (µM)	Fold Induction Over 0.1% DMSO Control			
	GS-9350	RTV	β-Naphthoflavone	Omeprazole
0.1			2.17	
1	1.12	0.80	5.91	
3	1.28	0.69		
5			17.72	
10	1.60	0.80	27.31	
25				8.16
50				13.46
100				27.34
200				67.33

5. Conclusions

Based on the in vitro experiments, both PXR and AhR are induced by cobicistat. However, with the exception of a higher degree of cobicistat induction with AhR over the range of 1 µM to 10 µm compared to ritonavir, the degree of induction of

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cobicistat induction was lower when compared to ritonavir and the positive control compounds. The results support the conclusion that for cytochrome P450 enzymes that are induced through the pregnane X receptor (PXR) or the aryl hydrocarbon receptor (AhR), cobicistat is anticipated to demonstrate minimal induction effects.

<u>AD-216-2028</u>

1. Title

Inhibition of Human CYP3A Activity by GS-9350 In Vitro

2. Objectives

The primary objective of the in vitro study was to evaluate cobicistat's CYP3A inhibition kinetics and to compare to ritonavir's CYP3A inhibition kinetics.

3. Methods

The inhibitory effect of cobicistat (concentration not specified) in human liver microsomes was evaluated against the following substrates metabolized by CYP3A: a) midazolam (2.5 µM), b) testosterone (50 µM), c) terfenadine (2.5 µM), c) elvitegravir (concentration not specified), d) atazanavir (1 µM), and e) telaprevir (1 µM). The inhibitory effects of ritonavir were also evaluated but the specific concentration was not provided.

With the exception of atazanavir and telaprevir, LC/MS/MS was used to quantitate metabolite concentrations. For atazanavir and telaprevir, the rate of loss for the parent compound was evaluated.

A two step incubation process was used for the experiments evaluating K_{inact} and K_I. After the first order rate constant was derived, K_{inact} and K_I were calculated using a one site binding hyperbola model (Kitz-Wilson plot).

The IC_{50} values were determined using non linear regression. The reaction velocities for conversion to the metabolites were determined using linear regression. Log-linear regression was used to obtain the rate of loss of the atazanavir and telaprevir parent drug.

4. Results

Similar IC_{50} values in human liver microsomes measuring CYP 3A activity were observed for cobicistat and ritonavir (see Table 1).

Table 1-Cobicistat and ritonavir IC$_{50}$ values in human liver microsomes measuring CYP 3A activity

Activity	Calculated IC$_{50}$ (μM)	
	GS-9350	RTV
Midazolam 1'-hydroxylase	0.15	0.11
Testosterone 6β-hydroxylase	0.15	0.12
Terfenadine *t*-butyl-hydroxylase	0.29	0.28
Elvitegravir Hydroxylase	0.03	0.03
Atazanavir Oxidation	0.04	0.04
Telaprevir Oxidation	0.03	0.02

In general, the cofactor dependent inhibition effects of cobicistat were within the range of observed CYP 3A inhibition values when compared to ritonavir and positive control compounds that are mechanism based CYP 3A inhibitors. With the exception of mifepristone's effect on midazolam 1'-hydroxylase, the percentage of cofactor dependent CYP3A inhibition was highest for cobicistat (see Table 2).

Table 2- Cofactor dependent CYP3A inhibition effects of cobicistat, ritonavir, and positive control compounds

Compound	Cofactor-Dependent Inhibition (%, Mean ± SD, n = 2)					
	Midazolam 1'-Hydroxylase			Testosterone 6β-Hydroxylase		
GS-9350	69.6	±	0.15	82.1	±	0.23
RTV	55.9	±	13.5	74.8	±	5.22
Mibefradil	67.3	±	4.87	80.3	±	1.12
Mifepristone	83.9	±	1.56	71.2	±	3.69

In comparison to ritonavir, cobicistat's CYP 3A K$_{inact}$ and K$_I$ values were higher in human liver microsomes (see Table 3).

Table 3-Cobicistat and ritonavir CYP3A K$_{inact}$ and K$_I$ values in human liver microsomes

Parameter	Inhibitor	
	GS-9350	Ritonavir
K$_I$ (μM)	1.07	0.26
k$_{inact}$ (min^{-1})	0.47	0.23

Inactivation kinetics were determined using midazolam 1'-hydroxylase as the probe activity.

5. Conclusions

Based on the in vitro experiments, cobicistat and ritonavir have similar inhibitory IC_{50} values when measuring their effects on CYP 3A activity in human liver microsomes. The results of the experiments evaluating cofactor dependent CYP3A inhibition effects support the observation that cobicistat demonstrates mechanism based CYP 3A inhibition. When compared to ritonavir and positive control compounds, the percentage of cofactor dependent CYP3A inhibition measuring the effects on midazolam 1'-hydroxylase and testosterone 6β-hydroxylase was highest for cobicistat with exception of mifepristone's effect on midazolam 1-hydroxylase. Cobicistat's CYP 3A K_{inact} and K_I values of 1.07 µM and 0.47 min^{-1} were higher than the K_{inact} and K_I values for ritonavir of 0.26 µM and 0.23 min^{-1} in human liver microsomes.

1. Title

In Vitro Assessment of Human Liver Cytochrome P450 Inhibition Potential of GS-9350

2. Objectives

The primary objective of the in vitro study was to evaluate cobicistat's inhibitory effects on CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A.

3. Methods

0.05 μM to 25 μM of cobicistat was incubated with human liver microsomes and NADPH and cytochrome P450 substrates. The following metabolites and substrates were evaluated: a) CYP1A2: resorufin from 7-ethoxyresorufin (0.5 μm), b) CYP2C9: 4-hydroxytolbutamide from tolbutamide (120 μm), c) CYP2C19: 4'-hydroxymephenytoin from S-mephenytoin (25 μm), d) CYP2D6: dextrorphan from dextromethorphan (5 μm), and e) CYP3A: 1'-hydroxymidazolam from midazolam (2.5 μM) and 6β-hydroxytestosterone from testosterone (50 μM). Positive controls for CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A were also included as part of the experiments. Ritonavir's inhibitory effects on CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A were also evaluated.

LC/MS/MS was used to determine concentrations of the metabolites except for CYP1A2. The CYP1A2 reaction was analyzed through quantification of the fluorescence for a metabolite. The reaction velocities were determined based on the rate of conversion to the metabolites and compared to vehicle controls (100% activity). IC_{50} values were derived using log-linear interpolation of the enzyme activity versus inhibitor concentration information.

4. Results

At concentrations up to 25 μM, no inhibition of CYP 1A2, CYP 2C9, and CYP2C19 by cobicistat was observed. Based on the C_{max} concentration for cobicistat 150 mg once daily in healthy subjects of approximately 1560 ng/mL (2 μM), cobicistat may inhibit CYP2D6 and would be anticipated to inhibit CYP 3A.

Table 1-Cobicistat and ritonavir IC$_{50}$ values in human liver microsomes measuring CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A activity

Enzyme	Activity	Calculated IC50 (μM)		
		Control Inhibitor*	RTV	GS-9350
CYP1A2	Ethoxyresorufin O-deethylase	0.03	> 25	> 25
CYP2C9	Tolbutamide hydroxylase	1.58	3.85	> 25
CYP2C19	S-Mephenytoin 4'-hydroxylase	10.8	> 25	> 25
CYP2D6	Dextromethorphan O-demethylase	0.04	3.40	9.17
CYP3A	Midazolam 1'-hydroxylase	0.07	0.104	0.154
CYP3A	Testosterone 6β-hydroxylase	0.09	0.113	0.151

*Control Inhibitors: CYP1A2, α-Napthoflavone (0–100 μM); CYP2C9, Sulfaphenazole (0–10 μM); CYP2C19, Tranylcypromine (0–100 μM); CYP2D6, Quinidine (0–10 μM); CYP3A4/5, Ketoconazole (0–10 μM).

5. Conclusions

Based on the in vitro experiments conducted with cobicistat concentrations up to 25 μM, only inhibition of CYP2D6 (IC$_{50}$ of 9.17 μM) and CYP3A ((IC$_{50}$ of 0.151 μM for 6β-hydroxytestosterone and IC$_{50}$ of 0.154 μM for 1'-hydroxymidazolam) was observed. When compared to a C$_{max}$ concentration for cobicistat 150 mg once daily in healthy subjects of approximately 2 μM, cobicistat would be anticipated to inhibit CYP3A and potentially CYP2D6.

<u>AD-216-2030</u>

1. Title

Interaction of GS-9350 and Ritonavir with MRP1, MRP2, and Pgp

2. Objectives

The primary objective of the in vitro study was to evaluate the effects of cobicistat on MRP1, MRP2, and P-gp. The effects of ritonavir on these transporters were also evaluated.

3. Methods

MDCKII cells were maintained at 37ºC in 96 well plates. The test compounds were then incubated in cell culture medium that included 10 µM of calcein AM from 1 to 2.5 hours for the various transfected MDCKII cells. The wells were subsequently evaluated for calcein fluorescence with an excitation of 494 nm and an emission of 517 nm. The following equations were used in calculating the percentage inhibition and the percentage viability:

$$\textbf{Ratio (R)} = \textbf{TF}^{\textbf{MDR1}}/\textbf{TF}^{\textbf{WT}}, \textbf{TF}^{\textbf{ABCC1}}/\textbf{TF}^{\textbf{WT}} \textbf{ or TF}^{\textbf{ABCC2}}/\textbf{TF}^{\textbf{WT}}.$$

$$\textbf{Inhibition} = ((\textbf{R}^{\textbf{I}}\textbf{-RNI}) / (1\textbf{-R}^{\textbf{NI}})) \times 100\%$$

$$\textbf{Viability} = [(\textbf{total fluorescence WT}_{\textbf{treated}}) / (\textbf{total fluorescence WT}_{\textbf{untreated}})] \times 100\%$$

Where,

TF is total fluorescence

R^I and R^{NI} represent the ratio observed in the presence and absence of test compound, respectively.

4. Results

Based on the information in the report, the concentrations that were reported in the tables (see below) for the study are protein free concentrations.

The results of the in vitro experiments support the conclusion that cobicistat inhibits MRP1, MRP2, and P-gp based on the increasing percentage inhibition that was observed for all three transporters as concentrations increased to 90 µM (see Tables 1 through 3). The total C_{max} concentration for cobicistat 150 mg once daily in healthy subjects was approximately 1560 ng/mL (2 µM). Based on plasma protein binding of greater than 90% that has been reported for cobicistat

in humans, protein free concentrations of less than 2 µM cobicistat would not be predicted to result in significant inhibition of MRP1, MRP2, and P-gp.

For ritonavir, the results of the in vitro experiments support the conclusion that ritonavir inhibits MRP1 and P-gp based on the increasing percentage inhibition that was observed for MRP1 and P-gp but not MRP2 as concentrations increased to 20 µM (see Tables 4 through 6).

Table 1-Cobicistat inhibitory effects on MRP1 dependent calcein efflux

Dosing (µM)	% Viability	% Inhibition	Inhibition
L	104	0	Low
2.81	94.9	1.6	Low
5.63	105	2.6	Low
11.3	90.9	26.8	Medium
22.5	95.8	36.3	Medium
45.0	104	48.7	Medium
90.0	91.9	65.7	Medium

Table 2-Cobicistat inhibitory effects on MRP2 dependent calcein efflux

Dosing (µM)	% Viability	% Inhibition	Inhibition
1.41	93.4	5.7	Low
2.81	80.0	11.6	Low
5.63	80.0	11.5	Low
11.3	73.4	13.7	Low
22.5	78.4	19.7	Low
45.0	96.5	28.8	Medium
90.0	81.1	55.1	Medium

Table 3-Cobicistat inhibitory effects on P-gp dependent calcein efflux

Dosing (µM)	% Viability	% Inhibition	Inhibition
1.41	119	1.50	Low
2.81	105	2.10	Low
5.63	101	0.70	Low
11.3	88.5	6.20	Low
22.5	90.0	14.9	Low
45.0	94.2	57.1	Medium
90.0	107	88.8	High

Table 4-Ritonavir inhibitory effects on MRP1 dependent calcein efflux

Dosing (µM)	% Viability	% Inhibition	Inhibition
0.31	107	1.60	Low
0.63	82.8	11.1	Low
1.25	79.4	11.3	Low
2.50	70.7	20.2	Medium
5.00	62.9	29.4	Medium
10.0	87.2	23.2	Medium
20.0	77.2	52.9	Medium

Table 5-Ritonavir inhibitory effects on MRP2 dependent calcein efflux

Dosing (µM)	% Viability	% Inhibition	Inhibition
0.31	136	0.4	Low
0.63	117	2.4	Low
1.25	129	1.5	Low
2.50	107	3.3	Low
5.00	120	2.9	Low
10.0	145	2.4	Low
20.0	141	5.5	Low

Table 6-Ritonavir inhibitory effects on P-gp dependent calcein efflux

Dosing (µM)	% Viability	% Inhibition	Inhibition
0.31	86.8	3.50	Low
0.63	91.8	4.90	Low
1.25	96.5	5.90	Low
2.50	94.8	3.70	Low
5.00	81.3	12.0	Low
10.0	109	28.8	Medium
20.0	104	69.3	High

5. Conclusions

Based on the in vitro experiments, cobicistat inhibits MRP1, MRP2, and P-gp. However, protein free cobicistat concentrations of less than 2 µM would not be predicted to result in significant inhibition of MRP1, MRP2, and P-gp, with percentage inhibition values of less than 1.6%, 11.6%, and 2.10%, respectively.

<u>AD-216-2038</u>

1. Title

Identification of Major Metabolites of GS-9350 In Vitro

2. Objectives

The primary objective of the in vitro study was to determine the metabolites of cobicistat in humans, rats, and dogs.

3. Methods

Human metabolites were identified in cryopreserved human hepatocytes and Sprague Dawley rat, beagle dog, and human metabolites were identified in pooled hepatic microsomal fractions.

For the experiments using microsomes, 2.5 µL of a 10 mM cobicistat stock solution were added to microsomes for a final protein concentration of 1 mg/mL. The reactions were started by adding NADPH cofactor. 50 µM of cobicistat was evaluated for the reactions with sampling at 0, 2, 5, 10, 15, 30, 45, and 60 minutes.

For the experiments using cryopreserved human hepatocytes, 250 µL of 100 µM cobicistat solution were added to hepatocytes. 50 µM of cobicistat was evaluated with sampling at 0, 1, 3, and 6 hours. 7-hydroxycoumarin and testosterone were used as positive controls at concentrations of 2 µM.

Samples were analyzed using LC/MS/MS.

4. Results

The cobicistat metabolites that were identified in hepatic microsomes and cryopreserved human hepatocytes are displayed in Table 1 and Table 2.

Table 1-Metabolites identified in hepatic microsomes

Metabolites		Ret. Time (Min)	Molecular Ion [M+H]+	Proposed Metabolite Structure	Identification[a]		
GS-9350	RTV[b]				Human	Rat	Dog
GS-9350		10.2	776		Yes	Yes	Yes
E1	M-1	7.4	635		Yes	Yes/ Major	Yes
E2	M-5	7.4	651		No	No	Yes
E3	M-2	9.0	792		Yes/ Major	Yes/ Major	Yes/ Major
E4		8.1	706		No	No	Yes
E5	M-11	8.1	637		Yes	Yes	Yes
E6		9.3	792		No	No	Yes
E7		9.7	762		No	No	Yes
E8		10.5	790		No	No	Yes
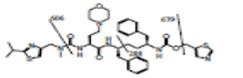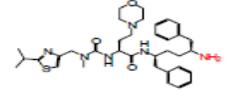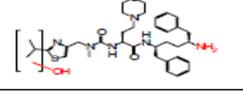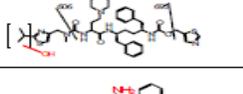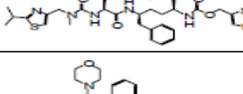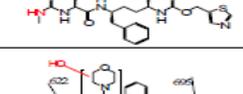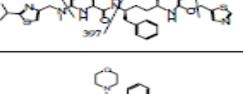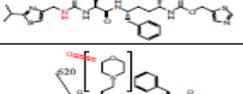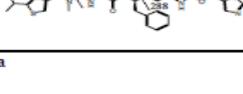
a   Major = > 25% of total metabolites by UV peak area
b   Equivalent ritonavir metabolite from reference (1)

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Table 2-Metabolites identified in cryopreserved human hepatocytes

Metabolites		Retention Time (Min)	Molecular Ion [M + H]+	Parent Structure/Proposed Metabolite
GS-9350	RTV[c]			
GS-9350		10.2	776	
E1	M-1	7.4	635	
E3	M-2	9.0	792	
E5	M-11	8.1	637	
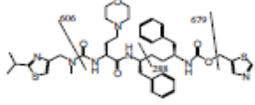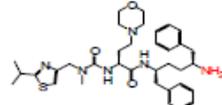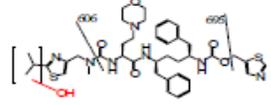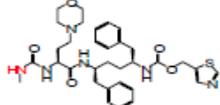
c   Equivalent ritonavir metabolite from reference (1)

5. Conclusions

Based on the in vitro experiments, in humans, the E1, E3, and E5 metabolites were identified, with the E3 metabolite present as more than 25% of the total metabolites when evaluating UV peak areas. There were no unique human metabolites that were identified.

<u>AD-216-2041</u>

1. Title

Drug Interaction Properties of Putative Human Metabolites of GS-9350

2. Objectives

The primary objective of the in vitro study was to evaluate the drug-drug interaction profiles for the three identified cobicistat metabolites in humans: E1 (GS-342006), E3 (GS-364751), and E5 (GS-341842).

3. Methods

For the cytochrome P450 inhibition experiments, the specific concentrations of the E1, E3, and E5 metabolite that were evaluated were not specified.

Using microsomes, the following metabolites and substrates were evaluated: a) CYP1A2: resorufin from 7-ethoxyresorufin (0.5 µm), b) CYP2C9: 4-hydroxytolbutamide from tolbutamide (120 µm), c) CYP2C19: 4'-hydroxymephenytoin from S-mephenytoin (25 µm), d) CYP2D6: dextrorphan from dextromethorphan (5 µm), and e) CY3A (in total 3 CYP3A substrates were evaluated): 1'-hydroxymidazolam from midazolam (2.5 µM), 6β-hydroxytestosterone from testosterone (50 µM), and hydroxy-terfenadine and fexofenadine from terfenadine (2.5 µm). Positive controls for CYP1A2, CYP2C9, CYP2C19, and CYP2D6, and were also included as part of the experiments (the report does not provide information on whether a positive control was used for CYP3A).

Samples were analyzed using LC/MS/MS for the cytochrome P450 inhibition experiments with the exception of CYP1A2. The CYP1A2 reaction was analyzed through quantification of the fluorescence (excitation of 535 nm and an emission of 595 nm) for a metabolite.

The IC_{50} values were determined using non linear regression. The reaction velocities for conversion to the metabolites were determined using linear regression. The rates were compared to vehicle controls (100% activity)

The in vitro cytochrome P450 induction experiments were conducted using Puracyp's hepatoma derived cell lines (DRE12.6 and DPX2). The effects of cobicistat's metabolites on the pregnane X receptor (PXR) or the aryl hydrocarbon receptor (AhR) were evaluated. PXR is involved in the induction of the CYP3A and CYP2C enzymes and AhR is involved in the induction of the CYP1A2 and CYP2B6 enzymes. Table 1 below provides information on the concentrations of the cobicistat metabolites (E1, E3, and E5) and the positive control compounds that were evaluated.

Table 1- Concentrations of the cobicistat metabolites (E1, E3, and E5) and the positive control compounds evaluated for the in vitro PXR and AXR induction experiments

| Inducing Agent | Concentrations Investigated (µM) | |
| | AhR Assessment | PXR Assessment |
	DRE12.6 Cells	DPX2 Cells
Rifampicin	—	10
Mifepristone	—	10
Androstanol	—	10
Omeprazole	25, 50, 100, 200	—
GS-341842	1, 3, 10	1, 3, 10
GS-342006	1, 3, 10	1, 3, 10
GS-364751	1, 3, 10	1, 3, 10

The cells were placed in a 96 well plate that were stored at 37ºC. The test compounds were added (three wells per compound), and the experimental conditions were maintained for 24 hours. After toxicity measurements were made, luminescence was measured using a BMG luminometer. Based on the toxicity assessment, the luminescence values were adjusted, the three values were averaged and the fold induction was determined by comparing to the concentrations of the DMSO vehicle control.

4. Results

Based on the in vitro data from the current study and the cobicistat IC_{50} values from the AD-216-2029 study that are displayed in Table 2, the following results were obtained:

a) When compared to cobicistat, only the E3 metabolite demonstrated greater inhibitory activity. Greater inhibitory activity was demonstrated for CYP2C19 and CYP2D6 for the E3 metabolite.

b) The E1 metabolite demonstrated inhibitory effects against CYP3A for midazolam but significant CYP3A inhibitory effects were not demonstrated for testosterone or terfenadine. A significant CYP2D6 inhibitory effect was not demonstrated for dextromethorphan.

c) The E3 metabolite demonstrated inhibitory effects against CYP3A for midazolam, testosterone, and terfenadine. CYPC19 and CYP2D6 inhibitory effects were also demonstrated by the E3 metabolite.

d) The E5 metabolite demonstrated inhibitory effects against CYP3A for midazolam and testosterone but significant CYP3A inhibitory effects were not

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demonstrated for terfenadine. A significant CYP2D6 inhibitory effect was not demonstrated for dextromethorphan.

Table 2-IC$_{50}$ values for cobicistat metabolites (E1, E3, and E5) in human liver microsomes measuring CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A activity

Enzyme	Activity	Calculated IC$_{50}$ (μM)			
		GS-9350	GS-342006 (E1)	GS-364751 (E3)	GS-341842 (E5)
CYP1A2	Ethoxyresorufin O-deethylase	> 25	> 25	> 25	> 25
CYP2C9	Tolbutamide hydroxylase	> 25	> 25	> 25	> 25
CYP2C19	S-Mephenytoin 4'-hydroxylase	> 25	> 25	2.95	> 25
CYP2D6	Dextromethorphan O-demethylase	9.17	> 5	0.21	> 5
CYP3A	Midazolam 1'-hydroxylase	0.154	2.41	0.179	0.23
CYP3A	Testosterone 6β-hydroxylase	0.151	> 5	0.287	0.71
CYP3A	Terfenadine oxidase	0.25	> 25	1.85	> 25

For the vitro cytochrome P450 induction experiments, based on the in vitro data from the current study and the cobicistat in vitro induction data from the AD-216-2027 study that are displayed in Table 3 and Table 4, the following results were obtained:

a) The induction effects of all three cobicistat metabolites were lower than the induction effects of cobicistat for both PXR and AhR.

b) The induction effects of both cobicistat and all three cobicistat metabolites were lower than the induction effects of the positive controls for both PXR and AhR.

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Table 3-Comparison of PXR induction with cobicistat, cobicistat metabolites (E1, E3, and E5), and positive control compounds

Conc.	Fold Induction Over 0.1% DMSO Control						
	GS-9350	E1	E3	E5	Rif[a]	Mif[a]	And[a]
1 μM	1.57	0.85	0.84	1.38		—	—
3 μM	1.61	1.50	0.92	1.17		—	—
10 μM	2.24	1.62	1.24	1.42	12.49[b]	7.10[b]	3.67[b]

a   Abbreviations: Rif, rifampicin; Mif, mifepristone; And, androstanol

b   Average of values from GIL-2007-107 and GIL-2007-108

Table 4-Comparison of AhR induction with cobicistat, cobicistat metabolites (E1, E3, and E5), and positive control compounds

Concentration (μM)	Fold Induction Over 0.1% DMSO Control				
	GS-9350	E1	E3	E5	Omeprazole[a]
1	1.12	0.86	0.93	0.81	
3	1.28	0.83	0.84	0.75	
10	1.60	0.83	0.76	0.68	
25					5.94
50					13.83
100					32.74
200					52.45

a   Average of values from GIL-2007-107 and GIL-2007-108

5. Conclusions

Based on the in vitro experiments, the following conclusions can be made:

a) All three cobicistat metabolites inhibited CYP 3A, however the specific CYP 3A substrates that were significantly inhibited differed for each metabolite. Additionally, CYPC19 (IC_{50} of 2.95 μM) and CYP2D6 (IC_{50} of 0.21 μM) were inhibited by the E3 metabolite.

b) For both PXR and AhR, lower cytochrome P450 induction effects were demonstrated for all three cobicistat metabolites when compared to cobicistat and lower cytochrome P450 induction effects were also demonstrated for all three cobicistat metabolites and cobicistat when compared to the positive controls.

AD-216-2070

1. Title

In Vitro Assessment of Human Liver CYP2B6 and CYP2C8 Inhibition Potential of GS-9350

2. Objectives

The primary objective of the in vitro study was to evaluate cobicistat's inhibitory effects on CYP2B6 and CYP2C8.

3. Methods

Cobicistat concentrations up to 30µM were incubated with human liver microsomes, NADPH and cytochrome P450 substrates. The following metabolites and substrates were evaluated: a) CYP2B6: 4-hydroxybupropion from bupropion (40 µm), and b) CYP2C8: 6α-hydroxypaclitaxel from paclitaxel (10 µm). Ritonavir's inhibitory effects on CYP2B6 and CYP2C8 were also evaluated.

Samples were analyzed using LC/MS/MS.

The reaction velocities were determined based on the rate of conversion to the metabolites and compared to vehicle controls (100% activity). IC_{50} values were derived using log-linear interpolation of the enzyme activity versus inhibitor concentration information.

4. Results

At concentrations up to 30 µM, inhibition of both CYP2B6 and 2C8 was observed. Based on the C_{max} concentration for cobicistat 150 mg once daily in healthy subjects of approximately 1560 ng/mL (2 µM), cobicistat may inhibit CYP2B6 and but would not be anticipated to inhibit CYP2C8.

Table 1-Cobicistat and ritonavir IC_{50} values in human liver microsomes measuring CYP2B6 and CYP2C8 activity

Enzyme	Activity	Calculated IC_{50} (µM)		
		Control Inhibitor[a]	Ritonavir	GS-9350
CYP2B6	Bupropion 4-hydroxylase	2.8	2.9	2.8
CYP2C8	Paclitaxel 6α-hydroxylase	0.06	5.5	30.1

a   Control Inhibitors: CYP2B6, Triethylenethiophosphoramide (0–30 µM); CYP2C8 Montelukast (0–30 µM)

5. Conclusions

Based on the in vitro experiments conducted with cobicistat concentrations up to 30 µM, inhibition of CYP2B6 (IC_{50} of 2.8 µM) and CYP2C8 ((IC_{50} of 30.1 µM) was observed. When compared to a C_{max} concentration for cobicistat 150 mg once daily in healthy subjects of approximately 2 µM, cobicistat may inhibit CYP2B6 and but would not be anticipated to inhibit CYP2C8.

AD-216-2071

1. Title

In Vitro Assessment of Human Liver CYP2B6 and CYP2C8 Inhibition Potential of GS-9350

2. Objectives

In Vitro Assessment of the Induction Potential of GS-9350 in Primary Cultures of Human Hepatocytes

3. Methods

Cobicistat concentrations of 1, 3, 10, and 30 µM were incubated with human hepatocytes from three different donors for three days. This range of values to evaluate cobicistat's in vitro induction effects is appropriate based on a C_{max} concentration for cobicistat 150 mg once daily in healthy subjects of approximately 1560 ng/mL (2 µM).

Positive controls were also included as part of the experiments to evaluate the induction effects on the following cytochrome P450 enzymes: a) CYP1A1/CYP1A2: 2 µM of   3-methylcholanthrene (3-MC), b) CYP2B: 1 mM of phenobarbital, and c) CYP 3A: 10 µM of rifampin.

The formation of metabolites in order to determine CYP1A2, CYP2B6, and CYP3A activity was analyzed using LC/MS/MS. The mRNA levels in CYP1A2, CYP2B6, and CYP3A, UGT1A1, and MDR1 were analyzed using quantitative Real Time-Polymerase Chain Reaction (qRT-PCR).

The following equations were used in calculating the relevant results (µ=mean):

A) Fold induction:

$$\frac{\mu_{(sample)}}{\mu_{(DMSO)}}$$

B) Percentage positive control (adjusted):

$$\frac{\mu_{(sample)} - \mu_{(DMSO)}}{\mu_{(positive\ control)} - \mu_{(DMSO)}} \times 100$$

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C) Relative fold change in mRNA ($\Delta C_{T=}$changes in mRNA content, and $\Delta\Delta C_T$=changes in MRNA content relative to endogenous control gene control and the 0.1% DMSO vehicle control)

$$2^{-\Delta\Delta C_T}$$

The applicant used the previous criteria of a 40% or greater change relative to the positive control as the degree of induction that warrants an in vivo trial.

The applicant did not calculate cobicistat E_{max} or EC_{50} values or provide R3 values for CYP1A2, CYP2B6, and CYP3A, UGT1A1, and MDR1. The R3 value is the currently recommended criteria for determining whether further evaluation is needed regarding the investigational drug's induction effects on a particular cytochrome P450 enzyme.

4. Results

Based on comparing the relative fold induction in mRNA levels for CYP1A2, CYP2B6, and CYP3A, UGT1A1, and MDR1 with cobicistat from 1 µM to 30 µM, increases in mRNA levels were only observed with CYP1A2 and CYP3A. Increases in mRNA levels were not observed with CYP2B6, UGT1A1, or MDR1 when comparing the relative fold induction in mRNA levels with cobicistat from 1 µM to 30 µM. At clinically relevant cobicistat concentrations ranging from 1 to 3 µM, there were minimal changes in the relative fold induction in mRNA levels for CYP1A2; however the changes in the relative fold induction in mRNA levels for CYP3A may be relevant. The results are displayed in Tables 1 and 2.

Table 1-Evaluation of cobicistat's induction effects using changes in mRNA levels (percentage adjusted positive control)

Treatment	CYP1A2			CYP2B6			CYP3A4			UGT1A1			MDR1		
	Hu790	Hu793	Hu8053	Hu790	Hu793	Hu8053	Hu790	Hu793	Hu8053	Hu790	Hu793	Hu8053	Hu790	Hu793	Hu8053
3-MC (2 µM)	100	100	100	3.1	-0.53	0.65	-0.97	-1.3	-2.8	140	81.2	83.6	-25.5	-33.1	-31.2
Phenobarbital (1000 µM)	0.14	0.34	0.15	100	100	100	61.0	79.6	47.8	124	127	107	89.8	98.9	106
Rifampicin (10 µM)	0.07	0.01	0.16	32.1	40.9	78.1	100	100	100	100	100	100	100	100	100
GS-9350 (1 µM)	0.08	0.05	-0.01	0.65	-0.67	6.2	7.0	14.4	10.0	3.4	7.6	8.4	-3.6	-35.8	-11.4
GS-9350 (3 µM)	0.15	-0.14	0.07	0.65	-0.02	13.2	17.6	25.2	32.0	3.4	10.6	17.6	-4.5	-21.5	8.0
GS-9350 (10 µM)	0.53	0.04	0.65	0.29	-0.12	8.9	22.9	27.9	31.3	0.37	10.2	18.4	14.8	-6.9	2.3
GS-9350 (30 µM)	1.4	3.0	1.7	-1.2	-1.2	-6.4	2.7	19.0	4.0	-4.4	6.2	-1.7	28.2	8.4	49.4

Table 2-Evaluation of cobicistat's induction effects using changes in mRNA levels (fold induction)

Treatment	CYP1A2			CYP2B6			CYP3A4			UGT1A1			MDR1		
	Hu790	Hu793	Hu8053	Hu790	Hu793	Hu8053	Hu790	Hu793	Hu8053	Hu790	Hu793	Hu8053	Hu790	Hu793	Hu8053
3-MC (2 µM)	400	406	565	3.10	0.825	1.07	0.270	0.528	0.227	12.0	9.63	3.94	0.693	0.573	0.687
Phenobarbital (1000 µM)	1.55	2.46	1.78	65.3	46.4	12.1	45.2	31.9	12.2	10.8	14.5	4.78	1.96	2.30	2.00
Rifampicin (10 µM)	1.26	1.15	1.85	21.7	19.6	9.64	73.5	39.7	24.6	8.85	11.6	4.53	2.07	2.31	1.94
GS-9350 (1 µM)	1.30	1.28	0.864	1.55	0.763	1.69	6.08	6.58	3.26	1.22	1.85	1.25	0.933	0.538	0.875
GS-9350 (3 µM)	1.58	0.522	1.33	1.54	1.06	2.46	13.8	10.8	8.48	1.22	2.16	1.58	0.923	0.724	1.06
GS-9350 (10 µM)	3.10	1.27	4.60	1.32	1.01	1.99	17.6	11.8	8.32	0.980	2.12	1.61	1.14	0.914	1.01
GS-9350 (30 µM)	6.62	13.3	10.4	0.377	0.501	0.293	2.91	8.36	1.85	0.603	1.70	0.890	1.28	1.11	1.45

Using the previous criteria of a 40% or greater change relative to the positive control (adjusted) as the degree of induction that warrants an in vivo trial, for CYP1A2, CYP2B6, and CYP3A, there were no changes exceeding this threshold with cobicistat from 1 µM to 30 µM. The results are displayed in Tables 3 and 4.

Table 3-Evaluation of cobicistat's induction effects using changes in enzyme activity (percentage adjusted positive control)

Treatment	CYP1A2			CYP2B6			CYP3A4		
	Hu790	Hu793	Hu8053	Hu790	Hu793	Hu8053	Hu790	Hu793	Hu8053
3-MC (2 µM)	100	100	100	4.8	0.10	4.7	0.01	-4.2	4.9
Phenobarbital (1000 µM)	3.3	6.1	-2.7	100	100	100	70.6	65.4	72.9
Rifampicin (10 µM)	2.5	3.7	-3.5	80.2	54.1	38.5	100	100	100
GS-9350 (1 µM)	0.54	-0.38	-5.8	8.4	1.2	5.2	-1.4	-6.7	-0.23
GS-9350 (3 µM)	0.26	-0.31	-5.1	3.5	-1.7	9.0	-1.7	-6.5	0.16
GS-9350 (10 µM)	1.7	1.3	-1.5	3.7	4.4	12.8	-1.6	-6.0	0.04
GS-9350 (30 µM)	0.61	4.2	-1.4	20.5	2.5	3.8	8.2	-6.5	-1.2

Table 4-Evaluation of cobicistat's induction effects using changes in enzyme activity (fold induction)

Treatment	CYP1A2			CYP2B6			CYP3A4		
	Hu790	Hu793	Hu8053	Hu790	Hu793	Hu8053	Hu790	Hu793	Hu8053
3-MC (2 µM)	102.6	42.5	15.5	1.2	1.0	1.8	1.0	0.6	2.2
Phenobarbital (1000 µM)	4.3	3.5	0.6	5.5	9.0	17.4	16.0	7.3	19.1
Rifampicin (10 µM)	3.5	2.5	0.5	4.6	5.3	7.3	22.2	10.6	25.9
GS-9350 (1 µM)	1.5	0.8	0.2	1.4	1.1	1.8	0.7	0.4	0.9
GS-9350 (3 µM)	1.3	0.9	0.3	1.2	0.9	2.5	0.6	0.4	1.0
GS-9350 (10 µM)	2.7	1.5	0.8	1.2	1.4	3.1	0.7	0.4	1.0
GS-9350 (30 µM)	1.6	2.7	0.8	1.9	1.2	1.6	2.7	0.4	0.7

5. Conclusions

Based on the in vitro experiments conducted, when comparing the relative fold induction in mRNA levels with cobicistat concentrations ranging from 1 µM to 30 µM, increases in mRNA levels were observed for CYP1A2 and CYP3A. Increases in mRNA levels were not observed with CYP2B6, UGT1A1, or MDR1 when comparing the relative fold induction in mRNA levels with cobicistat from 1 µM to 30 µM. At clinically relevant cobicistat concentrations ranging from 1 to 3

µM, the changes in the relative fold induction in mRNA levels for CYP3A may be relevant.

1. Title

Inhibition of P-glycoprotein-Dependent Bi-Directional Transport of Digoxin Through Monolayers of Caco-2 Cells by GS-9350

2. Objectives

The primary objective of the study was to determine if cobicistat is an intestinal inhibitor of P-glycoprotein (P-gp) by evaluating the bi-directional transport of digoxin, a P-gp substrate.

3. Methods

The experiments utilized Caco-2 cells. The test material in a buffer was added and 100 µL of solution was removed from the receiver compartment at one and two hours. Two separate wells were evaluated for each experiment. The monolayers were preincubated for 60 minutes with the compound being evaluated as a possible inhibitor to saturate transporter binding sites. The cells were tested either on the apical or basolateral side to obtain information on the forward (A to B) and reverse (B to A) permeability, respectively. LC/MS/MS was used to analyze the samples.

The following equations were used in deriving the results of the experiments:

$$P_{app} = (dR/dt) \times V_r/(A \text{ x } D_0)$$

$$\% \text{ Recovery} = 100 \times ((V_r \text{ x } R_{120}) + (V_d \times D_{120}))/(V_d \times D_0)$$

dR/dt is the slope of the cumulative concentration in the receiver compartment versus time in µM/s based on receiver concentrations measured at 60 and 120 minutes.

V_r and V_d is the volume in the receiver and donor compartment in cm^3, respectively.

A is the area of the cell monolayer (0.33 cm^2).

D_0 and D_{120} is the measured donor concentration at the beginning and end of the experiment, respectively.

R_{120} is the receiver concentration at the end of the experiment (120 minutes).

4. Results

The results of the experiments evaluating whether cobicistat is an intestinal inhibitor of P-gp is displayed in Table 1. In Caco-2 cells, the effects of cobicistat and two P-gp inhibitors (cyclosporine and ritonavir) on P-gp were evaluated. For all three compounds, to various degrees, the forward permeability was increased

and the reverse permeability was decreased for digoxin when compared to digoxin's forward permeability and reverse permeability by itself. This information supports the conclusion that cobicistat is a P-gp inhibitor, including in the intestine.

Table 1-Results of the in vitro experiments evaluating whether cobicistat is an intestinal inhibitor of P-gp in Caco-2 cells

Inhibitor	Inhibitor Conc. (µM)	Direction	Initial Digoxin Conc. (µM)	Digoxin Papp (10^{-6} cm/s)			Efflux Ratio
				R1	R2	Average	
None		Cell-Free	11.6	38.5		38.5	7.72
		Forward	13.6	1.07	1.53	1.30	
		Reverse	8.00	8.96	11.1	10.0	
Cyclosporin A	10	Cell-Free	9.30	47.0		47.0	1.68
		Forward	10.3	1.83	2.68	2.25	
		Reverse	10.0	3.56	4.00	3.78	
Ritonavir	20	Cell-Free	8.50	45.6		45.6	1.84
		Forward	10.4	2.77	3.56	3.17	
		Reverse	8.60	5.57	6.05	5.81	
GS-9350	90	Cell-Free	10.8	51.1		51.1	1.69
		Forward	11.9	2.74	1.74	2.24	
		Reverse	11.6	5.33	2.26	3.80	

5. Conclusions

The results of the vitro experiments support the conclusion that cobicistat is a P-gp inhibitor, including in the intestine.

AD-216-2075

1. Title

In Vitro Assessment of Human UGT1A1 Inhibition Potential of GS-9350

2. Objectives

The primary objective of the in vitro study was to evaluate cobicistat's inhibitory effects on UGT1A1.

3. Methods

The experiments utilized human hepatic microsomes and recombinant human UGT1A1 (Supersomes). Cobicistat concentrations up to 100 µM were evaluated. For UGT1A1, β-estradiol and β-estradiol-3-glucuronide were evaluated as the substrate and metabolite, respectively. Ritonavir's inhibitory effects on UGT1A1 were also evaluated. Atazanavir was used as a positive control because it inhibits UGT1A1.

Samples were analyzed using LC/MS/MS.

The reaction velocities were determined based on the rate of conversion to the metabolites and compared to vehicle controls (100% activity). IC_{50} values were derived using log-linear interpolation of the enzyme activity versus inhibitor concentration information.

4. Results

At concentrations up to 100 µM, cobicistat's IC_{50} value for UGT1A1 inhibition was higher than the positive control, atazanavir. The C_{max} concentration for cobicistat 150 mg once daily in healthy subjects is approximately 1560 ng/mL (2 µM). No specific criteria is included in the FDA's draft guidance for industry on drug interaction
studies (February 2012) based on cobicistat's IC_{50} value for UGT1A1 inhibition and cobicistat's C_{max} value for cobicistat 150 mg once daily to determine whether further evaluation is needed regarding cobicistat's inhibitory effects on UGT1A1.

Table 1-Cobicistat and ritonavir IC_{50} values in human liver microsomes measuring UGT1A1 activity

Enzyme	Activity	Calculated IC_{50} (µM)		
		Control Inhibitor[a]	Ritonavir	GS-9350
UGT1A1	β-Estradiol-3-glucuronidation	0.83	4.73	16.3

a   Control Inhibitor: Atazanavir (0–100 µM)

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5. Conclusions

Based on the in vitro experiments conducted with cobicistat concentrations up to 100 µM, cobicistat's IC_{50} value for UGT1A1 inhibition was higher than the positive control, atazanavir.   No specific criteria is included in the FDA's draft guidance for industry on drug interaction studies (February 2012) based on cobicistat's IC_{50} value for UGT1A1 inhibition and cobicistat's C_{max} value for cobicistat 150 mg once daily to determine whether further evaluation is needed regarding cobicistat's inhibitory effects on UGT1A1.

1. Title

In Vitro Interaction Studies of GS-9350 with Human OCT2 Uptake Transporter

2. Objectives

The primary objective of the in vitro study was to evaluate whether cobicistat and ritonavir inhibit the OCT2 transporter.

3. Methods

The experiments utilized Chinese hamster ovary (CHO) cells that expressed the human OCT2 transporter, and non transfected parent CHO cells. The effects of cobicistat and ritonavir (up to 100 µM were evaluated for both compounds) and 100 µM of verapamil on 2 µM of ^{14}C metformin were evaluated. Samples were analyzed using liquid scintillation counting.

The following equation was used to evaluate the OCT2 transport activity:

$$Activity\% = \frac{OCT2_{test} - CHO_{test}}{OCT2_{vehicle} - CHO_{vehicle}} \times 100\%$$

The IC$_{50}$ values were determined using non linear regression by evaluating the OCT2 transport activity of the test compounds compared to vehicle control (100% activity).

4. Results

Table 1- OCT2 IC$_{50}$ values for cobicistat and ritonavir

Compound	Assay Code	IC$_{50}$ (µM)	Inhibitory Efficacy (%)[a]
GS-9350	GS-340649	8.24	88
Ritonavir	GS-017415	22.6	85
Cimetidine	GSJ-000072	44.5	70
Trimethoprim	GSJ-000073	29.4	90

a   Maximum concentrations tested were 100 µM (GS-9350 and ritonavir) or 300 µM (cimetidine and trimethoprim)

Verapamil inhibited metformin transport by ≥ 91.5%.

5. Conclusions

Cobicistat's IC_{50} value for OCT2 inhibition is 8.24 μM. The total C_{max} concentration for cobicistat 150 mg once daily in healthy subjects is approximately 1560 ng/mL (2 μM). Based on the derived cobicistat IC_{50} value for OCT2 inhibition, and cobicistat human plasma protein binding of greater than 90%, a human drug-drug interaction trial evaluating cobicistat's OCT2 inhibition effects is not necessary.

1. Title

In Vitro Interaction Studies of GS-9350 with Human MATE1 and MATE2-K Efflux Transporters

2. Objectives

The primary objective of the in vitro study was to evaluate whether cobicistat and ritonavir inhibit MATE1 and MATE2-K transporters.

3. Methods

The experiments utilized human embryonic kidney cells (HEK293) that expressed MATE1, MATE2-K or empty vector. 5 µM of [1-^{14}C] tetraethyl ammonium (TEA) bromide was used as a substrate in evaluating cobicistat and ritonavir concentrations up to 100 µM. Cimetidine (10 µM or 100 µM) was used as the positive control (though Tables 1 and 2 in the study report indicate that concentrations of 300 µM were evaluated). Samples were analyzed using liquid scintillation counting and protein concentrations were derived using colorimetric methods.

The following equation was used in evaluating the fractional transport activity:

$$\text{Cleared volume} = \frac{\text{Uptake amount (dpm/well)}}{\text{Protein amount (mg/well)} \bullet \text{Concentration (dpm/}\mu L)}$$

The fractional transport activity was derived from the cleared volume of the test compounds compared to vehicle control (100% activity). The IC$_{50}$ values were determined by evaluating the fractional transport activity of the test compounds.

4. Results

Table 1-MATE1 IC$_{50}$ values for cobicistat and ritonavir

Compound	Assay Code	IC$_{50}$ (µM)[a]
GS-9350	GS-340649	1.87
Ritonavir	GS-017415	1.34
Cimetidine	GSJ-000072	1.64
Trimethoprim	GSJ-000073	6.35

a   Maximum concentrations tested were 100 µM (GS-9350 and ritonavir) or 300 µM (cimetidine and trimethoprim)

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Table 2-MATE2-K IC$_{50}$ values for cobicistat and ritonavir

Compound	Assay Code	IC$_{50}$ (μM)^a
GS-9350	GS-340649	33.5
Ritonavir	GS-017415	100
Cimetidine	GSJ-000072	43.4
Trimethoprim	GSJ-000073	1.38

a   Maximum concentrations tested were 100 μM (GS-9350 and ritonavir) or 300 μM (cimetidine and trimethoprim)

5. Conclusions

No specific criteria exist for determining whether further evaluation of cobicistat's inhibition of MATE1-and MATE2-K is necessary. The cobicistat IC$_{50}$ values that were derived were 1.87 μM for MATE1 and 33.5 μM for MATE2-K.

1. Title

Assessment of the Potential for GS-9350 and Ritonavir to be Substrates of the Human OCT2 Uptake Transporter

2. Objectives

The primary objective of the in vitro study was to evaluate whether cobicistat and ritonavir are OCT2 substrates.

3. Methods

The experiments utilized Chinese hamster ovary (CHO) cells that expressed the human OCT2 transporter, and non transfected parent CHO cells. 2 µM of ^{14}C metformin was used as a positive control. [N-^{14}CH$_3$] cobicistat concentrations ranging from 2 µM to 20 µM and [^{3}H] ritonavir concentrations ranging from 10 µM to 100 µM with incubation times of 2 minutes and 20 minutes, respectively, were evaluated. Samples were analyzed using liquid scintillation counting. 100 µM of the OCT2 inhibitor verapamil was also evaluated subsequent to the observation that quick and generally concentration independent uptake of both cobicistat and ritonavir was occurring in wild type cells.

4. Results

Metformin demonstrated minimal accumulation in CHO wild type cells with significant accumulation occurring in CHO OCT2 expressing cells. Uptake of cobicistat and ritonavir were observed both in the CHO wild type cells and in the CHO OCT2 expressing cells. Further accumulation of both ritonavir and cobicistat was observed as the concentration of both substrates was increased.

Table 1-Cobicistat, ritonavir, and metformin accumulation in CHO OCT2 expressing cells and CHO wild type cells

Compound	Conc. (µM)	Time (min)	Accumulation (pmol/mg protein)		Ratio
			CHO-OCT2	CHO-K (wt)	OCT2/wt
GS-9350	2	2	401.9 ± 18.8	280.2 ± 1.7	1.43
		20	725.9 ± 22.9	508 ± 106.5	1.43
	20	2	3241.2 ± 184.2	2258.1 ± 330.9	1.44
		20	5872.5 ± 487.4	4614.6 ± 945.2	1.27
RTV	10	2	1872.8 ± 106.8	1119.7 ± 74.2	1.68
		20	1834.8 ± 190.4	903.3 ± 352.3	2.03
	100	2	7682.8 ± 337	5684.0 ± 485.0	1.35
		20	11454.7 ± 1380.7	6347.8 ± 1686.0	1.80
Metformin	2	10	215.2 ± 17.0	6.0 ± 1.3	35.9

When the effects of 100 µM of verapamil, an OCT2 inhibitor, were evaluated, significant inhibition of metformin accumulation was observed in the OCT2 expressing cells but not in the wild type cells. Similar findings were not observed with cobicistat or ritonavir. For cobicistat, verapamil did not result significantly inhibit the accumulation of cobicistat OCT2 expressing cells and appeared to have greater inhibitory effects on cobicistat with wild type cells. For ritonavir, verapamil's inhibitory effects on OCT2 expressing cells were greater than the effects on cobicistat but significantly less than the effects on metformin and appeared to have comparable inhibitory effects on ritonavir with wild type cells.

Table 2-Verapamil inhibitory effects on cobicistat, ritonavir, and metformin accumulation in CHO OCT2 expressing cells and CHO wild type cells

Compound	Verapamil (100 µM)	Accumulation (pmol/mg protein)		Ratio
		CHO-OCT2	CHO-K (wt)	OCT2/wt
GS-9350 (2 µM)	-	401.9 ± 18.8	280.2 ± 1.7	1.43
	+	375.8 ± 17.8	235.4 ± 3.5	1.60
	Δ	6.5%	16.0%	
RTV (10 µM)	-	1872.8 ± 106.8	1119.7 ± 74.2	1.68
	+	1373.7 ± 155.1	798.1 ± 89.0	1.72
	Δ	26.6%	28.7%	
Metformin (2 µM)	-	215.2 ± 17.0	6.0 ± 1.3	35.9
	+	5.6 ± 1.1	6.6 ± 1.1	0.9
	Δ	97.4%	-10.0%	

5. Conclusions

The results of the experiments evaluating whether cobicistat and ritonavir are OCT2 substrates were inconclusive. When comparing the accumulation in OCT2 expressing cells to wild type cells, at cobicistat concentrations ranging from 2 µM to 20 µM, an increase in accumulation of up to 44% was observed, and at ritonavir concentrations ranging from 10 µM to 1000 µM, an increase in accumulation of up to 103% was observed in contrast to the approximately 3500% increase with 2 µM of metformin. The significant inhibitory effect of verapamil on OCT2 expressing cells with metformin (97.4% decrease in accumulation) was not observed with cobicistat (6.5% decrease in accumulation) or ritonavir (26.6% decrease in accumulation).

1. Title

In Vitro Interaction Studies of Cobicistat and Ritonavir with Human OCTN1 Transporter

2. Objectives

The primary objective of the in vitro study was to evaluate whether cobicistat and ritonavir inhibit human OCTN1.

3. Methods

The experiments utilized S_2 cells that were empty vectors or transfected with human OCTN1. 5 µM of [1-^{14}C] tetraethyl ammonium (TEA) bromide was used as a substrate in evaluating cobicistat and ritonavir concentrations up to 100 µM. Verapamil (100 µM) was used as the positive control. Samples were analyzed using liquid scintillation counting and protein concentrations were derived using colorimetric methods.

The following equation was used in evaluating the fractional transport activity:

$$\text{Cleared volume} = \frac{\text{Uptake amount (dpm/well)}}{\text{Protein amount (mg/well)} \bullet \text{Concentration (dpm/}\mu\text{L)}}$$

The fractional transport activity was derived from the cleared volume of the test compounds compared to vehicle control (100% activity). The IC_{50} values were determined by evaluating the fractional transport activity of the test compounds.

4. Results

Table 1-OCTN1 IC_{50} values for cobicistat and ritonavir

Compound	Assay Code	IC_{50} (µM)	Maximal inhibition [a] (%)
Cobicistat	GS-340649	2.49	~94
Ritonavir	GS-017415	2.08	~91

a   Maximum concentrations tested were 100 µM

5. Conclusions

No specific criteria exist for determining whether further evaluation of cobicistat's inhibition of OCTN1 is necessary. The cobicistat IC_{50} value that was derived was 2.49 µM for OCTN1.

<u>AD-216-2099</u>

1. Title

In Vitro Assessment of Cobicistat and Ritonavir Inhibition of Human Breast Cancer Resistance Protein

2. Objectives

The primary objective of the in vitro study was to evaluate whether cobicistat and ritonavir inhibit BCRP.

3. Methods

The experiments utilized MDCKII cells that were wild type cells or transfected with BCRP. MDCKII cells were maintained in 96 well plates. The test compounds (the study report did not specify the concentrations that were evaluated) were then incubated in cell culture medium that included 10 µM of Hoechst 33342 for 3 hours. The wells were subsequently evaluated for Hoechst 33342 fluorescence with an excitation of 353 nm and an emission of 460 nm. The following equations were used in calculating the percentage viability and the percentage transport inhibition:

$$\% \text{ viability} = [(\text{TF WT}_\text{I}) / (\text{TF WT}_\text{NI})] \times 100 \%$$

Where,

WT_I represents the fluorescence in the presence of test article for wild type cells

WT_NI represents the fluorescence in the absence of test article for wild type cells

$$\text{Ratio} = \text{TF}_\text{Transfected} / \text{TF}_\text{WT}$$

$$\% \text{ Inhibition} = ((\text{Ratio}_\text{I} - \text{Ratio}_\text{NI}) / (1 - \text{Rratio}_\text{NI})) \times 100\%$$

where TF=total fluorescence

IC_{50} values were derived using as a sigmoidal plot of the percentage inhibition versus concentration with a variable Hill coefficient.

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4. Results

Table 1-BCRP IC$_{50}$ values for cobicistat and ritonavir

Test compound	IC$_{50}$ (μM)	Maximal inhibition (%)
Cobicistat	59 ± 28	~ 73
Ritonavir	> 20	~ 25

Figure 1-Inhibition of BCRP transport of Hoechst 33342 by cobicistat and ritonavir
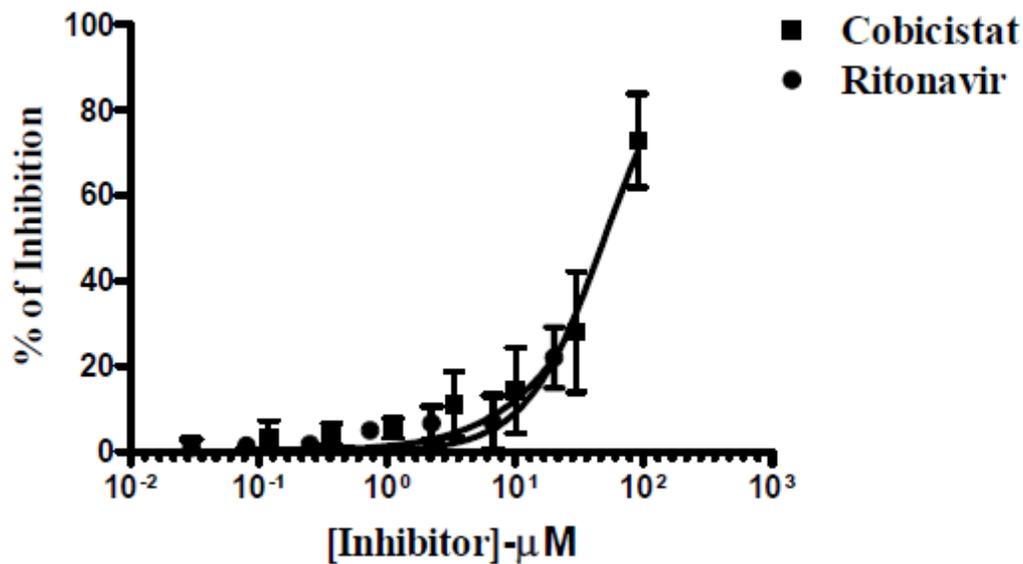
5. Conclusions

Based on the I$_1$/IC$_{50}$ calculation that was less than 0.1 and the I$_2$/IC$_{50}$ calculation that was greater than 10, a human drug-drug interaction trial should be conducted evaluating the BCRP inhibitory effects of cobicistat.

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1. Title

In Vitro Assessment of Cobicistat and Ritonavir Inhibition of Human OATP1B1 and OATP1B3

2. Objectives

The primary objective of the in vitro study was to evaluate whether cobicistat and ritonavir inhibit the OATP1B1 and OATP1B3 transporters.

3. Methods

The experiments utilized Chinese hamster ovary (CHO) cells that expressed the human OATP1B1 and OATP1B3 transporters and wild type CHO cells. CHO cells were maintained in 96 well plates. Cobicistat (at concentrations ranging from 0.135 µM to 90 µM) and ritonavir (at concentrations ranging from 0.027 µM to 20 µM) were then pre-incubated in cell culture medium that included 2 µM of Fluo 3 for 1 hour. The wells were subsequently evaluated for Fluo 3 fluorescence with an excitation of 485 nm and an emission of 530 nm. The following equations were used in calculating the percentage viability and the percentage transport inhibition:

A) Percentage viability

$$\% \text{ viability} = [(TF\ WT_I) / (TF\ WT_{NI})] \times 100\ \%$$

Where,

WT_I represents the fluorescence in the presence of test article for wild type cells

WT_{NI} represents the fluorescence in the absence of test article for wild type cells.

B) Percentage transport inhibition

$$\% \text{ inhibition} = (1 - ((OATP_I - WT_{NI}) / (OATP_{NI} - WT_{NI})))*100$$

Where,

$OATP_I$ represents the fluorescence in the presence of test article for either OATP1B1 or OATP1B3 overexpressing cells.

$OATP_{NI}$ represents the fluorescence in the absence of test article for either OATP1B1 or OATP1B3 overexpressing cells.

WT_{NI} represents the fluorescence in the absence of test article for wild type cells.

IC_{50} values were derived using as a sigmoidal plot of the percentage inhibition versus concentration with a variable Hill coefficient.

4. Results

Table 1- OATP1B1 and OATP1B1 IC_{50} values for cobicistat and ritonavir (average of two experiments)

Test Articles	Transporters	IC_{50} (µM)	Maximal inhibition (%)
Cobicistat	OATP1B1	3.50 ± 0.72	~ 98.5
	OATP1B3	1.88 ± 0.76	~ 99.5
Ritonavir	OATP1B1	2.05 ± 1.33	~ 98.7
	OATP1B3	1.83 ± 1.13	~ 99.1

Figure 1-Inhibition of OATP1B1 transport of Fluo 3 by cobicistat and ritonavir
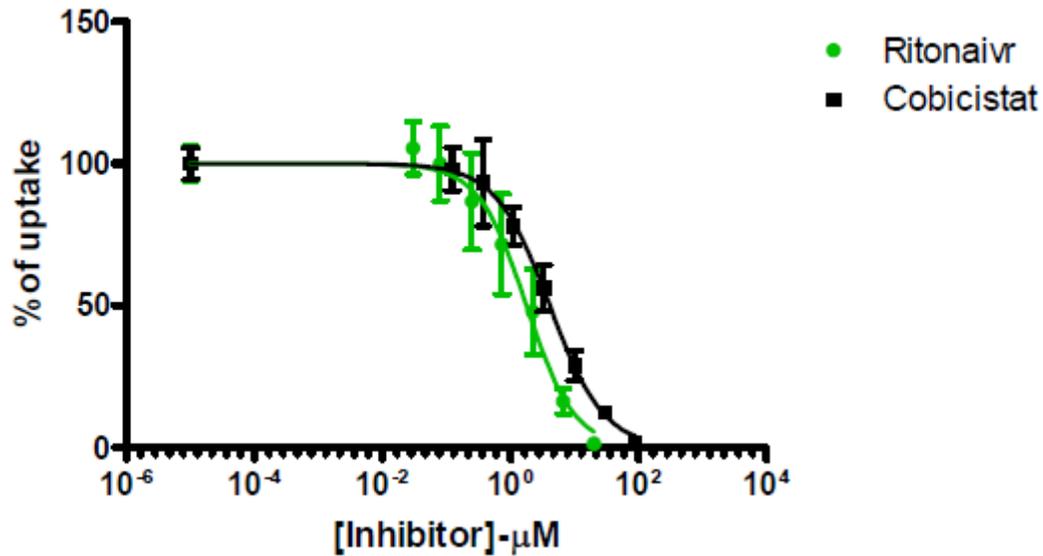
Figure 2-Inhibition of OATP1B3 transport of Fluo 3 by cobicistat and ritonavir
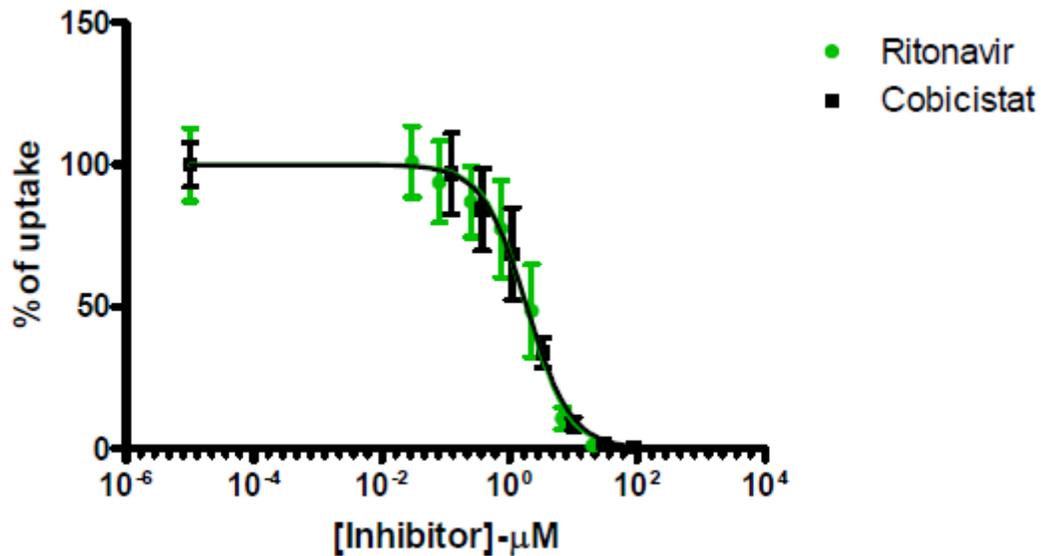
5. Conclusions

The cobicistat IC_{50} values that were derived were 3.5 µM for OATP1B1 and 1.88 µM for OATP1B3. Based on the total C_{max}/IC_{50} calculations and the R value calculations for OATP1B1 and OAT1B3, cobicistat's inhibitory effects on OATP1B1 and OATP1B3 should be further evaluated in a human drug-drug interaction trial.

1. Title

Bi-Directional Permeability of Cobicistat Through Monolayers of P-glycoprotein and BCRP Overexpressing Cells

2. Objectives

The primary objective of the study was to determine if cobicistat is a substrate of P-glycoprotein (P-gp) and the Breast Cancer Resistance Protein (BCRP).

3. Methods

The test material in a buffer was added and 100 µL of solution was removed from the receiver compartment at one and two hours. Two separate wells were evaluated for each experiment. The cells were tested either on the apical or basolateral side to obtain information on the forward (A to B) and reverse (B to A) permeability, respectively. LC/MS/MS was used to analyze the samples.

4. Results

The results of the experiments evaluating whether cobicistat is a P-gp substrate is displayed in Table 1. In MDCKII-MDR1 cells, the efflux ratio was ≥ 2, and when cyclosporine, a P-gp inhibitor was administered, the efflux ratio decreased by 94%. The draft February 2012 guidance to industry on drug interaction studies defines significant inhibition of a substrate's efflux by a P-gp inhibitor as a decrease in the flux ratio of > 50% or to unity.

Table 1-Results of the in vitro experiments evaluating whether cobicistat is a P-gp substrate in MDCK II cells

Cell type	Direction	Initial Conc. (µM)	Recovery (%)	P_{app} (x 10^{-6} cm/s)			Efflux Ratio
				R1	R2	Average	
MDCKII-WT	Cell-Free	9.85	110.12	28.88		28.88	
	Forward	10.18	81.81	3.26	3.27	3.26	3.7
	Reverse	10.06	97.65	10.51	13.88	12.19	
MDCKII-MDR1	Cell-Free	10.19	103.94	33.68		33.68	
	Forward	10.34	85.84	0.32	0.32	0.32	60.9
	Reverse	9.72	102.22	17.56	21.20	19.38	
MDCKII-MDR1 (10 µM Cyclosporin A)	Cell-Free	11.2	102.90	26.84		26.84	
	Forward	11.2	91.24	3.40	3.66	3.53	3.8
	Reverse	10.1	94.85	12.41	14.00	13.21	

The results of the experiments evaluating whether cobicistat is a BCRP substrate is displayed in Table 2. In MDCKII-BCRP cells, the efflux ratio was ≥ 2, and when Ko134, a compound used by the applicant as a BCRP inhibitor, was evaluated in MDCKII-BCRP cells, the efflux ratio decreased by 76%. The draft February 2012 guidance to industry on drug interaction studies defines significant inhibition of a substrate's efflux by a BCRP inhibitor as a decrease in the flux ratio of > 50% or to unity.

Table 2-Results of the in vitro experiments evaluating whether cobicistat is a BCRP substrate in MDCK II cells

Cell type	Direction	Initial Conc. (µM)	Recovery (%)	P_{app} (x 10^{-6} cm/s)			Efflux Ratio
				R1	R2	Average	
MDCKII-WT	Cell-Free	9.49	100.66	30.35		30.35	
	Forward	9.35	78.66	1.71	2.27	1.99	7.0
	Reverse	8.60	102.55	13.43	14.62	14.03	
MDCKII-BCRP	Cell-Free	9.15	96.97	26.81		26.81	
	Forward	9.29	79.49	1.54	1.50	1.52	13.4
	Reverse	8.43	110.12	19.15	21.50	20.33	
MDCKII-BCRP (10 µM Ko134)	Cell-Free	8.90	102.29	31.72		31.72	
	Forward	9.12	76.97	3.54	4.04	3.79	3.2
	Reverse	9.14	87.59	11.22	12.67	11.94	

5. Conclusions

The results of the vitro experiments support the conclusions that cobicistat is a P-gp and a BCRP substrate.

1. Title

Inhibition of Breast Cancer Resistance Protein-Dependent Bi-Directional Transport of Prazosin through Monolayers of Caco-2 Cells by Cobicistat

2. Objectives

The primary objective of the in vitro study was to evaluate whether cobicistat inhibits the BCRP transporter by evaluating the bi-directional transport of prazosin, which the study report indicates is a BCRP substrate. The BCRP inhibitory effects of ritonavir were also evaluated.

3. Methods

The experiments utilized Caco-2 cells. The test material in a buffer was added and 100 µL of solution was removed from the receiver compartment at one and two hours. Two separate wells were evaluated for each experiment. The monolayers were preincubated for 60 minutes with the compound being evaluated as a possible inhibitor to saturate transporter binding sites. The cells were tested either on the apical or basolateral side to obtain information on the forward (A to B) and reverse (B to A) permeability, respectively. LC/MS/MS was used to analyze the samples.

The following equations were used in deriving the results of the experiments:

$d\mathbf{R}/d\mathbf{t}$ is the slope of the cumulative concentration in the receiver compartment versus time in µM/s based on receiver concentrations measured at 60 and 120 minutes.

$\mathbf{V_r}$ and $\mathbf{V_d}$ is the volume in the receiver and donor compartment in cm^3, respectively.

$\mathbf{A}$ is the area of the cell monolayer (0.33 cm^2).

$\mathbf{D_0}$ and $\mathbf{D_{120}}$ is the measured donor concentration at the beginning and end of the experiment, respectively.

$\mathbf{R_{120}}$ is the receiver concentration at the end of the experiment (120 minutes).

4. Results

The results of the experiments evaluating whether cobicistat is an intestinal inhibitor of BCRP is displayed in Table 1. For FTC (which the study report indicates is a BCRP inhibitor), ritonavir, and cobicistat, the mean forward permeability was increased and the mean reverse permeability was decreased for prazosin when compared to prazosin's forward permeability and reverse

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permeability by itself. This information supports the conclusion that cobicistat is a BCRP inhibitor, including in the intestine.

Table 1-Results of the in vitro experiments evaluating whether cobicistat is an intestinal inhibitor of BCRP in Caco-2 cells using 10 µM of prazosin as a BCRP substrate

Inhibitor	Inhibitor Conc. (µM)	Direction	Prazosin Papp (10^{-6} cm/s)					Efflux Ratio
			R1	R2	R3	R4	Average	
None		Cell-Free	49.84		24.04		36.94	5.1
		Forward	1.59	2.05	2.8	3.56	2.50	
		Reverse	9.12	13.91	12.92	15.08	12.78	
FTC	2	Cell-Free	58.80		34.70		46.75	2.6
		Forward	3.51	3.95	4.97	5.33	4.44	
		Reverse	11.55	12.45	11.18	11.14	11.58	
Ritonavir	20	Cell-Free	51.30		28.44		39.87	2.8
		Forward	3.22	3.49	4.17	4.98	4.00	
		Reverse	9.98	12.62	9.93	12.49	11.26	
Cobicistat	90	Cell-Free	41.75		34.90		38.33	2.4
		Forward	3.36	3.57	5.78	6.24	4.74	
		Reverse	11.34	12.50	9.68	11.29	11.20	

5. Conclusions

The results of the vitro experiments support the conclusion that cobicistat is a BCRP inhibitor, including in the intestine.

1. Title

In Vitro Inhibition Studies of Cobicistat and Ritonavir with Human OAT1, OAT3 and MRP4 Transporters

2. Objectives

The primary objective of the in vitro study was to evaluate whether cobicistat and ritonavir inhibits the OAT1, OAT3, and MRP4 transporters.

3. Methods

Cobicistat concentrations up to 100 µM were evaluated.

For OAT1, the experiments utilized Chinese hamster ovary (CHO) cells and for OAT3, the experiments utilized Human Embryonic Kidney (HEK293) cells. ^{3}H-estrone-3-sulfate (E3S) and ^{3}H- para-aminohippuric acid (PAH) were evaluated as substrates. Table 1 summarizes the substrate and inhibitor information for the OAT1 and OAT3 experiments. Parenteral cells without transporters were used as a comparator. Samples were analyzed using liquid scintillation counting.

Table 1-Substrate and inhibitor information for the OAT1 and OAT3 experiments

Transporter	Incubation time (min)	Probe substrate (µM)	Reference inhibitor (µM)
Human OAT1	3	PAH (0.5 µM)	benzbromarone (200 µM)
Human OAT3	5	E3S (0.2 µM)	probenecid (200 µM)

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The following equation was used in evaluating the fractional transport activity for OAT1 and OAT3:

$$Activity\ \% = \frac{A-B}{C-D} \times 100$$

Legend:

A: translocated amount of substrate in the presence of TA on transfected cells

B: translocated amount of substrate in the presence of TA on parental cells

C: translocated amount of substrate in the presence of solvent on transfected cells

D: translocated amount of substrate in the presence of solvent on parental cells

For MRP4, membrane vesicle preparations (total protein 50 µg/well) were incubated with cobicistat, ritonavir, and 0.02 µM of the substrate (DHEAS (dehydroepiandrosterone-sulfate) with or without 4 mM of ATP. Control membranes without MRP4 were used as the negative control and MK571 was used as a positive control. Samples were analyzed using liquid scintillation counting.

The following equation was used in evaluating the fractional transport activity for MRP4:

$$Activity\ \% = \frac{A-B}{C-D} \times 100$$

Legend:

A: translocated amount of substrate in the presence of TA and ATP

B: translocated amount of substrate in the presence of TA

C: translocated amount of substrate in the presence of solvent and ATP

D: translocated amount of substrate in the presence of solvent

The IC_{50} values were determined by evaluating the fractional transport activity of the test compounds.

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4. Results

Table 2-OAT1, OAT3, and MRP4 IC$_{50}$ values for cobicistat and ritonavir

Compound	Transporter	Probe substrate	IC$_{50}$ (μM)	Maximum inhibition[a] (%)
Cobicistat	OAT1	PAH	> 100	140 % activation
	OAT3	E3S	> 100	ND
	MRP4	DHEAS	20.7	~ 92
Ritonavir	OAT1	PAH	> 20	ND
	OAT3	E3S	8.46	~ 62
	MRP4	DHEAS	> 20	~ 15

a   Maximum concentrations tested were 100 μM for cobicistat, and 20 μM for ritonavir

ND Not Determined

5. Conclusions

Cobicistat's IC$_{50}$ value for both OAT1 and OAT3 inhibition was greater than 100 μM. The total C$_{max}$ concentration for cobicistat 150 mg once daily in healthy subjects is approximately 1560 ng/mL (2 μM). Based on the derived cobicistat IC$_{50}$ values for OAT1 and OAT3 inhibition, and cobicistat human plasma protein binding of greater than 90%, the unbound C$_{max}$/IC$_{50}$ calculation that was less than 0.1, and a human drug-drug interaction trial evaluating the OAT1 or OAT3 inhibitory effects of cobicistat is not necessary.

No specific criteria exist for determining whether further evaluation of cobicistat's inhibition of MRP4 is necessary. For MRP4, the cobicistat IC$_{50}$ value was 20.7 μM.

4.2 PHARMACOMETRICS REVIEW

OFFICE OF CLINICAL PHARMACOLOGY:
PHARMACOMETRIC REVIEW

Pharmacometrics Reviewer: Jeffry Florian

Pharmacometrics Team Leader: Yaning Wang

Clinical Pharmacology Reviewer: Vikram Arya

Clinical Pharmacology Team Leader: Kellie Reynolds

1 SUMMARY OF FINDINGS

1.1 Key Review Questions

The purpose of this review is to address the following key questions.

1.1.1 Are the population PK parameters reported in the label (race, gender, geriatric, hepatitis B/C virus co-infections) supported by the population PK analysis submitted by the applicant?

The pharmacokinetic parameters for elvitegravir (EVG) 150 mg for adults in special populations (race and gender) supports the applicants claim of no difference in exposure with regards to race and gender. EVG AUC, C_{max}, and C_{0h} means (% confidence value) were in good agreement with the applicant's results. Shown below are the applicant's results for race (Table 1) and gender (Table 2).

Table 1: Population Pharmacokinetic Estimates of Elvitegravir 150 mg q.d. in Antiretroviral Treatment Naïve HIV-1-Infected Subjects (Comparison Between Races)			
Parameter	Asian (n=18)	Black (n=90)	White (n=295)
AUC_τ (ng·h/mL)	26,700 (33)	23,400 (33)	22,500 (30)
C_{max} (ng/mL)	1,970 (22)	1,720 (24)	1,710 (21)
C_{0h} (ng/mL)	528 (57)	478 (53)	435 (55)

Table 2: Population Pharmacokinetic Estimates of Elvitegravir 150 mg q.d. in Antiretroviral Treatment Naïve HIV-1-Infected Subjects (Comparison Between Gender)		
Parameter	Female (n=35)	Male (n=384)
AUC_τ (ng·h/mL)	25,800 (41)	22,700 (31)
C_{max} (ng/mL)	1,930 (26)	1,710 (22)
C_{0h} (ng/mL)	523 (79)	445 (54)

In addition, the applicant claims that hepatitis B/C infection has no impact on EVG exposure (n=24) is supported based on population PK analysis. Also, the applicant's claim of limited PK in elderly subjects (65 years and older) is supported by the Phase III

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data (only three subjects were 65 years or older). No definitive claims with regards to age effects on EVG exposure can be concluded based on the Phase III data. Finally, there is insufficient data on cobicistat (Cobi) exposure to support any labeling claims due to the limited number of subjects from Phase II/III with sampling available (n=61).

1.1.2 Does the EVG exposure-response relationship with virologic outcome (percent of subjects <50 HIV-1 copies/mL at week 48) support the selected 150 mg dose?

A flat exposure-response relationship was observed between EVG (C_{0h} and AUC) versus the primary study endpoint of percentage of subjects achieving virologic success (plasma viral load <50 HIV-1 RNA copies/mL) at Week 48 (Figure 1). This assessment was based on data available from 373 subjects administered the elvitregravir/cobicistat/emtracitabine/tenofovir disoproxil fumarate (E/C/T/F) fixed-dose combination (FDC) from Phase III (GS-US-236-0102; GS-US-236-0103).

Figure 1:   **Percentage of Subjects Achieving Virologic Success (<50 Copies/mL) Versus Elvitegravir C_{0h} (left) and AUC_τ (right) from Phase III.**
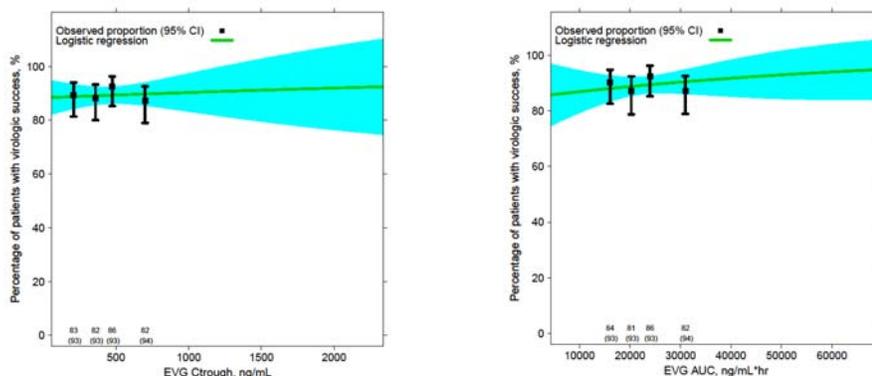
To identify if there was any loss in efficacy towards lower exposures, the exposure response relationship was further explored by separating the groups into deciles. It was seen that the observed percentage of subjects achieving virologic success was flat across all deciles (Table 3).   The median C_{0h} in the lowest decile was 156 ng/mL and corresponded to a virologic response of 87%. Virologic success in deciles with higher C_{0h} values (234–916 ng/mL) ranged between 84–97%. A similar trend was observed for $AUC_{tau;}$ though the response in the lowest decile was lower than 8 of the 9 other groups.

Table 3: Treatment-Naïve Subjects with Virologic Success by C_{0h} Decile (Q1-Q10)

	n	Median C_{0h}	% subjects with virologic success	n	Median AUC_{tau}	% subjects with virologic success
Q1	38	156	87	38	13,394	82
Q2	37	235	92	37	16,991	95
Q3	37	296	95	37	18,410	89

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Q4	37	342	84	37	19,816	89
Q5	37	402	86	37	21,425	89
Q6	38	438	87	38	22,721	87
Q7	37	498	97	37	24,340	95
Q8	37	561	92	37	26,522	95
Q9	37	674	81	37	30,113	81
Q10	38	916	92	38	36,780	92

No significant covariates, such as age, gender, race, or baseline viral load were identified as predictive of virologic success based on a generalized additive model (GAM) analysis. A trend of lower virologic response was observed in subjects with baseline viral load (subjects <100,000 copies/mL: 92% (n=214); subjects 100,000-500,000 copies/mL: 87% (n=135); subjects >500,000 copies/mL: 79% (n=24)). The virologic response rates between subjects with viral load <100,000 copies/mL and 100,000-500,000 copies/mL was similar.

1.1.3 Is there evidence of EVG (and cobicistat) exposure-safety relationships for headache, nausea, or diarrhea (adverse events of interest from the Phase III trials)?

An exposure-response relationship could not be established for headache, nausea, or diarrhea and EVG exposures. Logistic regression models were evaluated for EVG C_{max}, C_{0h}, and AUC_τ with no significant relationships identified. Modeling results for adverse event rates versus EVG AUC_τ are shown below in the reviewer's analysis.

Exposure-safety analyses for cobicistat were based on binned comparison of AUC_{tau} (and C_{max}) from 61 subjects with PK sampling from Phase II/III. Similar cobicistat exposures were observed between subjects with and without the key adverse events of interest.

1.1.4 Is the decrease in creatinine clearance following treatment with cobicistat dependent on baseline renal function?

Subjects with lower baseline renal function were also those subjects that experienced the smallest changes from baseline in the estimated glomerular filtration rate calculated using the Cockcroft-Gault equation (eGFR (C-G) – referred to as eGFR within this review). All E/C/T/F-treated subjects from the two Phase III pivotal trials (GS-US-236-0102; GS-US-236-0103) were pooled for assessing the relationship between on-treatment maximum decrease and baseline eGFR. A total of 759 subjects were included in this analysis.

Figure 2 shows the relationship between on treatment maximum decrease and baseline eGFR. A trend of smaller maximum decreases from baseline dependent on baseline eGFR was observed between subjects with normal renal function and mild renal impairment. There were insufficient subjects with baseline moderate renal function to determine the impact on eGFR in these subjects (n=4; screening eGFR ≤70 mL/min was an exclusion criteria in the Phase III trials). The mean maximum decrease in eGFR for subjects with baseline mild renal function was less than that observed for subjects with baseline normal renal function (Table 4).

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Figure 2: Maximum on Treatment Change in eGFR Versus Baseline eGFR in Subjects Administered E/C/T/F from GS-US-236-0102 and GS-US-236-0103
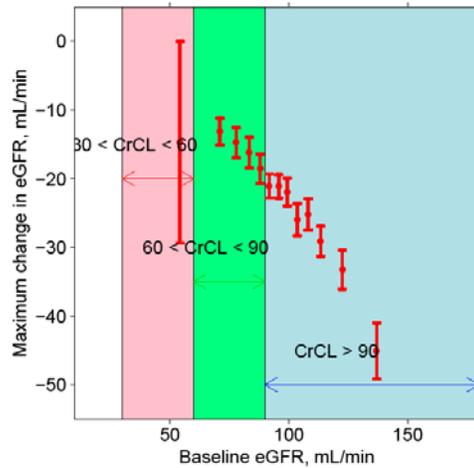
Table 4: Mean Maximum Change in eGFR Grouped by Baseline Renal Function for Subjects Treated with E/C/T/F from GS-US-236-0102 and GS-US-236-0103

eGFR Category, mL/min	Count, n	Mean max change in eGFR, mL/min
30-60 (moderate)	4	-16.0
60-90 (mild)	223	-13.4
>90 (normal)	532	-28.4

1.1.5 Is there evidence of an exposure-safety relationship for specific renal adverse events?

An imbalance in renal adverse events was observed between the E/C/F/T treatment arms and active comparator arms from Phase III (9 subjects on an E/C/F/T regimen, 2 subjects on Atripla, and 1 subject on atazanvir/ritonavir). Of these subjects, 6 discontinued E/C/F/T therapy due to renal-associated events. No exposure-response relationship regarding renal adverse events between EVG exposures could be identified from the available data. In addition, exposure-response relationships for other components of the

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E/C/F/T regimen could not be evaluated due to the limited number of subjects with cobicistat, tenofovir disoproxil fumarate, or emtricitabine pharmacokinetic data available (n=61).

1.1.6 Is a baseline eGFR ≥ 70 mL/min an appropriate criterion for initiating treatment with E/C/F/T?

The proposed criterion of baseline eGFR ≥ 70 mL/min is appropriate for initiating treatment with E/C/F/T. Using this criterion, <1% of subjects in the Phase III trials had minimum on-treatment eGFR <50 mL/min, which requires dose adjustment of tenofovir disoproxil fumarate and is not feasible for the E/C/F/T FDC.

The sponsor limited enrollment in Phase III to subjects with screening eGFR ≥ 70 mL/min due to observations from early phase development that Cobi reduces eGFR. The appropriateness of this criterion for maintaining subjects with eGFR ≥ 50 mL/min was assessed by grouping all subjects administered E/C/F/T from the two Phase III pivotal trials (GS-US-236-0102; GS-US-236-0103) according to baseline eGFR. Subjects were further grouped according to the lowest observed on-treatment eGFR. In total, assessments were available from 701 subjects, of which six subjects had a lowest on treatment eGFR <50 mL/min. Of these subjects, four had baseline eGFR <70 mL/min and none of the subjects had a baseline eGFR ≥ 90 mL/min. A greater percentage of subjects with baseline eGFR <70 mL/min had a lowest on-treatment eGFR <50 mL/min (29%, n=2/7) than subjects with baseline eGFR 70-<80 mL/min (4%, n=1/28) or eGFR 80-<90 mL/min (2%, n=1/58) supporting eGFR ≥ 70 mL/min as an appropriate criterion for initiating treatment with E/C/T/F.

Table 5: Shift Table of Baseline eGFR Versus Lowest On-Treatment eGFR for Subjects Treated with E/C/F/T from GS-US-236-0102 and GS-US-236-0103

E/C/F/T (# subjects)			Lowest On-Treatment eGFR (C-G), mL/min (n=701)					
			≥90	80–<90	70–<80	60–<70	50–<60	<50
Baseline eGFR (C-G), mL/min (n=701)	≥90	(n=606)	342	137	99	26	2	0
	80–<90	(n=58)	0	10	26	17	4	1
	70–<80	(n=28)	0	0	6	14	7	1
	60–<70*	(n=7)	0	0	0	3	2	2
	50–<60*	(n=2)	0	0	0	0	0	2
	<50*	(n=0)	0	0	0	0	0	0

*Nine patients had baseline eGFR <70 mL/min at baseline but had was screening eGFR ≥ 70 mL/min

1.1.7 Is on-treatment serum creatinine increase sensitive for identifying subjects treated with E/C/F/T who experience renal adverse events?

Confirming serum creatinine increase of ≥0.4 mg/dL from baseline (e.g., two consecutive measurements) identifies the four subjects administered E/C/F/T that developed proximal renal tubular dysfunction while limiting the number of subjects who may be prematurely discontinued from treatment.

Administration of Cobi is associated with an increase in serum creatinine. The sponsor performed an iohexol substudy which evaluated actual glomerular filtration rate (aGFR) and showed that while eGFR increased with following Cobi administration, aGFR was unchanged from baseline following administration of Cobi, ritonavir, or placebo over two weeks. Based on these findings, the sponsor hypothesizes that the decrease in eGFR (increase in serum creatinine) observed during Phase III may be attributed Cobi inhibiting tubular secretion of creatinine. However, this increase in on-treatment serum creatinine confounds evaluation of on-treatment renal function.

Multiple serum creatinine increase thresholds and scenarios were evaluated to determine criteria that would identify subjects who experienced on treatment renal adverse events while limiting the number of subjects who may be prematurely discontinued from treatment (Table 6) due to this hypothesized effect of Cobi on tubular secretion of creatinine. The metrics included changes occurring at a single treatment visit, multiple treatment visits, or multiple consecutive visits. A metric based on a single serum creatinine measure ≥0.4 mg/dL identified 81 subjects (11% of Phase III subjects administered E/C/F/T) and was deemed too insensitive for identifying subjects with renal adverse events. Increasing the metric to larger serum creatinine changes (≥0.5 or ≥0.6 mg/dL) increases sensitivity, but there were concerns that the magnitude of increase was so large that some subjects experiencing a renal adverse event may be excluded. Alternatively, using a lower metric (serum creatinine ≥0.4 mg/dL) but confirmed at consecutive clinical visits provided an appropriate balance between identifying subjects with renal adverse events and limiting the number of subjects who may be prematurely discontinued from treatment due to increases in serum creatinine from Cobi.

Table 6: Number and Percentage of Subjects Treated with E/C/F/T from GS-US-236-0102 and GS-US-236-0103 With Single, Multiple, or Consecutive Serum Creatinine Increases Exceeding Thresholds

E/C/F/T (n=701)	Serum Creatinine Increase (single measurement) (mg/dL)			Serum Creatinine Increase (multiple measurements) (mg/dL)			Serum Creatinine Increase (two consecutive measurements) (mg/dL)		
	≥0.4	≥0.5	≥0.6	≥0.3	≥0.4	≥0.5	≥0.3	≥0.4	≥0.5
	81 (11%)	27 (3.9%)	11 (1.6%)	108 (15%)	28 (4.0%)	9 (1.3%)	62 (8.9%)	17 (2.4%)	8 (1.1%)

1.2 Recommendations

1.3 Label Statements

Labeling statements to be removed are shown in ~~red strikethrough font~~ and suggested labeling to be included is shown in <u>underline blue font</u>.

2 PERTINENT REGULATORY BACKGROUND

This application contains the data in support of a new drug application for a single-tablet regimen (STR) that contains a fixed-dose combination of elvitegravir (EVG), cobicistat (Cobi), emtricitabine (FTC, Emtriva®) and tenofovir disoproxil fumarate (TDF, Viread®): the EVG/COBI/FTC/TDF (150/150/200/300 mg) tablet (referred to as E/C/F/T throughout this document).

The proposed indication for the E/C/F/T tablet is for use once daily as a complete regimen for the treatment of human immunodeficiency virus type 1 (HIV-1) infection in adults aged 18 years and over who are antiretroviral (ARV) treatment naive or have no known substitutions associated with resistance to the individual components.

EVG is a new chemical entity that belongs to the new class of HIV-1 integrase strand-transfer inhibitors (INSTIs) that prevent integration of HIV-1 genetic material into the host-cell genome. Cobicistat is a new chemical entity and structural analogue of ritonavir (RTV, r) without ARV activity. It is a more specific, mechanism-based cytochrome P450 3A (CYP3A) inhibitor than RTV that enhances or "boosts" the exposure of CYP3A substrates, including EVG.

Gilead Sciences, Inc. (Gilead) has developed EVG and COBI for use within a new 4-drug fixed-dose combination tablet (the E/C/F/T FDC) that also contains the current standard-of-care dual nucleoside/nucleotide reverse transcriptase inhibitor (NRTI/NtRTI) backbone FTC/TDF (Truvada® [TVD]).

3 RESULTS OF SPONSOR'S ANALYSIS

3.1 Introduction

The applicant developed a population pharmacokinetic model to explore the impact of intrinsic and extrinsic factors on EVG exposure. In addition, pharmacokinetic parameters were used by the applicant to explore exposure-response analyses between EVG and selected efficacy endpoints (e.g. HIV-1 plasma viral load <50 copies/mL) and key adverse events (e.g. headache, nausea, diarrhea).

3.2 Population Pharmacokinetic Model

Report 5.3.3.5 Population Pharmacokinetic Report: Population Pharmacokinetics of Cobicistat (Cobi)-Boosted Elvitegravir (EVG)

3.2.1 Data

The model was developed based on six phase 1 studies (236-0101, 236-0105, 236-0106, 236-0110, 216-0116, and 216-0123) in healthy subjects, one phase 2 study (236-0104) in

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HIV-1 infected subjects, and a 2 phase 3 studies (236-0102 and 236-0103) in HIV-1 infected subjects.

Three data sets were tested in the population analyses.

- poppkdata.csv included all PK observations with after EVG/Cobi or E/C/F/T with 100 mg and 150 mg doses of Cobi. Data from Phase 3 Study 236-0103 represented all subjects up to Week 40 as well as partial Week 48 data. It consisted of 8273 measured EVG concentrations from 580 subjects.

- intenspkwithdose150.csv included intensive PK sampling data after EVG/COBI or E/C/F/T with 150 mg doses of Cobi only (sparse sampling was included for the subset of subjects that participated in the PK Substudies in 236-0103 and 236-0104). This data set was created to test the covariate effect of Cobi AUC in subjects that received 150 mg Cobi only, the clinically relevant Cobi dose. It consisted of 4805 measurable EVG concentrations from 223 subjects.

- The final data set, finalpoppk150mg.csv included all PK observations after combination therapies with 150 mg Cobi. Data from Phase 3 Study 236-0103 represented data through Week 48. It consisted of 7783 EVG concentration data from a total of 580 subjects across 9 clinical studies.

3.2.2 Methods

Structural Model Development

Plasma concentration-time data from all individuals were pooled and analyzed simultaneously, using different estimations methods implemented in NONMEM.

Different structural PK models, including but not limited to 1-, 2-, or 3-compartment models with a 1_{st} order absorption rate constant and with or without absorption lag time parameters were tested on normal and log-transformed data. Inter-individual variability terms were included on the PK model parameters, where supported by data. Additive, proportional, and a combination of both were tested in the error model development. Furthermore, inclusion of an inter-individual variability term on the error model parameters, as well as different errors for the early and late time concentrations were tested.

Estimation methods FOCE INTER and Stochastic Approximation Expectation Maximization method (SAEM), as implemented in NONMEM were used to estimate the model parameters. However, the FOCE INTER option generally resulted in aborted model runs and hence the SAEM option was used for the main part of the modeling work.

Covariate Model Development

The following subjects' demographics data were available and obtained directly from the clinical data: age, gender, race, health status (healthy volunteers vs. HIV subject), weight, body mass index (BMI), body surface area (BSA), creatinine clearance (estimated GFR), and formulation (E/C/F/T versus EVG/COBI). Demographic data on hepatitis B and hepatitis C virus infection was available from subjects in Studies 236-0102 and 236-0103. Furthermore, COBI AUC_{tau} and C_{0h} concentrations were available in subjects who participated in intensive PK sampling periods of their studies. Missing individual covariates were replaced with median value for the whole data set.

Final Model Evaluation

The final model was evaluated using general goodness of fit graphs, where plots of population predicted values and individual predicted values versus observed concentrations were examined. Residuals versus time and residuals versus predicted concentrations were examined to determine if a model bias existed and the precision of the model parameter estimates was evaluated. Finally, a visual predictive check of expected 90% confidence interval was utilized, based on 300 simulations of the entire data set and comparison with the actual observations.

3.2.3 Results

3.2.3.1 Observed Concentration-Time Profiles

PK data in finalpoppk150mg.csv are shown in Figure 1. It depicts the EVG plasma concentrations vs. time since the last dose (TSLD) across the 9 studies. The plots demonstrated a fast absorption phase and a bi-phasic disposition profile for Cobi-boosted EVG following oral administration.

Figure 3: EVG Plasma Concentrations vs. Time Since the Last Dose (TSLD) Across the 9 Studies Used in the Analysis.
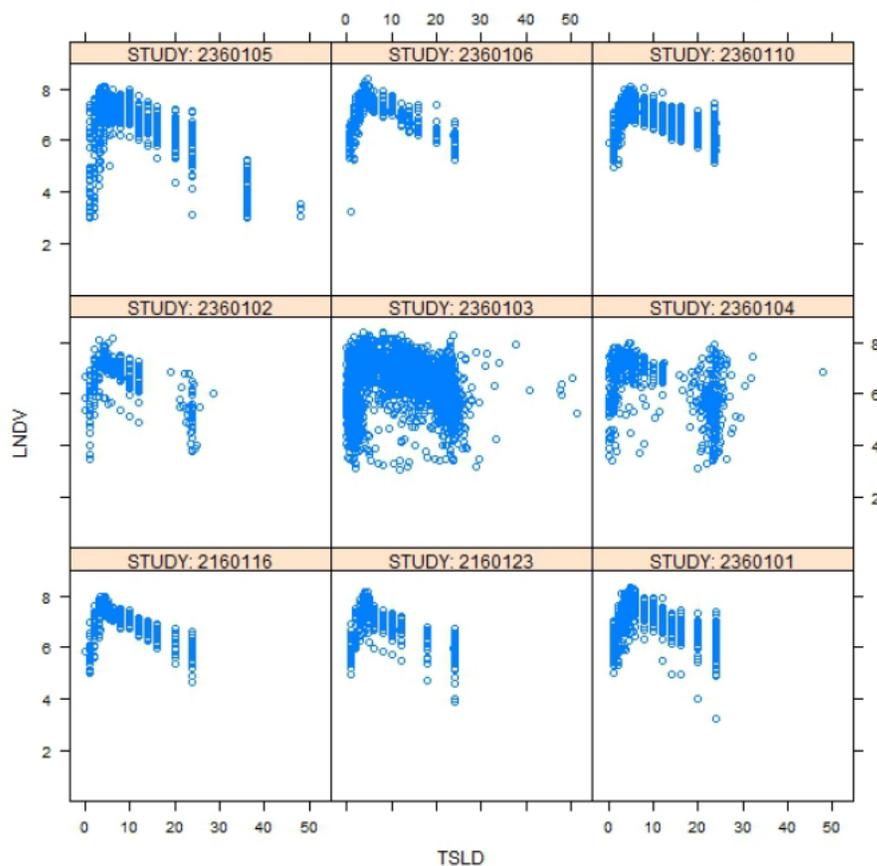
Sponsor's population PK report: report-body.pdf, pg 15

3.2.3.2 Population PK Model Results

A base model was established using a data set containing all PK observations from Studies 216-0116, 216-0123, 236-0101, 236-0102, 236-0104, 236-0105, 236-0106, and 236 0110.

The final base model was based on the log-normalized data set (finalpoppk150mg.csv) and consisted of a 2-compartment mammalian model with 1_{st} order absorption rate and an absorption lag-time. Inter-individual variability terms were included on all structural model parameters as well as the error estimate. The error model consisted of two proportional terms, one for the early time intervals (<2.5 hours) and one for the later time observations (>2.5 hours).

Cobi C_{0h} and AUC were tested for their relationship to EVG clearance. As expected, when the full dataset, including data from subjects receiving 100 and 150 mg Cobi (poppkdata.csv), was tested an effect of Cobi AUC was observed on EVG clearance. However, such an effect was not observed when evaluating data from Cobi dose of 150 mg (intenspkwithdose150.csv). Therefore, the final model is built on EVG/Cobi and E/C/F/T data with the 150 mg COBI dose only, as all clinical development of Cobi is based on a dose of 150 mg. This is in line with the clinical data, where the effect of Cobi on EVG clearance is at its maximum at the 150 mg Cobi dose level.

After addition of BSA effect on EVG clearance, model ETAs were plotted versus the covariates to visualize any residual trend between covariates and parameters. Based on these plots, the effect of Cobi AUC on EVG CL was formally tested in the model.
Inclusion of this variable and the addition of 1 extra parameter in the model resulted in an increase in the OFV by 84 points and hence Cobi AUC was not included as a covariate in the final model. No other covariates were tested in the model.

The final model was tested on EVG concentration-time data in combination with 150 mg Cobi doses (finalpoppk150mg.csv). The model described the PK profiles well. The final parameters and parameter precisions are listed in Table 7. The goodness-of-fit plots are presented in Figure 4.

Figure 4: Goodness of Fit Plots for the Applicant's Final Population PK Model
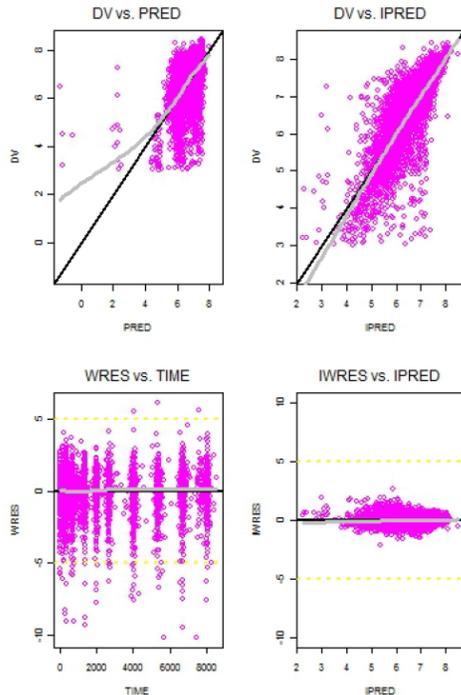
Sponsor's population PK report: report-body.pdf, pg 24

Table 7: Final Model Parameter Estimates Using finalpoppk150mg.csv

Parameter	Population Mean		IIV	
	Estimate	RSE (% CV)	Estimate	RSE (% CV)
CL/F (L/h)	6.55	2.0	31.6	8.0
CL~BSA	0.91	16.6	-	-
Vc/F (L)	12.9	6.4	49.1	22.7
Q/F (L/h)	3.58	9.6	63.3	18.5
Vp/F (L)	125	25.6	246	19.1
k_a (1/h)	0.134	3.5	22.1	25.4
t_{lag} (h)	1.55	4.3	64.8	11.1
σ1 (proportional)	30.9	6.8	65.0	8.5
σ2 (proportional) absorption phase	56.3	8.2		

Sponsor's population PK report: report-body.pdf, pg 23

While BSA was found to have a statistically significant effect on EVG clearance the effect, however, was modest and the resulting decrease in the inter-individual variability term associated with EVG clearance was 13%. Relative to the median BSA of 1.92 m^2 in the study population the range of observed BSA of 1.62 m^2 – 2.92 m^2 (5th to 95th percentile) corresponded to differences of only -15% and +18% in EVG CL/F, respectively. Such changes are not deemed to be clinically relevant. Thus, this covariate

is considered to have no clinical relevance and no dose individualization based on BSA is deemed necessary.

Reviewer's Comments: The population pharmacokinetic model development by the applicant was sufficient to describe the time course of EVG exposure. The applicant's label claims of no clinically relevant impact of gender, race, age (between 18 and 65 years), and hepatitis infection status on EVG exposure is supported by their population PK modeling results as these covariates were not found to significantly alter EVG PK during model development.

The applicant evaluated various covariate effects for significance in the course of model development. Cobi AUC was identified as a significant covariate when data from all phases of development were included. This was not unexpected as doses of Cobi <150 mg resulted in EVG exposures less than that achieved with EVG with ritonavir. Subsequent analysis using only data with EVG 150 mg and Cobi 150 mg resulted in Cobi AUC no longer being a significant predictor of EVG clearance, which supports the applicant's claim that doses of Cobi 150 mg are required for boosting EVG exposures.

Body surface area was identified as a significant covariate during model development. However, the impact on EVG clearance was modest and would not alter EVG clearance by more than 20% for subjects with high or low BSA. As such, no dose adjustments are recommended based on body surface area.

3.3 Efficacy of Elvitegravir in HIV-1 Treatment Naïve Subjects

Report 2.7 summary-clin-pharm.pdf: Clinical Pharmacology Summary

An E_{max} dose-response model for EVG using cumulative data from the monotherapy study (GS-US-183-0101) and the Phase 2 dose-ranging study (GS-US-183-105) in HIV-1 infected subjects evaluating antiviral activity as a function of steady-state trough EVG concentrations is shown in Figure 5. Mean C_{0h} values from EVG 150 mg represent the plateau range of this relationship providing near-maximal antiviral activity.

Figure 5: EVG Dose-Response Model Based on Monotherapy and Phase 2 Dose-Ranging Studies
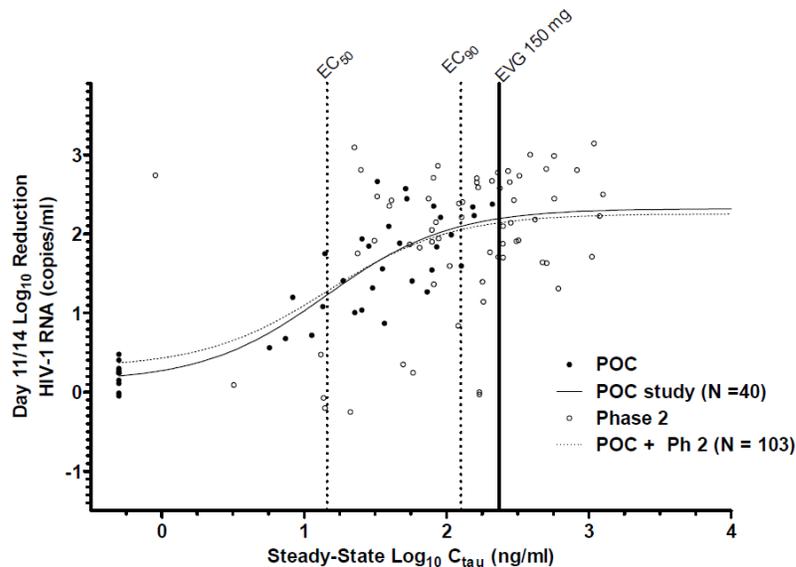
Sponsor's clin-pharm-summary.pdf, pg 210

The PK-PD analyses of EVG exposure-efficacy relationship were performed in treatment-naive HIV-1 infected subjects in Phase 3 studies using EVG exposures derived from population PK modeling versus efficacy based on HIV-1 RNA <50 copies/mL using PVR criteria by Week 48. Pure virologic response was defined as:

- Had achieved a confirmed suppression (ie, HIV-1 RNA <50 copies/mL on 2 consecutive visits) prior to or on the upper limit of the Week 48 analysis window
- Had not had a confirmed rebound (ie, HIV-1 RNA ≥50 copies/mL on 2 consecutive visits or the last available HIV-1 RNA ≥50 copies/mL during study followed by premature discontinuation of study) by the upper limit of the Week 48 analysis window after achieving confirmed suppression.

Elvitegravir PK-PD analyses using EVG C_{0h}-PVR results using different quantiles of PK exposure are presented in Table 8.

Table 8: Percentage of Pure Virologic Responders Across Quantiles of EVG Exposure (N = 373) (EVG PK/PD Analysis Set)

EVG C_{tau} Quartile (ng/mL)	PVR (%)	EVG C_{tau} Quintile (ng/mL)	PVR (%)	EVG C_{tau} Octile (ng/mL)	PVR (%)
N = 93 to 94		N = 74 to 75		N = 46 to 47	
58 to < 296	89	58 to < 264	89	58 to < 208	87
296 to < 423	88	264 to < 383	89	208 to < 296	92
423 to < 560	93	383 to < 456	87	296 to < 359	94
560 to 2341	87	456 to < 610	95	359 to < 423	83
		610 to 2341	87	423 to < 475	89
				475 to < 561	96
				561 to < 703	85
				703 to 2341	89

Pure virologic responder was not defined in Study GS-US-236-0104, so subjects (n = 46) in GS-US-236-0104 were excluded from this analysis.

Sponsor's clin-pharm-summary.pdf, pg 211

Similar results were obtained using the snapshot analysis with virologic success rates of 89% and 92% at the lowest and highest C_{0h} quartiles, respectively.

Figure 6: Percentage of Virologic Success at Week 48 (HIV-1 RNA <50 copies/mL, Snapshot Analysis) by EVG C_{0h} (ng/mL) Quartile Subgroup (EVG PK/PD Analysis Set)
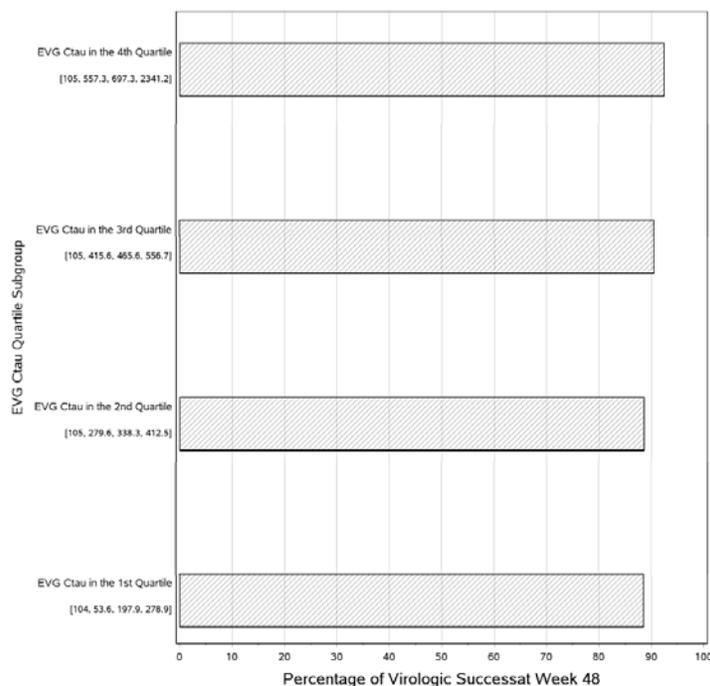
numbers presented in the square bracket were the sample size, minimum, median, and maximum of EVG C_{tau} for subjects included in that subgroup.

Sponsor's clin-pharm-summary.pdf, pg 213

Reviewer's comments: The applicant performed an exposure-response analysis of virologic response based on EVG exposures and identified no significant relationships. However, no evaluation of key subject demographics or factors was performed by the sponsor. The reviewer's analysis did not identify race, gender, or age as significant predictors of virologic success. Subjects with higher baseline viral load trended towards lower likelihood of virologic success, but these parameters were not significant during GAM analysis.

## 3.4 Safety of EVG and Cobi in HIV-1 Treatment Naïve Subjects

Report 2.7 summary-clin-pharm.pdf: Clinical Pharmacology Summary

The applicant performed binned analyses of adverse events versus EVG and Cobi.
Elvitegravir
Pharmacokinetic-pharmacodynamic analyses of the EVG exposure-safety relationship were performed in treatment-naive HIV-1 infected subjects in the E/C/F/T STR Phase 2 (GS-US-236-0104) and Phase 3 (GS-US-236-0102 and GS-US-236-0103) studies using EVG exposures derived from population PK modeling (n=419). The relationships between EVG exposures (AUC_{tau} or C_{max}) and incidence of adverse events (present/absent) are shown Figure 7. EVG exposures were comparable in subjects who experienced a key adverse event and those who did not.

Figure 7: Boxplot of EVG AUC_{tau} (ng•h/mL) Versus Incidence of Selected Adverse Events (EVG PK/PD Analysis Set)
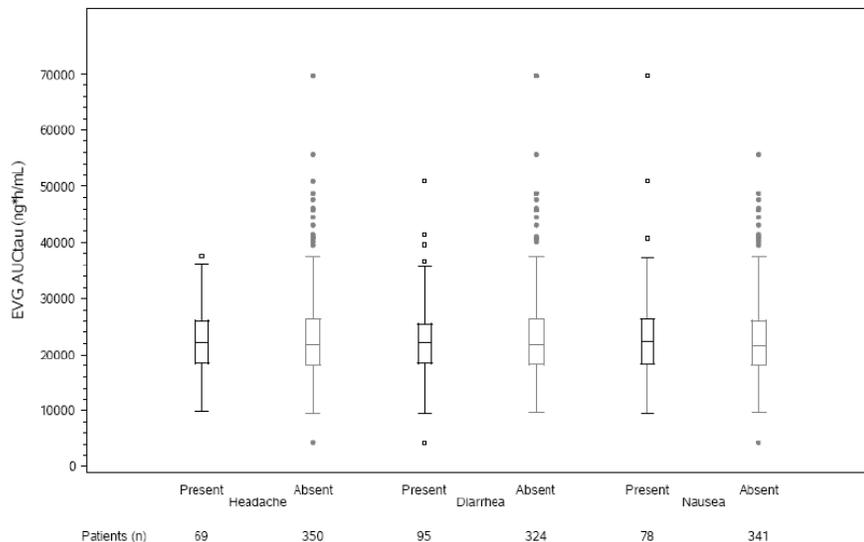
Sponsor's clin-pharm-summary.pdf, pg 219

Reviewer's comments: No relationship between the most common adverse events and EVG exposure were observed for headache, diarrhea, or nausea based on the applicant's analysis. Similar results were obtained during the reviewer's analysis.

Cobicistat
Following Cobi dosing, mean (SD) estimated glomerular filtration rate based on the Cockcroft-Gault equation ($eGFR_{CG}$) values decreased 9.9 (13.14) mL/min and

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11.9 (6.97) mL/min relative to baseline following 7 days of dosing in subjects with baseline eGFR$_{CG}$ ≥80 mL/min and 50 to 79 mL/min, respectively, but were reversible and returned to baseline values following a 7-day washout period (GS-US-216-0121). The time to onset, magnitude, and time to resolution of the observed changes in eGFR$_{CG}$ are consistent with the inhibition of tubular secretion of creatinine by Cobi, with the relative contribution of secretion to total clearance increasing with a decline in renal function. In contrast, glomerular filtration probe agent iohexol-based GFR was unchanged on Days 7 and 14 relative to Day 0 following Cobi dosing. Similar results were obtained using cysGFR.

Pharmacokinetic-pharmacodynamic analyses of the Cobi exposure-safety relationship were performed in treatment-naive HIV-1 infected subjects in the E/C/F/T STR Phase 2 (GS-US-236-0104) and Phase 3 (GS-US-236-0102 and GS-US-236-0103) studies using Cobi exposures derived from intensive PK substudies versus safety parameters that included commonly observed adverse events, namely headache, nausea, or diarrhea (Figure 8). Similar Cobi exposures were observed between those subjects experiencing a key adverse event during the studies and those who did not.

Figure 8: Boxplot of Cobi AUC$_{tau}$ (ng•h/mL) Versus Incidence of Selected Adverse Events (COBI PK/PD Analysis Set)
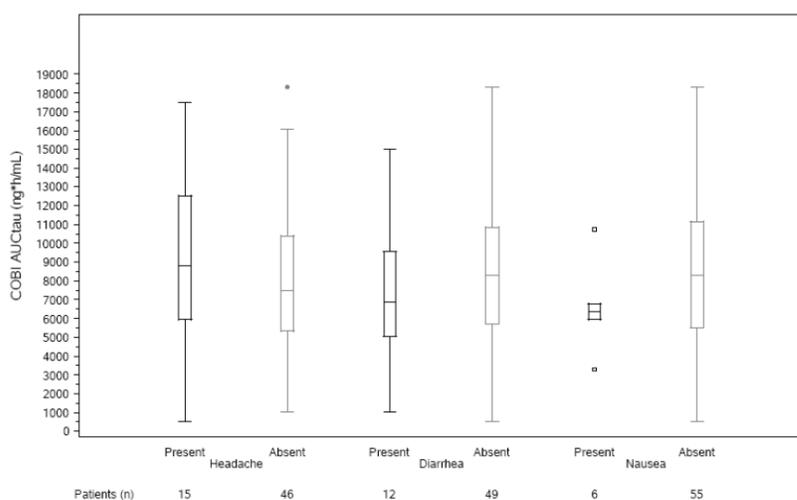
Sponsor's clin-pharm-summary.pdf, pg 224

A similar analysis was performed with respect to Cobi AUC$_{tau}$ quartiles and maximum decrease in eGFR$_{CG}$ (Figure 9). A non-significant trend of decreasing eGFR$_{CG}$ with increasing Cobi AUC$_{tau}$ was observed, though this analysis is limited by the number of subjects with Cobi exposure data available.

Figure 9: Boxplot of Maximum Decrease from Baseline in eGFR$_{CG}$ (mL/min) by Cobi AUC$_{tau}$ (ng•h/mL) (COBI PK/PD Analysis Set)
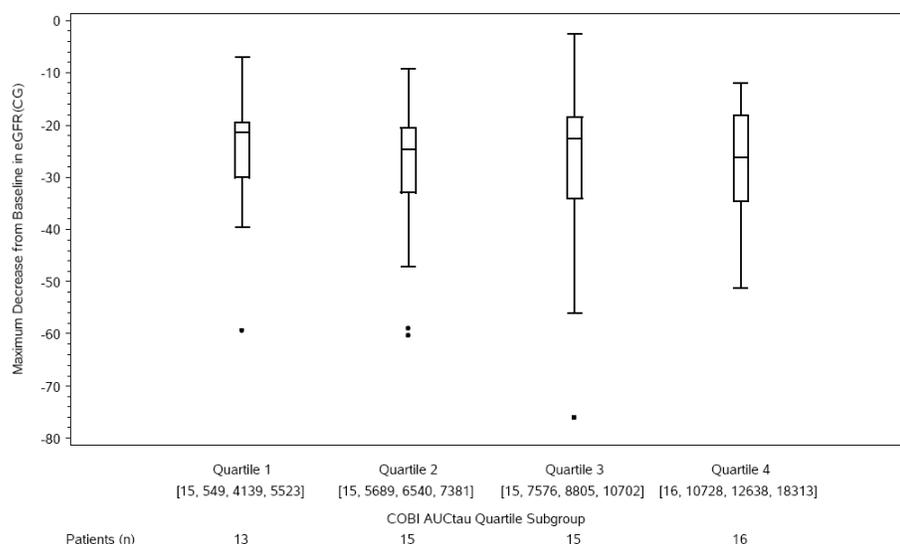
Sponsor's clin-pharm-summary.pdf, pg 227

Reviewer's comments: No relationship between the most common adverse events and Cobi exposure were observed for headache, diarrhea, or nausea based on the applicant's analysis.

The sponsor presented two analyses to support the hypothesis that increases in serum creatinine were due to inhibition of tubular secretion of creatinine. The first analysis was a dedicated iohexol which is described in detail in the Clinical Pharmacology Review above. The sponsor's second analysis evaluated changes in eGFR$_{CG}$ based on Cobi exposures. There was substantial overlap between subjects in all exposure quartiles, but there was a trend of greater decrease in eGFR$_{CG}$ in subjects with higher Cobi exposure. This analysis is limited due to the number of subjects with Cobi PK data available (n=61). The reviewer further evaluated the relationship between baseline eGFR$_{CG}$ and changes in baseline eGFR$_{CG}$ which demonstrated that subjects with higher subject function also had the largest decreases in eGFR$_{CG}$ from baseline. This supports the sponsor's hypothesis of Cobi inhibition of tubular secretion of creatinine increasing serum creatinine.

An additional observation of increased renal adverse events in subjects receiving E/C/F/T treatment versus comparator arms was observed from the Phase III studies. No exposure-response relationship regarding renal adverse events between exposures for EVG, Cobi, TDF, or FTC could be identified from the available data. However, this analysis is limited by the small number of events and the limited number of subjects with Cobi and TDF data available.

4 REVIEWER'S ANALYSIS

4.1 Introduction

The aim of this review to examine whether the label claims and proposed dose are justified by the existing PK, efficacy, and safety data using both population PK and exposure-response analyses.

4.2 Objectives

Analysis objectives are:

1. Determine if the label claims regarding elvitegravir (EVG) population PK parameters and covariates are accurate

2. Determine the impact of EVG and cobicistat (Cobi) exposure on common adverse events

4.3 Methods

4.3.1 Data Sets

Data sets used are summarized in Table 9.

Table 9. Analysis Data Sets

Study Number	Name	Link to EDR
PK-PD	adpkpd.xpt	\\Cdsesub1\evsprod\NDA203100\0000\m5\datasets\pk-pd\analysis\adam\datasets
Pop-PK	Finalpoppk150mg.xpt, intensepkwithdose150.xpt, poppkdata.xpt	\\Cdsesub1\evsprod\NDA203100\0000\m5\datasets\pop-pk\analysis\legacy\datasets
Pop-PK	Run1.ctl.txt, run41.ctl.txt	\\Cdsesub1\evsprod\NDA203100\0000\m5\datasets\pop-pk\analysis\legacy\programs

4.3.2 Software

Estimation and simulation were performed NONMEM 7.2 on the Pharmacometrics Group Linux cluster using the front end manager Perl Speaks NONMEM (PsN). Diagnostic graphs, model comparison, and statistical analysis were performed in R (version 10.1).

4.3.3 Models

4.3.3.1 Population Pharmacokinetics

Reevaluation of the sponsor's population PK model resulted in the same structure identified by the sponsor as described in Section 3.

4.3.3.2 Logistic Regression: Safety Exposure-Response Relationships

Logistic regression models for common adverse events were performed using the applicant's Phase III trial data. Three independent variables were used for developing logistic regression plots: steady-state AUC (AUC_τ), maximum concentration (C_{max}), and trough concentration (C_{0h}). AUC_τ and C_{0h} were calculated for each subject using empirical Bayes' estimates from the population PK model. C_{max} was obtained from subjects with intensive PK sampling during Phase III.

4.4 Results

Impact of Renal Impairment on EVG and Cobi Exposure

Estimated glomerular filtration rate (eGFR) was calculated from the Phase III subjects using the Cockcroft-Gault equation and available subject covariate data. Of the Phase III subjects with Cobi exposure data available, 18 subjects had calculated eGFR ranging from 60-<90 mL/min (mild renal impairment) and 42 subjects had eGFR >90 mL/min. Of the Phase II/III subjects with EVG exposure data available, 5 had calculated eGFR ranging from 30-<60 mL/min (moderate renal impairment), 136 subjects had calculated eGFR ranging from 60-<90 mL/min (mild renal impairment), and 339 subjects had eGFR >90 mL/min. No clinically significant differences in Cobi or EVG exposure were observed between subjects with normal or mild renal impairment (Table 10 and Table 11). The limited number of subjects with moderate renal impairment precluded a definitive comparison from being made regarding whether the EVG exposure in this group differs from subjects with normal renal function or subjects with mild renal impairment. However, E/C/F/T will not be administered to subjects with eGFR <70 mL/min. No subjects with severe renal impairment or end-stage renal disease were included in the Phase III trials.

| Table 10: Non-compartmental Pharmacokinetic Estimates of Cobicistat in Antiretroviral Treatment Naïve HIV-1-Infected Subjects (Comparison of Subjects with eGFR ≥ 90 mL/min and 60< eGFR <90 mL/min)		
Cobi PK	60 mL/min < eGFR <=90 mL/min	eGFR > 90 mL/min
Number of subjects	18	42
AUC, mean (SD) (range)	8889 (4177)	7910 (3614)
	(3961; 18313)	(549; 16058)
Cmax, mean (SD) (range)	1160 (394)	1120 (420)
	(510; 1809)	(103; 2141)
C_{0h}, mean (SD) (range)	43 (58)	50 (154)

Table 11: Population Pharmacokinetic Estimates of EVG in Antiretroviral Treatment Naïve HIV-1-Infected Subjects (Comparison of Subjects with eGFR ≥ 90 mL/min, 60< eGFR <90 mL/min, and 30< eGFR <60 mL/min)

EVG PK	30 mL/min < eGFR <=60 mL/min	60 mL/min < eGFR <=90 mL/min	eGFR > 90 mL/min
Number of subjects	5	136	339
AUC, mean (SD) (range)	17471 (3715)	22922 (8344)	22489 (7349)
	(12911; 22404)	(7667; 69754)	(2828; 55684)
Cmax, mean (SD) (range)	1417 (236)	1762 (524)	1740 (403)
	(1124; 1768)	(729; 3981)	(377; 3386)
C_{0h}, mean (SD) (range)	291 (118)	451 (275)	423 (258)
	(194; 424)	(45; 2341)	(46; 1601)

4.4.1 Exposure-Response for Safety: Other Adverse Events

Logistic regression models were evaluated for EVG C_{0h}, C_{max}, and AUC_τ with no significant relationships identified. Modeling results for adverse event rates versus EVG AUC_τ (Figure 10) indicate no significant relationship.

Figure 10: Percentage of Subjects with Headache (top left), Diarrhea (top right), and Nausea (bottom left) Adverse Events Versus EVG AUC_τ for All Treatment Naïve Subjects with PK Sampling (n = 373)
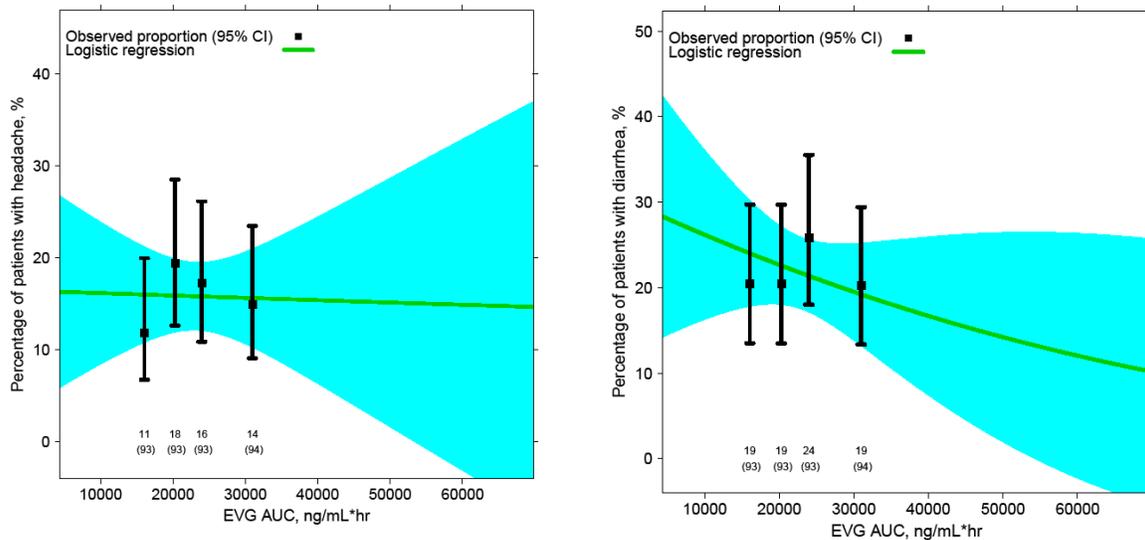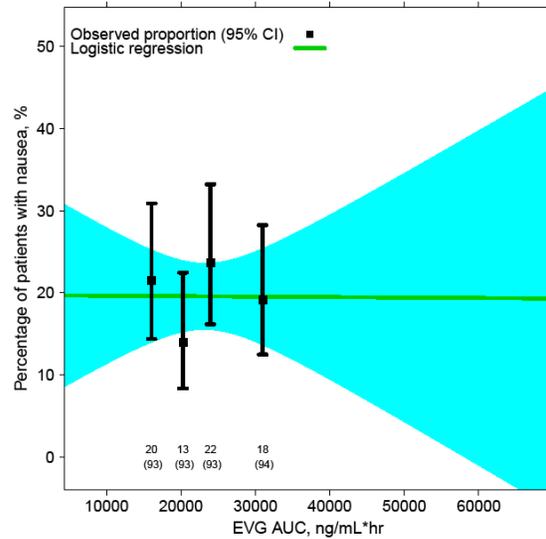
5 LISTING OF ANALYSES CODES AND OUTPUT FILES

File Name	Description	Location in \\cdsnas\pharmacometrics\
ER_Analyses.R	Exposure-response analysis for efficacy and safety for EVG, Cobi, TDF, and FTC	Reviews\Ongoing PM Reviews\Elvitegravir_Cobicistat_NDA203100_JAF\ER Analyses
Creatinie_TimeCourse.R	Generate serum creatinine and eGFR timecourse plots from Phase III subjects	Reviews\Ongoing PM Reviews\Elvitegravir_Cobicistat_NDA203100_JAF\ER Analyses
Renal_Function_TimeCourse.R	Shift table analysis of Phase III subjects for protein and glucose in the urine	Reviews\Ongoing PM Reviews\Elvitegravir_Cobicistat_NDA203100_JAF\ER Analyses

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6   APPENDIX

6.1   Shift Tables Using Body Surface Area Normalized MDRD

In addition to the eGFR assessment performed in Section 1, a sensitivity analysis was performed by the reviewer evaluating shift in patient estimated glomerular filtration rate based on the MDRD equation and corrected for individual body surface area ($MDRD_{ind}$). As noted previously, the sponsor limited enrollment in Phase III to subjects with screening eGFR $\geq$ 70 mL/min due to observations from early phase development that Cobi reduces eGFR. Similar eGFR results to those presented in Question 1.1.6 were obtained using $MDRD_{ind}$.

As before, all subjects administered E/C/F/T from the two Phase III pivotal trials (GS-US-236-0102; GS-US-236-0103) were grouped according to baseline eGFR. Subjects were further grouped according to the lowest observed on-treatment eGFR. Eleven subjects had a lowest on treatment eGFR ($MDRD_{ind}$) <50 mL/min. Of these subjects, four had baseline eGFR ($MDRD_{ind}$) <70 mL/min and one of the subjects had a baseline eGFR ($MDRD_{ind}$) $\geq$ 90 mL/min. A greater percentage of subjects with baseline eGFR ($MDRD_{ind}$) <70 mL/min had a lowest on-treatment eGFR ($MDRD_{ind}$) <50 mL/min (25%, n=2/8) than subjects with baseline eGFR ($MDRD_{ind}$) 70-<80 mL/min (17%, n=5/30) or eGFR ($MDRD_{ind}$) 80-<90 mL/min (2%, n=1/59).

Table 12: Shift Table of Baseline eGFR ($MDRD_{ind}$) Versus Lowest On-Treatment eGFR ($MDRD_{ind}$) for Subjects Treated with E/C/F/T from GS-US-236-0102 and GS-US-236-0103

E/C/F/T (# subjects)			Minimum On-Treatment eGFR ($MDRD_{ind}$), mL/min (n=701)					
			$\geq$90	80–<90	70–<80	60–<70	50–<60	<50
Baseline eGFR ($MDRD_{ind}$), mL/min (n=701)	$\geq$90	(n=602)	257	154	150	37	3	1
	80–<90	(n=59)	0	6	20	23	9	1
	70–<80	(n=30)	0	0	4	13	8	5
	60–<70*	(n=8)	0	0	0	4	2	2
	50–<60*	(n=1)	0	0	0	0	0	1
	<50*	(n=1)	0	0	0	0	0	1

*Ten patients had baseline eGFR (MDRDind) <70 mL/min at baseline but had was screening eGFR $\geq$ 70 mL/min

4.3 OCP FILING FORM

4 Page(s) has been Withheld in Full immediately following this page as duplicate copy of OCP Filing & Review Form 12.23.11

Application No.:	NDA 203-100	**Reviewer:** Deepika Arora Lakhani, PhD	
Submission Date:	Oct 27, 2011 Jan 20, 2012 Apr 27, 2012 June 13, 2012 June 20, 2012		
Division:	Division of Antiviral Products	**Team Leader (acting):** Sandra Suarez Sharp, PhD	
Sponsor:	Gilead Sciences, Inc.	**Biopharmaceutics Supervisor (Acting):** Richard Lostritto, PhD	
Trade Name:	-Stribild- (under review)	**Date Assigned:**	Nov 1, 2011
Generic Name:	Elvitegravir, Cobicistat, Emtricitabine and Tenofovir Disoproxil Fumarate Tablets	**Date of Review:**	June 27, 2012
Indication:	HIV Infection	**Type of Submission:** New Drug Application 505b(1)	
Formulation/ strengths	IR Tablet; 150mg EVG/150mg COBI/200mg FTC/300mgTDF		
Route of Administration	Oral		

SUMMARY OF BIOPHARMACEUTICS FINDINGS:

The submission is a 505(b)(1) application for a film coated single tablet regimen (STR) that contains the active substances elvitegravir (EVG), cobicistat (COBI), emtricitabine (FTC), and tenofovir disoproxil fumarate (tenofovir DF, TDF). The EVG/COBI/FTC/TDF tablet is an immediate-release tablet containing EVG 150 mg, COBI 150 mg, FTC 200 mg and TDF 300 mg. EVG/COBI/FTC/TDF tablets are bilayer tablets with EVG and COBI in one layer referred to as the EVG/COBI layer (Layer 1) and the other layer contains FTC and TDF and is referred to as the FTC/TDF layer (Layer 2).

Elvitegravir (EVG, GS-9137, JTK-303) and Cobicistat (COBI, GS-9350) are agents of the integrase strand transfer inhibitor (INSTI) class and cytochrome P450 3A (CYP3A) inhibitor class, respectively, developed by Gilead for treatment of HIV infection. Emtricitabine and TDF are also approved for the treatment of HIV-1 infection in a fixed-dose combination (FDC) product Truvada® ([FTC/TDF]; NDA 21-752 for tablet formulation approved on 02 August 2004). Emtricitabine and TDF are approved in FDC products with efavirenz (EFV) and rilpivirine (RPV), both nonnucleoside reverse transcriptase inhibitors (NNRTI). These FDC products are Atripla® (efavirenz/emtricitabine/tenofovir DF [EFV/FTC/TDF]; approved for use in the US on 12 July 2006), and Complera™ (emtricitabine/rilpivirine/tenofovir DF [FTC/RPV/TDF]; approved for use in the US on 10 August 2011).

Some aspects of the product and process development of EVG/COBI/FTC/TDF tablet were conducted under a Quality by Design (QbD) paradigm to ensure desired product performance in terms of quality, safety, and efficacy.

This review focuses on: a) the acceptability of the dissolution method and acceptance criteria; b) the role of dissolution as a response parameter in design of experiments for optimization of EVG ^{(b) (4)} to eventually aid the construction of design space for the same; c) the role of dissolution as a response parameter in selection of tablet ^{(b) (4)} and; d) the role of dissolution in selection of a

Product Quality Review

NDA 203-100   **BIOPHARMACEUTICS NDA REVIEW**   2
Stribild (name under review) Tablet
Gilead Sciences Inc.

small design space for film coat [(b) (4)]

a) Dissolution Method and Acceptance Criteria:

A single combined method to assay the dissolution of EVG/COBI/FTC/TDF film-coated tablets was developed. The following acceptance criteria was recommended by the Agency and accepted by the Applicant on 11-June-2012.

Drug Name	Dosage Form	USP Apparatus	Speed (rpm)	Medium	Volume (mL)	Acceptance criteria
EVG, COBI, FTC, TDF	Tablet	II (paddle)	100	0.01 N HCl containing 2% w/w polysorbate 80 with helical stainless steel sinker	1000 mL, 37 °C	EVG, FTC, TDF: NLT [(b) (4)] (Q) dissolved at 30 minutes COBI: NLT [(b) (4)] (Q) dissolved at 10 minutes

The robustness of the dissolution method was evaluated by assessing the effect of small, deliberate changes to the surfactant (polysorbate 80) concentration and pH of the dissolution medium. The discriminating capacity of the dissolution method was evaluated [(b) (4)] [(b) (4)] The proposed dissolution method discriminates for formulation composition and EVG API particle size and has been deemed acceptable.

b) Dissolution as a response parameter in design of experiments to aid the construction of design space for EVG [(b) (4)]

The design space justification for EVG [(b) (4)] was based on evaluating [(b) (4)] and by providing the dissolution profiles for all DoE runs. During the review cycle, the Applicant was contacted to support the design space by providing the f2 comparison with respect to EVG [(b) (4)] design space. Data supported that the dissolution profiles of all batches defining the EVG [(b) (4)] design space met the f2 criteria when compared to the reference formulation, with the exception of one batch that does not meet the criterion for TDF dissolution, but is nearly similar with an f2 value of [(b) (4)]. Therefore, the proposed ranges [(b) (4)] [(b) (4)] are acceptable from Biopharmaceutics perspective.

c) Dissolution as a response parameter in the selection of tablet [(b) (4)]

The final tablet hardness ([(b) (4)] specification) is proposed [(b) (4)] The Applicant was contacted to support the proposed ranges by conducting f2 testing [(b) (4)] [(b) (4)] From the data provided, the reviewer recommends [(b) (4)] the proposed Layer 1 [(b) (4)] range [(b) (4)]

d) Dissolution as a response parameter for design space for film coat [(b) (4)]: The provided dissolution profile comparisons with f2 testing remain unchanged within the proposed small design space. The proposed range for film coat [(b) (4)] is deemed acceptable.

Product Quality Review

NDA 203-100   **BIOPHARMACEUTICS NDA REVIEW**   3
Stribild (name under review) Tablet
Gilead Sciences Inc.

RECOMMENDATION:

The ONDQA/Biopharmaceutics team has reviewed NDA 203-100 and its amendments submitted on Oct 27, 2011, Jan 20, 2012; Apr 27, 2012; June 13, 2012; and June 20, 2012. From Biopharmaceutics perspective NDA 203-100 for Stribild is recommended for approval.

The following dissolution method for the EVG/COBI/FTC/TDF is deemed acceptable:

Apparatus II , 2% Polysorbate 80, 0.01 N HCl, 1000 mL, 100 rpm

The following dissolution acceptance criteria has been recommended (and agreed by the Applicant, refer to submission dated 20-JUNE-2012) for the four APIs:

       EVG: Q= (b) (4) **at 30 mins**
      COBI: Q= (b) (4) **at 10 mins**
       FTC: Q= (b) (4) **at 30 mins**
       TDF: Q= (b) (4) **at 30 mins**

Comments to the CMC Reviewer

The following design space or target ranges proposed by the Applicant and recommended by the reviewer are provided below for each parameter:

- EVG   (b) (4) : The proposed design space is ACCEPTABLE
- Layer 1   (b) (4) range   (b) (4) : NOT ACCEPTABLE Recommend to be changed   (b) (4)
- The Applicant has not provided dissolution profiles for tablets prepared within the FTC/TDF   (b) (4) proposed ranges. The FTC/TDF   (b) (4) were manufactured   (b) (4) The Biopharmaceutics reviewer requests the CMC reviewer to evaluate the adequateness of the proposed   (b) (4) ranges.
- Design space for film coat   (b) (4) : ACCEPTABLE
- Recommend   (b) (4) the EVG particle size specification   (b) (4)

Deepika Arora Lakhani, PhD   Sandra Suarez Sharp, PhD
Biopharmaceutics Reviewer   Biopharmaceutics Team Leader (Acting)
Office of New Drugs Quality Assessment   Office of New Drugs Quality Assessment

cc. *on file; RLostritto; ADorantes*

INTRODUCTION

Elvitegravir (EVG, GS-9137, JTK-303) and Cobicistat (COBI, GS-9350) are agents of the integrase strand transfer inhibitor (INSTI) class and cytochrome P450 3A (CYP3A) inhibitor class, respectively, developed by Gilead for treatment of HIV infection. Emtricitabine (FTC) and tenofovir disoproxil fumarate (TDF). TDF are also approved for the treatment of HIV-1 infection in a fixed-dose combination (FDC) product Truvada® ([FTC/TDF]; NDA 21-752 for tablet formulation approved on 02 August 2004). The EVG/COBI/FTC/TDF tablet is an immediate-release tablet containing EVG 150 mg, COBI 150 mg, FTC 200 mg and TDF 300 mg. EVG/COBI/FTC/TDF tablets are bilayer tablets with EVG and COBI in one layer referred to as the EVG/COBI layer and the other layer contains FTC and TDF and is referred to as the FTC/TDF layer.

Bilayer Configuration

**Layer 2**
FTC/TDF: 652 mg

Layer 1
EVG/COBI: 698 mg
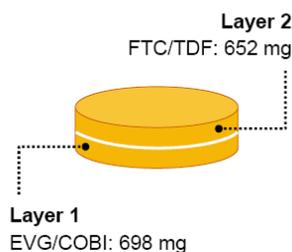
Drug Substances

Elvitegravir: EVG (Figure 1) is considered a BCS Class 2 drug with an intrinsic solubility of less than 0.001 mg/mL. Within the physiological pH range (1.2 to 6.8), the solubility of EVG is too low to satisfy the requirements for sink conditions and remains unaffected with pH.
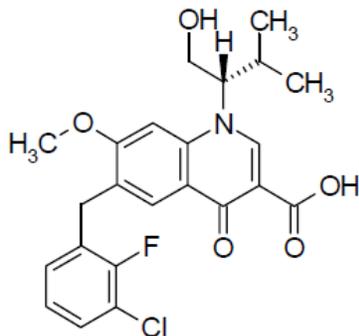

Figure 1. Chemical Structure of Elvitegravir

Product Quality Review

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Stribild (name under review) Tablet
Gilead Sciences Inc.

Cobicistat: COBI (Figure 2) is considered a BCS Class 2 drug due to the lower solubility at higher physiological pHs. COBI has three dissociation constants at 1.8, 2.5, and 6.4 and solubility is affected by pH.
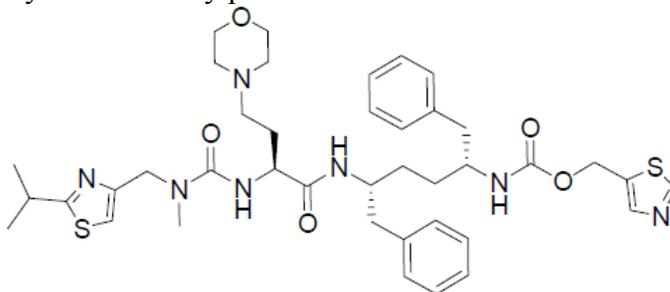

Figure 2. Chemical Structure of Cobicistat

The aqueous solubility of COBI (Figure 3) is low at basic pH (less than 1 mg/mL at pH >5) and is significantly enhanced under acidic conditions (over 70 mg/mL at pH 2.2).
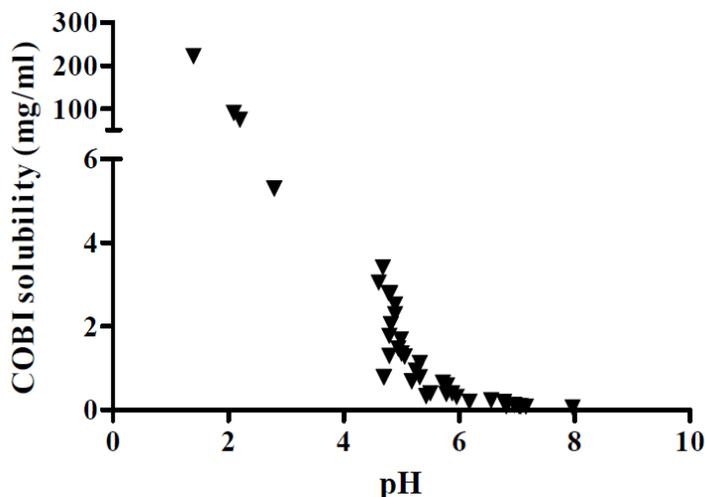
Figure 3. Aqueous Solubility-pH Profile of COBI (free base) at Room Temperature

Emtricitabine: FTC (Figure 4) is considered a BCS Class 1 drug. FTC has an intrinsic solubility of 119 mg/mL. The pKa of FTC is 2.65. The dose solubility volume for a 200 mg dose of FTC is less than 2 mL.
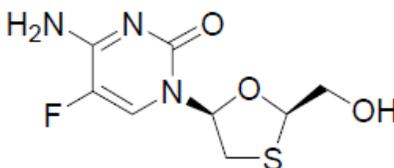

Figure 4. Chemical Structure of Emtricitabine

Tenofovir DF: TDF (Figure 5) is considered a BCS Class 3 drug with high solubility and low permeability. TDF is highly soluble, and sink conditions can be achieved across the physiological pH range of 1.2 to 6.8. The pKa of TDF is 3.75.
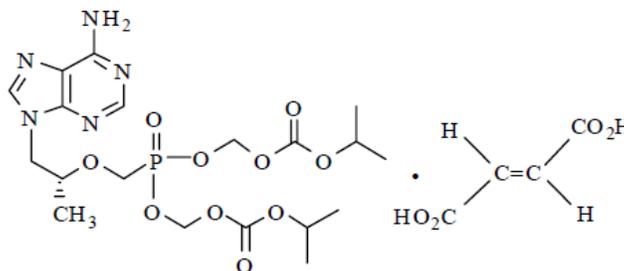
Figure 5. Chemical Structure of Tenofovir DF

The solubility of TDF is affected by pH as shown in Figure 6.
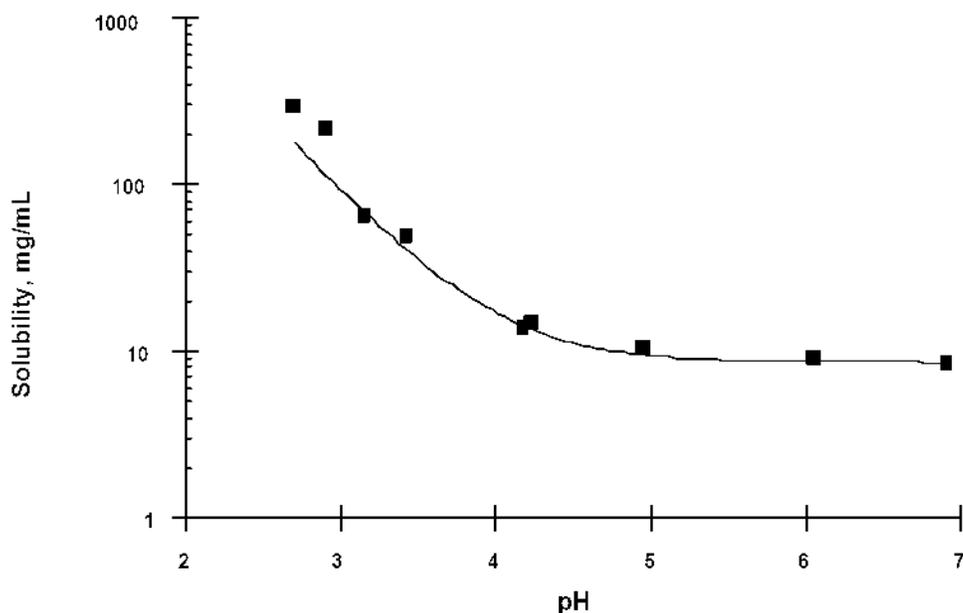
Figure 6. Aqueous Solubility-pH Profile of TDF at Room Temperature

Drug Product

EVG/COBI/FTC/TDF tablets are formulated for immediate release of the active ingredients for administration by the oral route, and contain 150 mg EVG, 150 mg COBI, 200 mg FTC, and 300 mg TDF in a bilayer tablet. The composition of the tablets is provided in Table 1.

Product Quality Review

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Stribild (name under review) Tablet
Gilead Sciences Inc.

Table 1. Composition of EVG/COBI/FTC/TDF Tablets

Components	% w/w	Unit Formula (mg/unit)	Quality Standard	Function
Elvitegravir	(b) (4)	150.0 [a]	In-House	Active
Cobicistat on Silicon Dioxide		(b) (4)	In-House	Active
Emtricitabine		200.0 [b]	In-House	Active
Tenofovir Disoproxil Fumarate		300.0 [b,d]	In-House	Active
Hydroxypropyl Cellulose		(b) (4)	NF, Ph. Eur.	(b) (4)
Sodium Lauryl Sulfate			NF, Ph. Eur., JP	
Silicon Dioxide			In-House (GSPEC-191-00) or NF, Ph. Eur.	
Lactose Monohydrate			NF, Ph. Eur., JP	
Microcrystalline Cellulose			NF, Ph. Eur., JP	
Croscarmellose Sodium			NF, Ph. Eur., JP	
Magnesium Stearate	(b) (4		NF, Ph. Eur., JP	
			USP, Ph. Eur.	
Film Coat	(b) (4)			
			In-House (GSPEC-183-00)	
			USP, Ph. Eur.	(b) (4)

Formulation Development

The formulations manufactured throughout the clinical development of the proposed product are summarized in Table 2.

Stribild (name under review) Tablet
Gilead Sciences Inc.
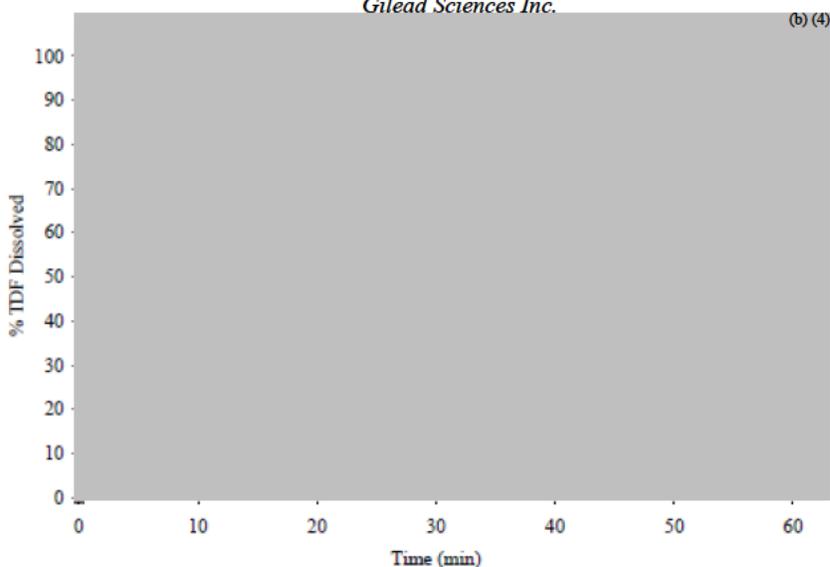
2% w/v polysorbate 80 in 0.01 N HCl (pH 2.0), 1000 mL, (b)(4) rpm paddle speed, and spiral sinkers

Figure 36. Effect of Hardness on the Dissolution of TDF from Film-coated
EVG/COBI/FTC/TDF Tablets

EVG and COBI showed no effect of final tablet hardness on dissolution. For FTC and
TDF, ⬚(b)(4)⬚ rate of dissolution at the 10 and 20 minute
time points was observed in tablets ⬚(b)(4)⬚
⬚ by 30 minutes. This ⬚(b)(4)⬚ dissolution performance is expected for FTC and
TDF due to their high aqueous solubility.

Reviewer's Conclusions for Discriminating Power of the Dissolution Method
The proposed dissolution method is acceptable since: 1) it is able to discriminate for
EVG particle size and 2) it is able to discriminate for tablets ⬚(b)(4)⬚
(FTC and TDF).
It must be noted that the proposed method failed to establish a discriminating method for
the API COBI. Nevertheless, the reviewer is deeming this method to be adequate as COBI
is combined with EVG in the first layer. Since the method is sensitive towards EVG, it is
expected that any aberrant manufacturing changes that can potentially impact the quality
of this layer will be picked up by EVG dissolution.

DISSOLUTION ACCEPTANCE CRITERIA
The following dissolution acceptance criteria was originally proposed by the Applicant as
a QC for the release of EVG/COBI/FTC/TDF IR, tablets:

Dissolution Acceptance Criteria
EVG, FTC and TDF Q= (b)(4) at 30 mins
COBI Q= (b)(4) at 30 mins

Further in a Quality Amendment, dated 20-APR-2012 that included the proposal to increase the paddle speed to 100 rpm, the Applicant had proposed to increase the FTC and TDF dissolution acceptance criterion to be Q= (b) (4) at 30 mins. According to the Applicant, this criterion is being proposed since the clinical batches dissolution data supports this (see discussion below about this issue).

Reviewer's Recommended Dissolution Acceptance Criteria
The following dissolution acceptance criteria is recommended as a QC for the release of EVG/COBI/FTC/TDF IR, tablets:

Dissolution Acceptance Criteria
EVG, FTC, TDF Q= (b) (4) at 30 min
COBI Q= (b) (4) at 10 min

The dissolution acceptance criteria of Q= (b) (4) in 30 min for EVG, FTC and TDF and of Q= (b) (4) at 10 mins for COBI was established based on the following information:

- Mean dissolution values from the clinical drug product release and the drug product stability testing

Dissolution Profiles from the Clinical and Stability Batches
Figures 37-40 show the mean dissolution profiles for about 10 batches, including clinical, commercial, and stability batches. The plot indicates that an acceptance criteria of Q= (b) (4) at 30 min is appropriate for EVG, FTC and TDF since all the mean values at 30 min were higher than (b) (4) and Q= (b) (4) at 10 min is appropriate for COBI since all the mean values at 10 min were higher than (b) (4). Please note the discussion regarding the two BA study batches (b) (4) for FTC and TDF (b) (4)

Product Quality Review

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Stribild (name under review) Tablet
Gilead Sciences Inc.
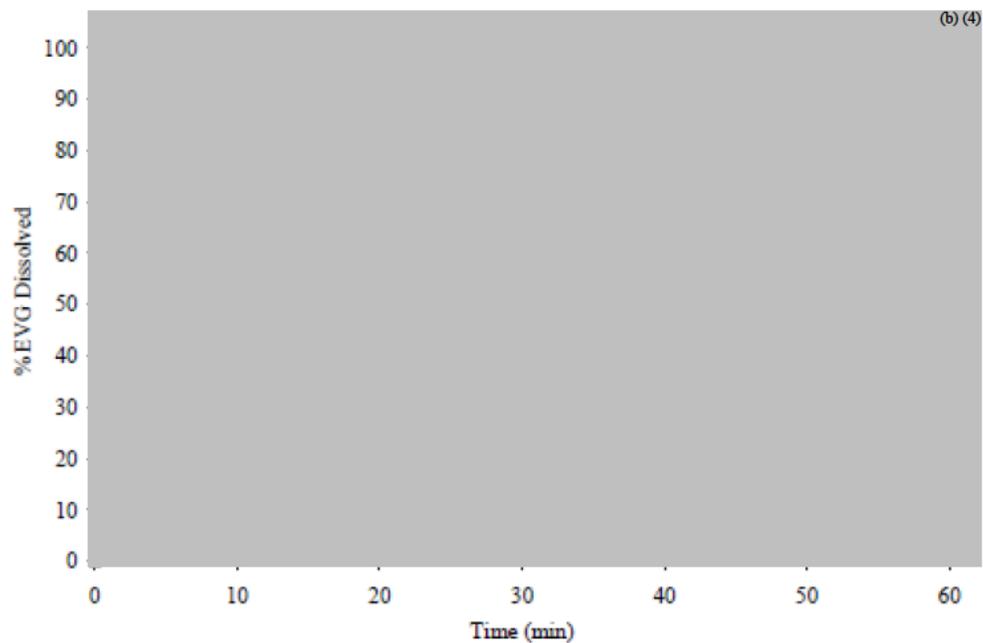
Figure 37. Dissolution Profiles of EVG from Clinical Lots of EVG/COBI/FTC/TDF Tablets
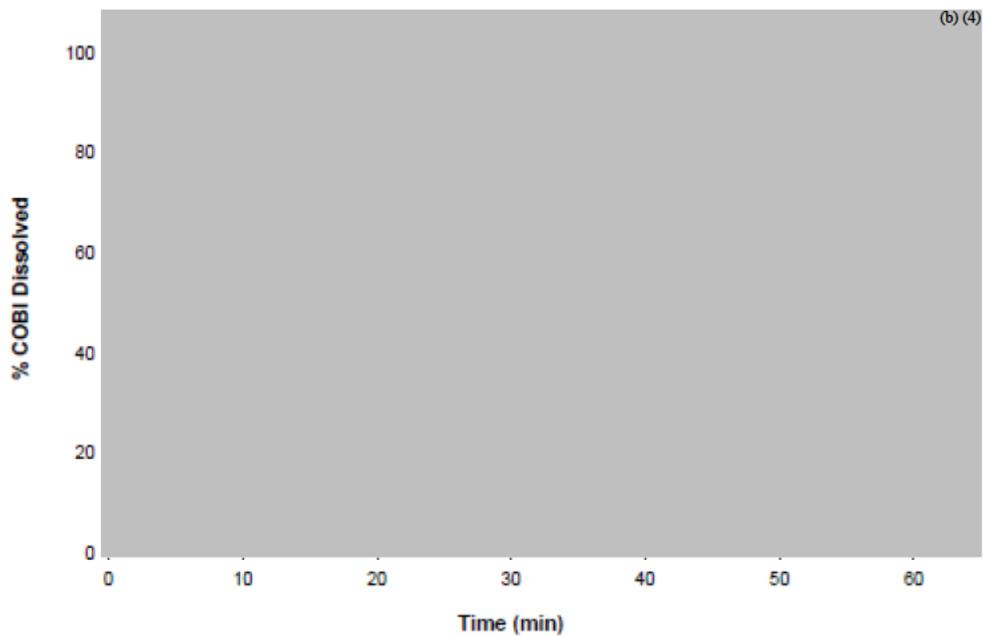
Figure 38. Dissolution Profiles of COBI from Clinical Lots of EVG/COBI/FTC/TDF tablets

2 Page(s) has been Withheld in Full immediately
following this page as B4 (CCI/TS)

Product Quality Review

NDA 203-100   **BIOPHARMACEUTICS NDA REVIEW**   35
Stribild (name under review) Tablet
Gilead Sciences Inc.

2. Therefore, the following dissolution acceptance criteria are recommended for your proposed product:

EVG

$Q=$ ▨ (b) (4) *at 30 min*

FTC

$Q=$ ▨ (b) (4) *at 30 min*

TDF

$Q=$ ▨ (b) (4) *at 30 min*

COBI

$Q=$ ▨ (b) (4) *at 10 min*

Our recommendation is based on the mean dissolution profiles observed for the pivotal phase 3 and stability batches. Revise the dissolution acceptance criteria accordingly and submit an updated sheet of specifications.

The Applicant responded to the IRs   (b) (4)

                          The Agency, upon

much deliberation rejected the Applicant's proposal   (b) (4)

                   The Applicant agreed to the Agency's recommendation and an amendment was filed with the final acceptance criteria (13-JUN-2012).

QUALITY-BY-DESIGN FOR EVG/COBI/TDF/FTC TABLET

In this submission the Applicant proposes to use dissolution as a response parameter in the construction of the design space for EVG ▨ (b) (4), selection of tablet ▨ (b) (4) and for establishing a small design space for the ▨ (b) (4) film coat. Please refer to Dr. Celia Cruz review for the details of the manufacturing process and QbD aspects to the manufacturing process development. The review below will focus on evaluating parameters where in dissolution was used as a response parameter.

Dissolution as a response parameter in design of experiments to aid the construction of design space for EVG ▨ (b) (4)

The design space justification for EVG ▨ (b) (4) was based on evaluating ▨ (b) (4) and by

Product Quality Review

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Gilead Sciences Inc.

providing the dissolution profiles for all DoE runs. The manufacturing process flow for this tablet is furnished below:

(b) (4)

Justification for the proposed design space for Layer 1 (EVG-COBI) was execution of a multivariate design of experiment   (b) (4)   , and the carry through of this expected variation   (b) (4)   of the bilayer tablets. The scale of this study was   (b) (4)   . The DoE was a reduced 11 run experiment; the summary of the experiments is provided below:

Table 3. Summary of DoE experiments   (b) (4) ; n = 11 runs.

(b) (4)

The impact of [   ] (b) (4) design space on dissolution of EVG, COBI, FTC and TDF was analyzed by providing profile for all DoE runs. However, f2 statistical analysis comparison was not provided for the profiles. The dissolution profiles for the 4 APIs from the 11 DoE runs is provided in Figures 41-44.
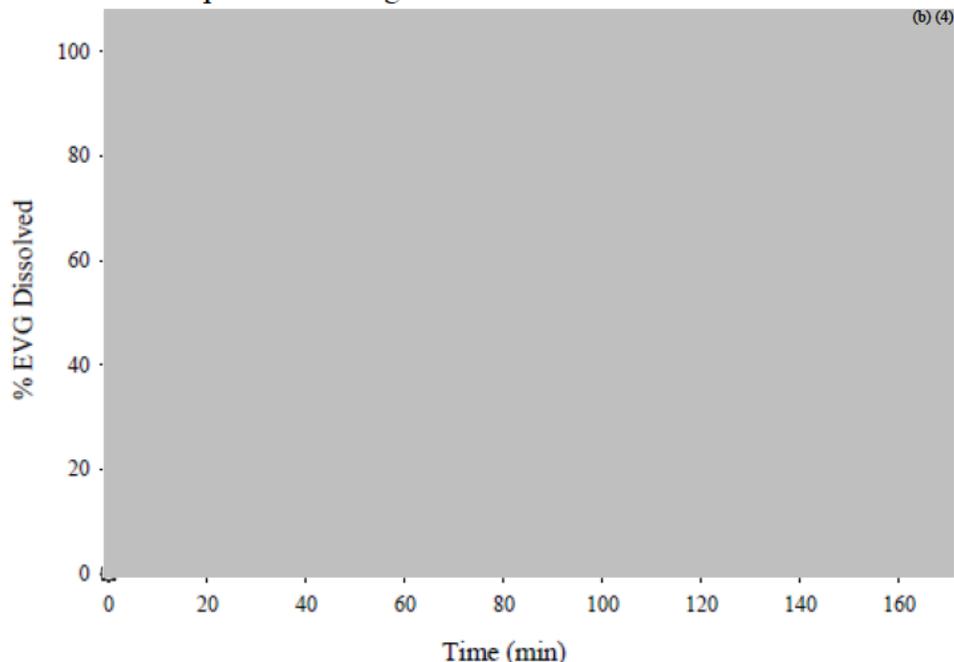
Figure 41. Dissolution Profiles of EVG for EVG/COBI/FTC/TDF Film-Coated Tablets Manufactured with Various EVG [   ] (b) (4) Process Parameters
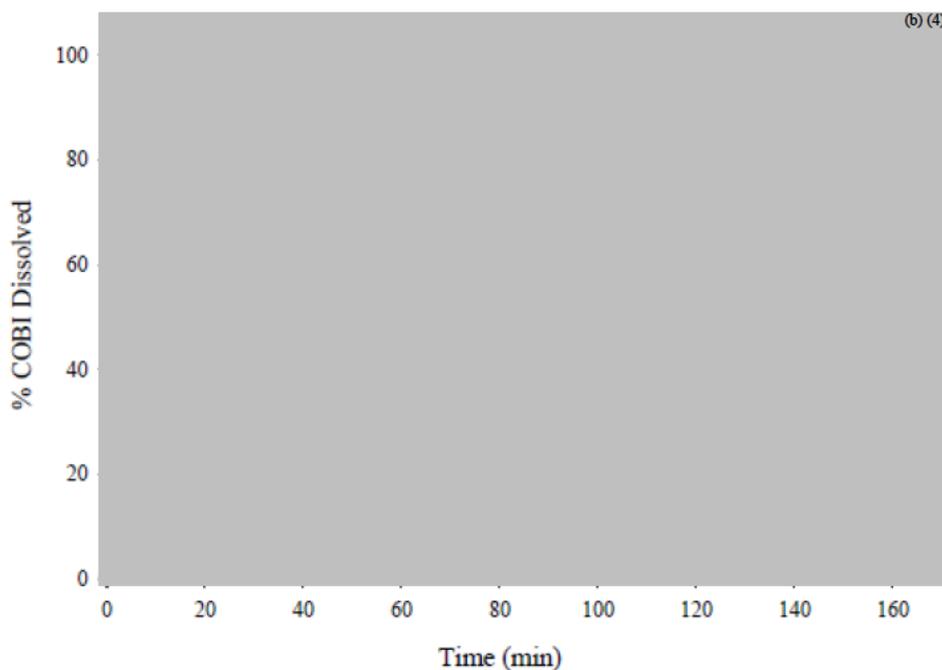
Figure 42. Dissolution Profiles of COBI for EVG/COBI/FTC/TDF Film-Coated Tablets Manufactured with Various EVG [   ] (b) (4) Process Parameters
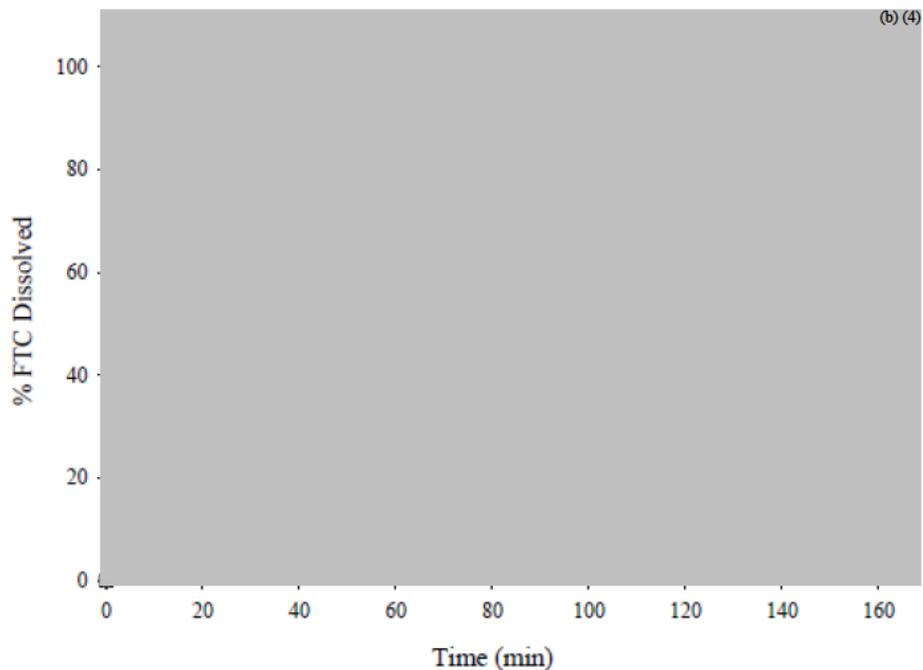
Figure 43. Dissolution Profiles of FTC for EVG/COBI/FTC/TDF Film-Coated Tablets Manufactured with Various EVG ⁽ᵇ⁾⁽⁴⁾ Process Parameters
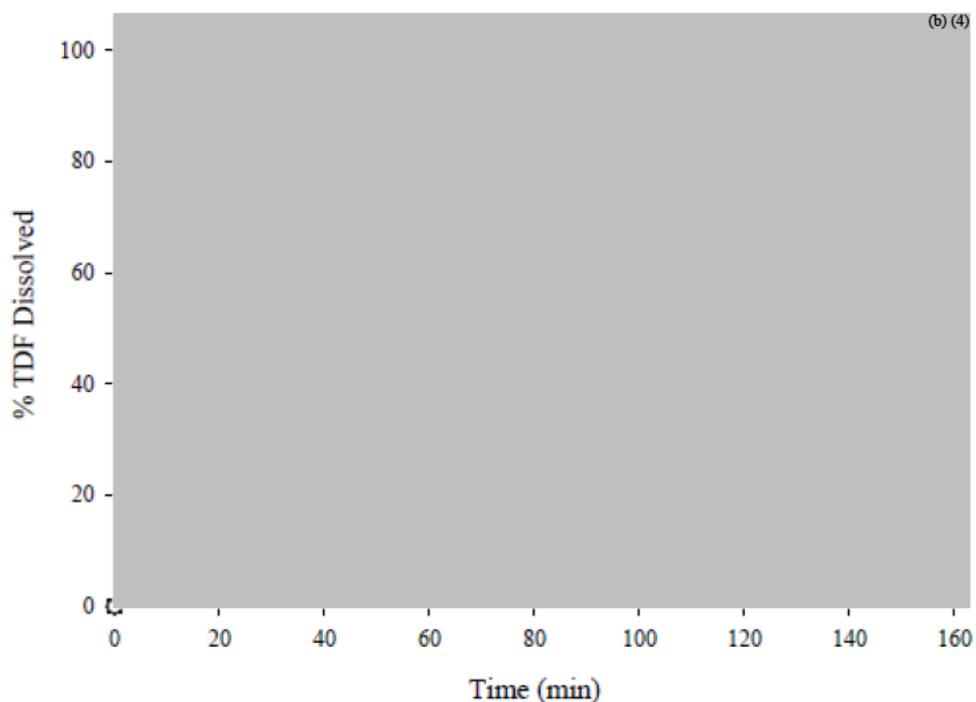
Figure 44. Dissolution Profiles of TDF for EVG/COBI/FTC/TDF Film-Coated Tablets Manufactured with Various EVG ⁽ᵇ⁾⁽⁴⁾ Process Parameters

Reviewer's Comments
*The following IR was sent to the Applicant requesting more data to support the design space on 23-MAR-2012:*

Product Quality Review

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Gilead Sciences Inc.

There are insufficient data (e.g. dissolution profiles comparison with f2 statistical testing, in-vitro in-vivo correlation (IVIVC models) or in-vivo bioequivalence studies) to determine whether batches manufactured throughout the proposed design space would result in products that are bioequivalent. Submit adequate justification, including (but not limited to) the following information:
• f2 statistical testing for FTC, TDF, and EVG dissolution profile comparisons of EVG/COBI/FTC/TDF tablets manufactured across the EVG ^{(b) (4)} *design space (Figure 5-8 in 3.3.P.2.3).*
• f2 statistical testing for FTC, TDF, and EVG dissolution profile comparisons of EVG/COBI/FTC/TDF tablets manufactured across the final hardness range (Figure 11-14 in 3.2.P.2.3).

If available, provide dissolution profiles and f2 statistical testing for FTC, TDF, and EVG dissolution profile comparisons of EVG/COBI/FTC/TDF tablets manufactured across ^{(b) (4)}

(ADDRESSED LATER IN THE REVIEW)

The Applicant responded with data for the above IR using the dissolution method at ^{(b) (4)} *rpm. Also for the reference batch for the calculation of the f2 values, the Applicant used the* ^{(b) (4)} *lot. A follow-up IR was sent to re-submit the above data generated using the dissolution method at 100 rpm (dated 22-MAY-2012).*

Refer to Page 40 of the Quality Information Amendment dated 20-April-2012 that contained responses to our IR dated 23-March-2012. Explain the use of the ^{(b) (4)} **lot as the reference lot for calculating f2 values. In addition, it is noted that the dissolution testing was conducted using** ^{(b) (4)} **rpm. Therefore, resubmit the calculations for Table 9, for each API, using a pivotal Phase 3 lot that is representative of the target manufacturing conditions as the reference lot and using 100 rpm for the paddle speed.**

The Applicant submitted the requested information on 29-MAY-2012, clarifying that in the design space experiments, varying EVG ^{(b) (4)} *process parameters were studied while using the same batch of FTC/TDF* ^{(b) (4)} *manufactured at target conditions in all experiments. In the bilayer configuration of the EVG/COBI/FTC/TDF tablets, the FTC/TDF portion is manufactured* ^{(b) (4)}

The f2 values for EVG, FTC and TDF submitted were calculated from dissolution data obtained at 100 rpm paddle speed using a Phase 3 clinical batch (Clinical Lot BK1005B) that is representative of the target manufacturing conditions as reference (Table 4).

Table 4. *f2 Statistical Testing for EVG, FTC, and TDF Dissolution Profile Comparison of EVG/COBI/FTC/TDF Tablets Manufactured Across the EVG* ⬛ (b) (4) *Design Space Using A Representative Phase 3 Clinical Batch as Reference*

Run No.	Lot No.	f2 Statistical Testing for EVG, FTC and TDF using Phase 3 Clinical Lot BK1005B as Reference		
		EVG	**FTC**	**TDF**
1	4871-03			
2A	4871-04			
2B	4871-04B			
3	4871-05			
4	4871-06			
5	4871-07			
6	4871-08			
7	4871-09			
8	4871-10			
9	4871-11			
10	4871-12			
11	4871-13			

As is evident from the above f2 analyses, the dissolution profiles of all batches from the EVG ⬛ (b) (4) *design space experiments are similar to EVG from the pivotal batch. f2 test failure is observed for FTC batches* ⬛ (b) (4)

⬛ *f2 analysis for FTC and TDF dissolution profiles are of limited relevance with respect to the EVG granulation process parameters. Because of this, the reviewer is not concerned with the FTC failure and deems the proposed design space for EVG* ⬛ (b) (4)

⬛

acceptable from Biopharmaceutics perspective.

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Stribild (name under review) Tablet
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Dissolution as a response parameter in selection of Layer 1 ░░░ (b) (4)
The design space justification ░░░ (b) (4) focused on determining the impact
of Layer 1 ░░░ (b) (4)

░░░ There was no information provided in the
original submission on the impact of ░░░ (b) (4) Layer 1 ░░░ (b) (4)
░░░

Reviewer's Comments
The final tablet hardness (░░░ (b) (4) specification) is proposed ░░░ (b) (4). The
Applicant was contacted to support the proposed ranges by f2 comparison ░░░ (b) (4)
░░░ On 23-MAR-2012, the following IR was sent:

If available, provide dissolution profiles and f2 statistical testing for FTC, TDF, and
EVG dissolution profile comparisons of EVG/COBI/FTC/TDF tablets manufactured
across

░░░ (b) (4)

The Applicant's response (summarized below) supported the proposed Layer 1
░░░ (b) (4) ranges.

f2 Comparison with Respect to Final Tablet Hardness
The f2 statistical testing for FTC, TDF, and EVG dissolution profile comparisons of
EVG/COBI/FTC/TDF tablets manufactured to the final target range of tablet hardness is
shown below. The dissolution profiles of all batches within the proposed hardness range
met the criteria of being similar in the f2 comparison to the reference formulation for
EVG dissolution. TDF dissolution for tablets manufactured with a tablet hardness of ░░░ (b) (4)
░░░ do not meet the criterion for similarity. In addition, the FTC dissolution for
tablets manufactured with a hardness of ░░░ (b) (4) also do not meet the criterion for
similarity, comparing to tablets ░░░ (b) (4). The data supports
the Applicant's proposed tablet hardness range ░░░ (b) (4).

Table 5. f2 Statistical Testing for EVG, FTC and TDF Dissolution Profile Comparison of
EVG/COBI/FTC/TDF Tablets Manufactured Across the Final Tablet Hardness Range

Lot No.	Final Tablet Hardness (kp)	f2 Statistical Testing for EVG, FTC and TDF using the ░░░ (b) (4) Final Hardness Lot (lot no. 4870-119-2) as Reference		
		EVG	**FTC**	**TDF**
4870-119-1	░░░ (b) (4)			
4870-119-2				
4870-119-3				
4870-119-4				

f2 Comparison with Respect to Layer 1 ▮▮▮ (b) (4)

The f2 statistical testing for FTC, TDF, and EVG dissolution profile comparisons of EVG/COBI/FTC/TDF tablets manufactured across a range of Layer 1 ▮▮▮ (b) (4) *is shown below. This f2 comparison included clinical and development batches. The Applicant's selection of Lot BK1002B as the reference lot is reasonable as it was* ▮▮▮ (b) (4) *close to the midpoint of the* target proposed range ▮▮ (b) (4) *The data supports that the dissolution profiles of all batches within the proposed Layer 1* ▮▮▮ (b) (4) *range met the criteria of being similar in the f2 comparison to the reference batch, with the exception of the development batches manufactured at* ▮▮▮ (b) (4) *and a final tablet hardness of* ▮▮ (b) (4)

Table 11. f2 Statistical Testing for EVG, FTC and TDF Dissolution Profile Comparison of EVG/COBI/FTC/TDF Tablets Manufactured Across the Layer 1 ▮▮ (b) (4) Range

Lot No.	(b) (4)
BK1101B	
BK1102B	
BK0904B	
BK1005B	
BK1104B	
BK1003B	
BK1103C	
BK1106B	
BK1002B	
BK1103D	
4870-30C	
4870-30A	
4870-30D	
4870-30B	

Although the Applicant has not provided data ▮▮▮ (b) (4) *the data above supported the* ▮▮ (b)(4) *range* ▮▮ (b)(4) **Hence, from a Biopharmaceutics perspective, only Layer 1 range** ▮▮ (b) (4) **can be granted, as supported by data.**

The Applicant has not provided dissolution profiles for tablets prepared within the FTC/TDF ▮▮▮ (b)(4) *proposed ranges. The FTC/TDF* ▮▮▮ (b)(4) *were manufacture* ▮▮▮ (b)(4) *The*

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Gilead Sciences Inc.

Biopharmaceutics reviewer requests the CMC reviewer to evaluate the adequateness of the proposed ▓▓▓ (b) (4) *ranges.*

Dissolution as a response parameter in small scale design space development for film coated (target film coat ▓▓▓▓▓▓ (b) (4) **)**
Data is provided supporting no change in dissolution profiles for any API within the proposed design space ranges (refer to data presented in Figures 22-25).

Reviewer's Comment
The small design space range of target film coat ▓▓▓▓▓▓▓▓ (b) (4) *is acceptable.*

CONCLUSIONS

The NDA is recommended for Approval from a Biopharmaceutics perspective. The following dissolution method for the EVG/COBI/FTC/TDF is deemed acceptable:
Apparatus II , 2% Polysorbate 80, 0.01 N HCl, 1000 mL, 100 rpm

The following dissolution acceptance criteria has been recommended (and agreed by the Applicant) for the four APIs:

$$\text{EVG: } Q = \text{▓▓ (b) (4)} \text{ at 30 mins}$$
$$\text{COBI: } Q = \text{▓▓ (b) (4)} \text{ at 10 mins}$$
$$\text{FTC: } Q = \text{▓▓ (b) (4)} \text{ at 30 mins}$$
$$\text{TDF: } Q = \text{▓▓ (b) (4)} \text{ at 30 mins}$$

Comments to the CMC Reviewer
The following design space or target ranges proposed by the Applicant and recommended by the reviewer are provided below for each parameter:

- EVG ▓▓▓▓ (b) (4) : The proposed design space is ACCEPTABLE
- Layer 1 ▓▓ (b) (4) range ▓▓▓▓ (b) (4) : NOT ACCEPTABLE Recommend to be changed ▓▓ (b) (4)
- The Applicant has not provided dissolution profiles for tablets prepared within the FTC/TDF ▓▓▓ (b) (4) proposed ranges. The FTC/TDF ▓▓▓ (b) (4) were manufactured ▓▓▓▓▓▓▓▓ (b) (4)

  ▓▓▓▓ The Biopharmaceutics reviewer requests the CMC reviewer to evaluate the adequateness of the proposed ▓▓▓ (b) (4) ranges.
- Design space for film coat ▓▓▓ (b) (4) : ACCEPTABLE
- Recommend ▓▓ (b) (4) the EVG particle size specification ▓▓▓▓ (b) (4)

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/s/

DEEPIKA LAKHANI
06/28/2012
Recommend Approval from Biopharmaceutics perspective.

SANDRA SUAREZ
06/28/2012

BIOPHARMACEUTICS FILING REVIEW **Office of New Drugs Quality Assessment**			
Application No.:	NDA 203-100	**Reviewer:** Deepika Arora Lakhani, PhD	
Submission Date:	Oct 27, 2011		
Division:	Division of Medical Imaging and Hematology Products	**Team Lead:** Angelica Dorantes, PhD	
Sponsor:	Gilead Sciences, Inc.	**Supervisor (Acting):** Angelica Dorantes, PhD	
Trade Name:	-Pending-	**Date Assigned:**	May 11, 2011
Generic Name:	Elvitegravir, Cobicistat, Emtricitabine and Tenofovir Disoproxil Fumarate Tablets	**Date of Review:**	Dec 27, 2011
Indication:	HIV Infection	**Type of Submission:** New Drug Application 505b(1)	
Formulation/ strengths	Tablet 150mg EVG/150mg COBI/200mg FTC/300mgTDF		
Route of Administration	Oral		

SUBMISSION:

The submission is a 505(b)(1) application for a film coated single tablet regimen (STR) that contains the active substances elvitegravir (EVG), cobicistat (COBI), emtricitabine (FTC), and tenofovir disoproxil fumarate (tenofovir DF, TDF). The EVG/COBI/FTC/TDF tablet is an immediate-release tablet containing EVG 150 mg, COBI 150 mg, FTC 200 mg and TDF 300 mg. EVG/COBI/FTC/TDF tablets are bilayer tablets with EVG and COBI in one layer referred to as the EVG/COBI layer and the other layer contains FTC and TDF and is referred to as the FTC/TDF layer.

Elvitegravir (EVG, GS-9137, JTK-303) and Cobicistat (COBI, GS-9350) are investigational agents of the integrase strand transfer inhibitor (INSTI) class and cytochrome P450 3A (CYP3A) inhibitor class, respectively, being developed by Gilead for treatment of HIV infection. Emtricitabine and TDF are also approved for the treatment of HIV-1 infection in a fixed-dose combination (FDC) product Truvada® ([FTC/TDF]; NDA 21-752 for tablet formulation approved on 02 August 2004). Emtricitabine and TDF are approved in FDC products with efavirenz (EFV) and rilpivirine (RPV), both nonnucleoside reverse transcriptase inhibitor (NNRTI). These FDC products are Atripla® (efavirenz/emtricitabine/tenofovir DF [EFV/FTC/TDF]; approved for use in the US on 12 July 2006), and Complera™ (emtricitabine/rilpivirine/tenofovir DF [FTC/RPV/TDF]; approved for use in the US on 10 August 2011).

BIOPHARMACEUTICS:

A single combined method to assay the dissolution of EVG/COBI/FTC/TDF film-coated tablets was developed using USP paddle apparatus at 75 rpm with 1000 mL of 0.01 N HCl containing 2% w/w polysorbate 80 maintained at 37 °C:

Apparatus: No. 2 (paddles)
Rotation Speed: 75 rpm
Dissolution Medium: 0.01 N HCl containing 2% w/w polysorbate 80
Medium Volume: 1000 mL
Medium Temperature: 37 °C
Sinker: helical stainless steel sinker
Sampling Volume: 10 ± 0.2 mL
Analysis: UPLC or HPLC

1

The robustness of the dissolution method was evaluated by assessing the effect of small, deliberate changes to the surfactant (polysorbate 80) concentration and pH of the dissolution medium.

The discriminating capacity of the dissolution method was evaluated   (b) (4)

                                                                The proposed dissolution method discriminated for formulation composition and EVG API particle size.

The applicant has proposed dissolution specification of NLT [(b) (4)] (Q) of EVG, FTC and TDF dissolved at 30 minutes, and NLT [(b) (4)] (Q) of COBI dissolved at 30 minutes.

FTC and TDF are high-solubility, BCS Class 1 and Class 3 compounds, respectively. EVG is a low solubility BCS Class 2 molecule and has low solubility throughout the physiological pH range. The solubility of COBI in water is 0.1 mg/mL at room temperature. The solubility of COBI in aqueous media is affected by pH and increases at pH values below the protonation of the morpholino group, pH 6.2. (b) (4)

Based upon the reviewer's initial analysis of the provided dissolution data from batch release and stability, the Applicant's proposed dissolution specifications for all APIs in the tablet [(b) (4)
   ] Initial data review easily supports the following revised dissolution specifications:
EVG, FTC and TDF: NLT [(b) (4)] (Q) dissolved at 30 mins
COBI: NLT [(b) (4)] (Q) dissolved at 10 mins
Representative dissolution profiles for the 4 APIs (clinical batches and stability samples) is provided below:

Figure 1. Dissolution of EVG in Clinical Batches and Stability Samples of EVG/COBI/FTC/TDF Tablets.
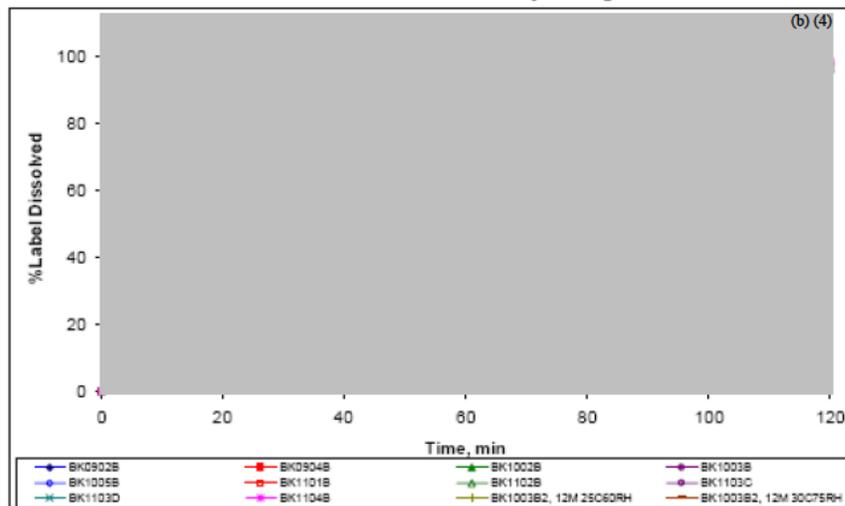
**Figure 2.** Dissolution of COBI in Clinical Batches and Stability Samples of EVG/COBI/FTC/TDF Tablets
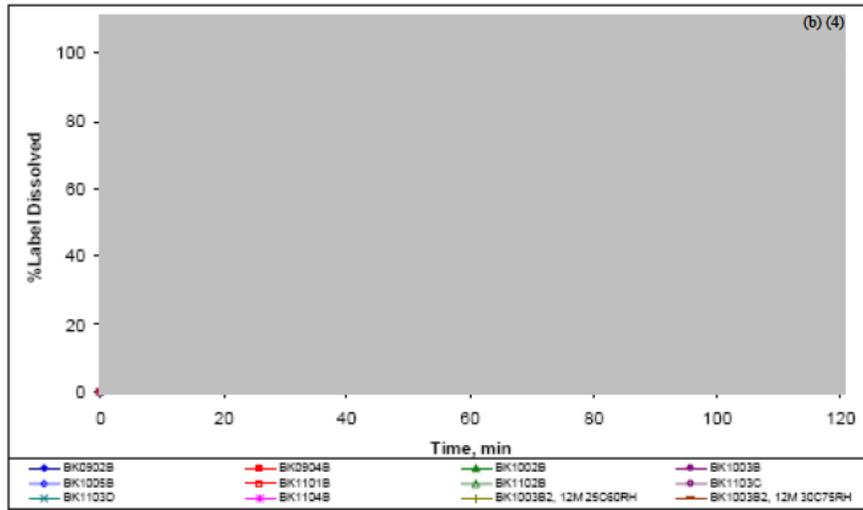
Figure 3. Dissolution of FTC in Clinical Batches and Stability Samples of EVG/COBI/FTC/TDF Tablets
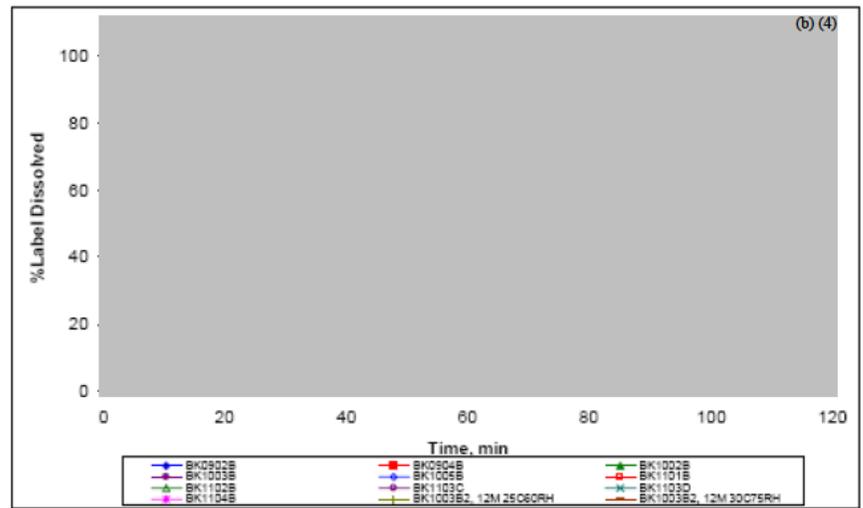
Figure 4. Dissolution of TDF in Clinical Batches and Stability Samples of EVG/COBI/FTC/TDF Tablets

3
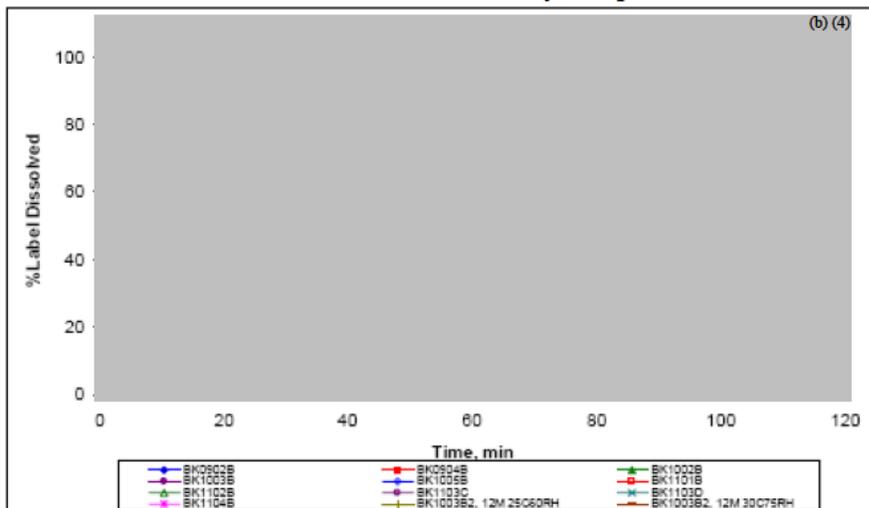
The application contains most of the information needed to justify the selection of the dissolution conditions; however, adequate justification for the use of high concentration of surfactant (2% polysorbate 80) in dissolution media as well as the effect of the presence of this surfactant on the dissolution of COBI is lacking. Also the proposed dissolution specifications seem ▩ (b) (4) Please note that the request for ▩ (b) (4) dissolution specifications is NOT being made following the filing review as the reviewer still needs to establish the suitability of the proposed dissolution testing conditions for the drug product. Clarification regarding the surfactant choice and its effect on the dissolution of COBI will be requested as a part of the comments to be communicated to the Applicant.

RECOMMENDATION:
The ONDQA/Biopharmaceutics team upon review of NDA 203-100 for filing purposes, found the application to be fileable, from Biopharmaceutics perspective. The below clarification comments need to be communicated to the applicant:

➢ Provide data to support selection of 2% polysorbate 80 as the most suitable surfactant concentration for the proposed dissolution medium.
➢ Provide data to support that dissolution of COBI is unaffected by the presence of 2% polysorbate 80 in the dissolution medium.

Deepika Arora Lakhani, Ph.D.   Angelica Dorantes, Ph.D.
Biopharmaceutics Reviewer   Biopharmaceutics Acting Supervisor
Office of New Drugs Quality Assessment   Office of New Drugs Quality Assessment

cc. Tapash Ghosh, Ph.D.

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/s/

DEEPIKA LAKHANI
01/03/2012
NDA is fileable from Biopharmaceutics perspective. Filing comments on Page 4 must be communicated to the applicant.

TAPASH K GHOSH
01/03/2012

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

Office of Clinical Pharmacology

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA/BLA Number	203100	Brand Name	
OCP Division (I, II, III, IV, V)	IV	Generic Name	Elvitegravir/Cobicistat/ Emtricitabine/Tenofovir
Medical Division	DAVP	Drug Class	Elvitegravir (EVG): Integrase Inhibitor Cobicistat (Cobi): Pharmacoenhancer Emtricitabine (FTC) and Tenofovir (TDF): NRTIs
OCP Reviewer	Vikram Arya, Ph.D.	Indication(s)	Treatment of HIV-1 Infection in Treatment Naïve HIV-1 Infected Adult Patients
OCP Team Leader	Kellie Reynolds, Pharm.D.	Dosage Form	Tablet
Pharmacometrics Reviewer	Jeffrey Florian, Ph.D.	Dosing Regimen	EVG/Cobi/FTC/TDF: 150/150/200/300 mg once daily
Date of Submission	10/26/2011	Route of Administration	Oral
Estimated Due Date of OCP Review	05/11/2012	Sponsor	Gilead Sciences Inc.
Medical Division Due Date	07/02/2012	Priority Classification	Standard
PDUFA Due Date	08/27/2012		

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X	3		
I. Clinical Pharmacology				
Mass balance:	X	2		
In Vitro absorption, isozyme and transporter characterization:	X	38		In vitro studies listed on pages 28, 29, 38 and 39 of the clinical pharmacology summary
Blood/plasma ratio:	X			
Plasma protein binding:	X	2		
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	X	3		
multiple dose:	X	4		
Patients-				

File name: 5_Clinical Pharmacology and Biopharmaceutics Filing Form/Checklist for NDA_BLA or Supplement 090808

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS
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single dose:				
multiple dose:	**X**	**2**		
Dose proportionality -				
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:	**X**	**10**		
In-vivo effects of primary drug:	**X**			
In-vitro:				
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				
geriatrics:				
renal impairment:	**X**	**2**		
hepatic impairment:	**X**	**1**		
PD -				
Phase 2:	**X**			
Phase 3:	**X**			
PK/PD -				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:	**X**	**3**		
Population Analyses -				
Data rich:	**X**			
Data sparse:	**X**			
II. Biopharmaceutics				
Absolute bioavailability				
Relative bioavailability -	**X**			
solution as reference:				
alternate formulation as reference:		**1**		
Bioequivalence studies -				
traditional design; single / multi dose:				
replicate design; single / multi dose:				
Food-drug interaction studies		**1**		
Bio-waiver request based on BCS				
BCS class				
Dissolution study to evaluate alcohol induced dose-dumping				
III. Other CPB Studies				
Genotype/phenotype studies				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies		**72**		

Reviewer's Note: The single tablet regimen contains 4 components, therefore, the number of studies shown in the table above for any given category represents the total number of studies conducted with either EVG, Cobi, EVG/Cobi or the single tablet regimen.

On **initial** review of the NDA/BLA application for filing:

	Content Parameter	Yes	No	N/A	Comment
Criteria for Refusal to File (RTF)					
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?			X	Pivotal Clinical trials used the to-be-marketed-formulation
2	Has the applicant provided metabolism and drug-drug interaction information?	X			
3	Has the sponsor submitted bioavailability data satisfying the	X			

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	CFR requirements?				
4	Did the sponsor submit data to allow the evaluation of the validity of the analytical assay?	X			
5	Has a rationale for dose selection been submitted?	X			
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive review to begin?	X			
7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can begin?	X			
8	Is the electronic submission searchable, does it have appropriate hyperlinks and do the hyperlinks work?	X			
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					
	Data				
9	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?	X			
10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?			X	
	Studies and Analyses				
11	Is the appropriate pharmacokinetic information submitted?	X			
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?			X	
13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?	X			
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?	X			
15	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?			X	
16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			X	
17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?	X			
	General				
18	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	X			
19	Was the translation (of study reports or other study information) from another language needed and provided in this submission?			X	

IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE?
Yes

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If the NDA/BLA is not fileable from the clinical pharmacology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Vikram Arya, Ph.D.	12/23/2011
Reviewing Clinical Pharmacologist	Date
Kellie Reynolds, Pharm.D.	12/23/2011
Team Leader/Supervisor	Date

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