CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

203100Orig1s000

PHARMACOLOGY REVIEW(S)

MEMO **FOOD AND DRUG ADMINISTRATION**

Division of Antiviral Products
Center for Drug Evaluation and Research

Date:	June 29, 2012
Reviewer:	Hanan Ghantous, PhD, DABT Supervisory Interdisciplinary Scientist
NDA #/SS#/date:	203-100/1/10/26/2011
Sponsor:	Gilead Sciences, Inc.
Drug Product:	Elvitegravir/Cobicistat/Emtricitabine/Tenofovir DF 50mg EVG/150mg COBI/200mg FTC/300mgTDF
Indication:	(b) (4)

Recommended Action: There are no nonclinical pharmacology and toxicology issues which would preclude the approval of NDA 203-100.

Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Disoproxil Fumarate (E/C/F/T) is a combination drug product consisting of an HIV-1 integrase strand transfer inhibitor (Elvitegravir, EVG), a pharmacoenhancer designed to inhibit CYP3A (Cobicistat, COBI), an HIV-1 reverse transcriptase inhibitor (Emtricitabine, FTC), and an HIV-1 reverse transcriptase inhibitor (Tenofovir disoproxil fumarate, TDF). E/C/F/T combination therapy is indicated for HIV-1 infection in adults ≥ 18 years of age who are antiretroviral treatment naïve or have no known substitutions associated with resistance to the individual components. The proposed daily clinical dose is 150 mg EVG, 150 mg COBI, 200 mg FTC, and 300 mg TDF.

EVG and COBI are new chemical entity, while FTC and TDF are current approved drug products. A combination of (FTC/TDF, Truvada) is also an approved drug product. Comprehensive programs of nonclinical studies with EVG, COBI, FTC, TDF and TDF/TFV have been conducted.

The nonclinical safety database on EVG, COBI, FTC and TDF, including combination toxicity studies with EVG and COBI, and with FTC and TDF, support the approval of the combination in a single dose tablet.

Conclusion: I concur with the primary Pharmacology/Toxicology reviewers, Drs. Pritam Verma, Laine Peyton Myers and Mark W. Powley that the nonclinical data support an approval action for E/C/F/T.

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/s/

HANAN N GHANTOUS
06/29/2012

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	203-100
Supporting document/s:	1
Applicant's letter date:	October 26, 2011
CDER stamp date:	October 27, 2011
Product:	Elvitegravir/Cobicistat/Emtricitabine/Tenofovir DF
	50mg EVG/150mg COBI/200mg FTC/300mgTDF
Indication:	Treatment of HIV-1 infection
Applicant:	Gilead Sciences, Inc. 333 Lakeside Drive Foster City, CA 94404
Review Division:	DAVP
Reviewer:	Pritam Verma, Ph.D.
	L. Peyton Myers, PhD.
	Mark Powley, Ph.D.
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1 Executive Summary

1.1 Introduction: Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Disoproxil Fumarate (E/C/F/T) is a combination drug product consisting of a novel HIV-1 integrase strand transfer inhibitor (Elvitegravir, EVG), a novel pharmacoenhancer designed to inhibit CYP3A (Cobicistat, COBI), an approved HIV-1 reverse transcriptase inhibitor (Emtricitabine, FTC), and an approved HIV-1 reverse transcriptase inhibitor (Tenofovir disoproxil fumarate, TDF). Gilead has submitted an NDA in support of E/C/F/T combination therapy to treat HIV-1 infection in adults ≥ 18 years of age who are antiretroviral treatment naïve or have no known substitutions associated with resistance to the individual components. The proposed daily clinical dose is 150 mg EVG, 150 mg COBI, 200 mg FTC, and 300 mg TDF.

Elvitegravir is a new chemical entity that belongs to the new class of HIV-1 integrase strand-transfer inhibitors that prevent integration of HIV-1 genetic material into the host-cell genome. Cobicistat is a new chemical entity and structural analogue of ritonavir. COBI is a pharmacoenhancer which inhibits CYP3A to enhance the exposure of CYP3A substrates, including EVG. Gilead has developed EVG and COBI for use as a fixed dose tablet with the approved drug products FTC and TDF.

1.2 Brief Discussion of Nonclinical Findings

Oral administration of E/C/F/T as independent agents as well as in combination resulted in similar exposures of all four agents.

Ample nonclinical safety information is currently available on EVG, COBI, FTC and TDF alone as well as combination toxicity studies (EVG+COBI and FTC+TDF), The 4 drugs, EVG, COBI, FTC, and TDF, exhibit different patterns of target organ toxicity. EVG caused changes in the cecum and upper small intestine in rats and dogs due to high local concentrations which were not considered adverse, COBI had dose-dependent effects on the liver (mouse, rat and dog) and thyroid (rat). These effects were considered adaptive and secondary to hepatic microsomal enzyme induction and thyroid hormone imbalance. The only notable effect of FTC was a minor anemia identified at dose levels constituting large clinical multiples. Extensive nonclinical investigations have shown that the target organs for TDF are distinctly different (GI, bone, and kidney). Administration of EVG+COBI or FTC+TDF in combination did not exacerbate the toxicities of the individual agents. Furthermore, no specific cause for concern has been identified in genotoxicity, carcinogenicity, and reproductive toxicity studies with the individual agents.

In addition, COBI alone was associated with nonadverse urinalysis and urine chemistry changes (diuretic effects) at high doses in rats and dogs, which were reversible. COBI also caused serum creatinine and BUN changes, but without morphological evidence of kidney damage. A potential COBI+TDF interaction was noted by a slight increase in few probable cases of renal damage in the COBI+TDF clinical trial arms compared to the RTV+TDF clinical trial arms. COBI may exacerbate the local exposures of TDF in the kidney and exacerbate TDF renal toxicity. (b) (4)

The lack of overlapping toxicity in animals, along with clinical data with EVG and COBI, and the E/C/F/T tablet, support the approval of E/C/F/T tablet.

1.3 Recommendations

1.3.1 Approvability: There are no nonclinical pharmacology and toxicology issues which would preclude the approval of E/C/F/T NDA.

1.3.2 Additional Non Clinical Recommendations: None

1.3.3 Labeling: E/C/F/T

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category B

There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, [TRADENAME] should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Antiretroviral Pregnancy Registry: To monitor fetal outcomes of pregnant women exposed to [TRADENAME], an Antiretroviral Pregnancy Registry has been established. Healthcare providers are encouraged to register patients by calling 1-800-258-4263.

Animal Data

Elvitegravir: Studies in animals have shown no evidence of teratogenicity or an effect on reproductive function. In offspring from rat and rabbit dams treated with elvitegravir during pregnancy, there were no toxicologically significant effects on developmental endpoints. The exposures (AUC) at the embryo-fetal No Observed Adverse Effects Levels (NOAELs) in rats and rabbits were respectively 23 and 0.2 times higher than the exposure in humans at the recommended daily dose of 150 mg.

Cobicistat: Studies in animals have shown no evidence of teratogenicity or an effect on reproductive function. In offspring from rat and rabbit dams treated with cobicistat during pregnancy, there were no toxicologically significant effects on developmental endpoints. The exposures (AUC) at the embryo-fetal NOAELs in rats and rabbits were respectively 1.8 and 4.3 times higher than the exposure in humans at the recommended daily dose of 150 mg.

Emtricitabine: The incidence of fetal variations and malformations was not increased in embryofetal toxicity studies performed with emtricitabine in mice at exposures (AUC) approximately 60 times higher and in rabbits at approximately 120 times higher than human exposures at the recommended daily dose.

Tenofovir Disoproxil Fumarate: Reproduction studies have been performed in rats and rabbits at doses up to 14 and 19 times the human dose based on body surface area comparisons and revealed no evidence of impaired fertility or harm to the fetus due to tenofovir.

(b) (4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

Elvitegravir: Long-term carcinogenicity studies of elvitegravir were carried out in mice (104 weeks) and in rats for up to 88 weeks (males) and 90 weeks (females). No drug-related increases in tumor incidence were found in mice at doses up to 2000 mg/kg/day alone or in combination with 25 mg per kg per day ritonavir at exposures 3- and 14-fold, respectively, the human systemic exposure at the recommended daily dose of 150 mg. No drug-related increases in tumor incidence were found in rats at doses up to 2000 mg/kg/day at exposures 12- to 27-fold, respectively in male and female, the human systemic exposure.

Elvitegravir was not genotoxic in the reverse mutation bacterial test (Ames test) and the rat micronucleus assay. In an *in vitro* chromosomal aberration test, elvitegravir was negative with metabolic activation; however, an equivocal response was observed without activation.

Elvitegravir did not affect fertility in male and female rats at approximately 16- and 30-fold higher exposures (AUC), respectively, than in humans at the therapeutic 150 mg daily dose.

Fertility was normal in the offspring of rats exposed daily from before birth (*in utero*) through sexual maturity at daily exposures (AUC) of approximately 18-fold higher than human exposures at the recommended 150 mg daily dose.

Cobicistat: The assessment of the carcinogenicity studies of cobicistat (b) (4) ongoing (b) (4)

Cobicistat was not genotoxic in the reverse mutation bacterial test (Ames test), mouse lymphoma or mouse micronucleus assays.

Cobicistat did not affect fertility in male or female rats at daily exposures (AUC) approximately 4-fold higher than human exposures at the recommended 150 mg daily dose.

Fertility was normal in the offspring of rats exposed daily from before birth (*in utero*) through sexual maturity at daily exposures (AUC) of approximately 1.2-fold higher than human exposures at the recommended 150 mg daily dose.

Emtricitabine: In long-term carcinogenicity studies of emtricitabine, no drug-related increases in tumor incidence were found in mice at doses up to 750 mg/kg/day (23 times the human systemic

exposure at the therapeutic dose of 200 mg/day) or in rats at doses up to 600 mg/kg/day (28 times the human systemic exposure at the therapeutic dose).

Emtricitabine was not genotoxic in the reverse mutation bacterial test (Ames test), mouse lymphoma or mouse micronucleus assays.

Emtricitabine did not affect fertility in male rats at approximately 140-fold or in male and female mice at approximately 60-fold higher exposures (AUC) than in humans given the recommended 200 mg daily dose. Fertility was normal in the offspring of mice exposed daily from before birth (*in utero*) through sexual maturity at daily exposures (AUC) of approximately 60-fold higher than human exposures at the recommended 200 mg daily dose.

Tenofovir Disoproxil Fumarate: Long-term oral carcinogenicity studies of tenofovir DF in mice and rats were carried out at exposures up to approximately 10 times (mice) and 4 times (rats) those observed in humans at the therapeutic dose for HIV 1 infection. At the high dose in female mice, liver adenomas were increased at exposures 10 times that in humans. In rats, the study was negative for carcinogenic findings at exposures up to 4 times that observed in humans at the therapeutic dose.

Tenofovir DF was mutagenic in the *in vitro* mouse lymphoma assay and negative in an *in vitro* bacterial mutagenicity test (Ames test). In an *in vivo* mouse micronucleus assay, tenofovir DF was negative when administered to male mice.

There were no effects on fertility, mating performance or early embryonic development when tenofovir DF was administered to male rats at a dose equivalent to 10 times the human dose based on body surface area comparisons for 28 days prior to mating and to female rats for 15 days prior to mating through day seven of gestation. There was, however, an alteration of the estrous cycle in female rats.

(b) (4)

2 Drug Information

Elvitegravir

2.1 Drug

CAS Registry Number (Optional): 697761-98-1
Generic Name: Elvitegravir

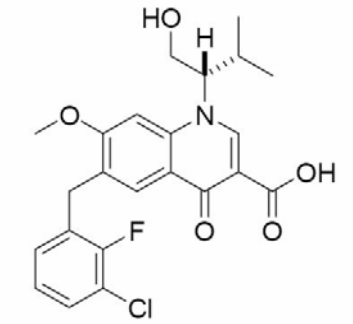
Code Name: GS-9137; JTK-303; EVG

Chemical Name: 3-Quinolinecarboxylic acid, 6-[(3-chloro-2-fluorophenyl)methyl]-1,4-dihydro-1-[(1S)-1-(hydroxymethyl)-2-methylpropyl]-7-methoxy-4-oxo-

Molecular Formula/Molecular Weight: $C_{23}H_{23}ClFNO_5$/447.88

Structure or Biochemical Description:

Figure 1 - EVG Structure



Pharmacologic Class: HIV-integrase inhibitor

Cobicistat

CAS Registry Number (Optional): 1004316-88-4

Generic Name: Cobicistat

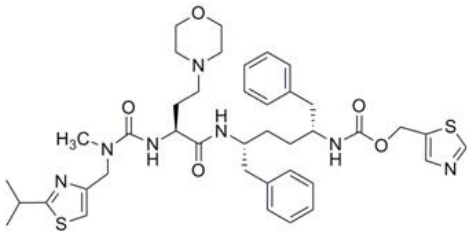
Code Name: COBI, GS-9350

Chemical Name: 1,3-thiazol-5-ylmethyl [(2*R*,5*R*)-5-{[(2*S*)-2-[(methyl{[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl}carbamoyl)amino]-4-(morpholin-4-yl)butanoyl]amino}-1,6-diphenylhexan-2-yl]carbamate

Molecular Formula/Molecular Weight: $C_{40}H_{53}N_7O_5S_2$/776.0

Structure or Biochemical Description:

Figure 2 - COBI Structure



Pharmacologic Class: Inhibition of CYP3A-mediated metabolism

Emtricitabine

CAS Registry Number (Optional): 143491-57-0

Generic Name: Emtricitabine

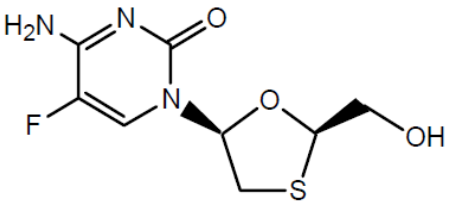
Code Name: FTC

Chemical Name: 5-fluoro-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine

Molecular Formula/Molecular Weight: $C_8H_{10}FN_3O_3$/ 247.24

Structure or Biochemical Description:

Figure 3 - FTC Structure



Pharmacologic Class: NRTI

Tenofovir disoproxil fumarate

CAS Registry Number (Optional): 202138-50-9

Generic Name: Tenofovir

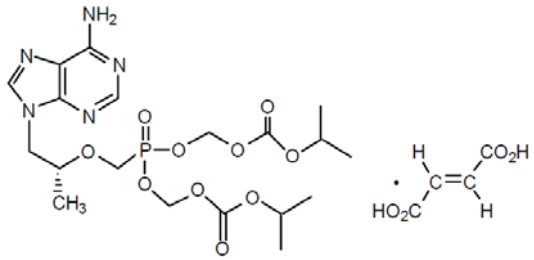
Code Name: TDF

Chemical Name: 9-[(*R*)-2- [[bis[[(isopropoxycarbonyl)oxy]methoxy]phosphinyl]methoxy] propyl]adenine fumarate (1:1)

Molecular Formula/Molecular Weight: $C_{23}H_{34}N_5O_{14}P$/ 635.52

Structure or Biochemical Description:

Figure 4 - TDF Structure



Pharmacologic Class: NRTI

2.2 Relevant INDs, NDAs, BLAs and DMFs: Elvitegravir: IND [(b) (4)], DMF 025187; Cobicistat: IND [(b) (4)], DMF 025188; Emtricitabine: IND 53,971, NDA 21-500, NDA 21-896; Tenofovir DF: IND 52,849, NDA 21-356, NDA 22-577; Emtricitabine/Tenofovir DF: IND 67,671 and NDA 21-752.

2.3 Drug Formulation: E/C/F/T tablets contain 150 mg of EVG, 150 mg of COBI, 200 mg of FTC, and 300 mg of TDF (as 245 mg tenofovir disoproxil).

2.4 Comments on Novel Excipients: With the exception of silicon dioxide, excipients are present at levels less than those listed for marketed oral products in the FDA's Inactive Ingredient Database (IID). Per the CMC reviewer, the levels of silicon dioxide may be up to 180 mg/day. This exposure is slightly higher than the maximum of 170 mg listed in the IID. Although there is a report of clinical sarcoidosis associated with ingested colloidal silicon dioxide (Sola et al., 2009), this excipient is generally considered to be relatively non-toxic (WHO, 1974; FDA, 1979). Given the indication and lack of overt toxicity associated with silicon dioxide, the proposed levels are considered acceptable.

2.5 Comments on Impurities/Degradants of Concern: Proposed specifications for degradant levels in cobicistat, emtricitabine, and tenofovir disoproxil fumarate were qualified by repeat dose general toxicology studies, in silico evaluation of mutagenic potential for cobicistat, or previous consideration of genotoxicity for the marketed products (i.e., emtricitabine and tenofovir disoproxil fumarate). Impurities are reviewed in Appendix C.

2.6 Proposed Clinical Population and Dosing Regimen: This NDA is 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 (E/C/F/T). 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.

2.7 Regulatory Background: This application is being submitted in support of a NDA for a film-coated single tablet regimen 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 referred as E/C/F/T. As the EVG and COBI components are new chemical entities, this NDA contains full data (except for carcinogenicity studies for COBI) on these two components. Per the agreement reached at the 13 July 2011 Type B (preNDA) meeting between Gilead and the FDA (refer to the Agency's comments, dated 08 July 2011; m1.6.3), results of nonclinical studies with FTC (IND 53,971, NDA 21-500 and NDA 21-896), TDF (IND 52,849, NDA 21-356 and NDA 22-577), and FTC/TDF (IND 67,671 and NDA 21-752) have been previously submitted to FDA and are incorporated by right of reference.

3 Studies Submitted: Comprehensive nonclinical toxicology programs were undertaken in support of EVG and COBI. These studies have characterized the acute toxicity, subchronic/ chronic toxicity, mutagenicity, carcinogenicity (studies with COBI are ongoing), and reproductive toxicity of each individual agent. Studies with EVG and COBI are reviewed in Appendix A and Appendix B, respectively. FTC and TDF are approved drug products. A combination of FTC/TDF, (Truvada) is also an approved drug product. Comprehensive nonclinical pharmacology, pharmacokinetic, and toxicology programs were undertaken in support of the registration of FTC, TDF and Truvada. Those studies are reviewed in their respective NDAs. Nonclinical studies on impurities of EVG. COBI, FTC and TDF are reviewed in Appendix C.

The absence of nonclinical safety studies with the E/C/F/T combination is in accordance with the ICH M3(R2) guidance, FDA Guidance for Industry, Nonclinical Safety Evaluation of Drug or Biologic Combinations, March 2006 and the CHMP Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/2005, January 2008). There are no anticipated relevant pharmacokinetic or toxicological interactions expected in the E/C/F/T combination beyond the anticipated pharmacokinetic boosting of EVG by COBI.

The well-characterized toxicity profiles of EVG, COBI, FTC, and TDF; the low potential for toxicologic interaction noted in the combination toxicology studies with EVG+COBI and FTC+TDF; along with the clinical safety data available for the approved agents FTC, TDF, the FTC/TDF (Truvada); and the clinical data from Phase 1, 2, and 3 studies conducted with the E/C/F/T combination jointly support a favorable benefit/risk profile for the proposed use of this new product for the treatment of HIV-1 infection in adults.

A small number of key studies were conducted using the combinations of EVG+ COBI, FTC+TDF, and E/C/F/T. The overall program, including the data from the combination and individual agent studies, is considered sufficient to support the safety of the E/C/F/T combination tablet.

3 Studies Submitted:

Pharmacokinetics/ADME/Toxicokinetics

Pharmacokinetics of EVG, FTC, TDF and GS-9350 after Oral Dosing in Various Formulations in Beagle Dogs (ad-216-2061)

3.1 Studies Reviewed

Above listed study was reviewed

3.3 Previous Reviews Referenced

Appendix A (EVG), Appendix B (COBI) and Appendix C (Impurities of EVG, COBI, FTC, and TDF) contain the relevant reviews for the NME drug products as well as drug impurities. FTC and TDF and FTC/TDF (Truvada) reviews in their respective NDAs.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Pharmacokinetics of EVG, FTC, TDF and GS-9350 after Oral Dosing in Various

Formulations in Beagle Dogs (AD-216-2061). The purpose of the present study was to evaluate the pharmacokinetics of EVG, FTC, TFV, and COBI following oral (po.) administration of solid dose forms of EVG (37.5 mg), FTC (50 mg), TDF (75 mg), and COBI (25 mg) in dogs. The comparative exposures of elvitegravir (EVG), emtricitabine (FTC), tenofovir disoproxil fumarate (TDF) and GS-9350(COBI) were evaluated by dosing the agents orally in pentagastrin pretreated male beagle dogs (6 animals/group). Pharmacokinetic data are shown in Table 1.

Table 1 - Pharmacokinetics of EVG, COBI, FTC, and TFV after Oral Dosing in Various Formulations in Beagle Dogs

Report Title				Study Type				Test Article				Report Number			
Pharmacokinetics of EVG, FTC, TDF, and COBI After Oral Dosing in Various Formulations in Beagle Dogs				Formulation Comparison (Combination tablet)				EVG, FTC, TDF, COBI				AD-216-2061			
Plasma Pharmacokinetic Parameters (Mean ± SD, n = 12)	EVG 37.5 mg, TDF 75 mg, FTC 50 mg, and COBI 25 mg Coadministered				EVG 37.5 mg, TDF 75 mg, FTC 50 mg, and COBI 25 mg (Bilayer Tablet)				EVG 37.5 mg, TDF 75 mg, FTC 50 mg, and COBI 25 mg (Trilayer Tablet)							
	EVG	COBI	FTC	TFV	EVG	COBI	FTC	TFV	EVG	COBI	FTC	TFV				
t_{max} (h)	2.5 ± 1.2	1.2 ± 0.9	1.5 ± 0.9	1.2 ± 1.0	2.7 ± 1.2	1.6 ± 0.5	1.5 ± 0.5	1.3 ± 0.6	2.8 ± 1.1	2.2 ± 2	2.3 ± 2	1.5 ± 0.9				
C_{max} (ng/mL)	192 ± 76	113 ± 115	2859 ± 1132	815 ± 574	230 ± 104	126 ± 122	3618 ± 1087	974 ± 497	273 ± 87	154 ± 138	2804 ± 1022	979 ± 596				
AUC_{0-t} (ng•h/mL)	1083 ± 427	225 ± 248	10,578 ± 3884	3266 ± 1891	1433 ± 721	265 ± 239	14,109 ± 5002	3608 ± 1199	1559 ± 641	292 ± 243	11,176 ± 2961	3459 ± 1609				

COBI = cobicistat; EVG = elvitegravir; FTC = emtricitabine; TDF = tenofovir disoprixil fumarate; SD = standard deviation; AUC = area under the plasma concentration-time curve; t_{max} = time to reach the maximum plasma concentration

Conclusion: Comparing oral coadministration of EVG, FTC, TDF, and GS-9350 as multiple independent tablets, and as bilayer (b) (4) single tablet combinations, reveals that similar exposures of all four agents were achieved.

11 Integrated Summary and Safety Evaluation

The E/C/F/T tablet contains the same dosages of FTC and TDF that are currently approved within Viread®, Emtriva®, Atripla®, and Truvada® for use in adults (200 mg of FTC and 300 mg of TDF). Comprehensive programs of nonclinical studies with EVG, COBI, FTC, TDF and TDF/TFV have been conducted. All nonclinical studies for EVG and COBI are reviewed and included in Appendix A (for EVG), Appendix B (for COBI) and Appendix C (for Impurities) with the exception of a combination toxicology study (included in this review). All of the definitive safety pharmacology, toxicology, and toxicokinetic studies for EVG, COBI, FTC, TDF, and FTC/TDF were conducted in accordance with guidelines issued by the International Conference on Harmonization and with Good Laboratory Practice.

Although COBI showed the potential for cardiotoxicity (decreases in LV function and prolong the PR interval) in isolated rabbit hearts, follow up data from clinical trials did not reveal clinically-significant changes in these parameters at the proposed dosage of COBI. Given the lack of effects for EVG, FTC and TDF on the cardiovascular system, the potential for cardiovascular effects with the E/C/F/T tablet is considered low.

The E/C/F/T combination is not anticipated to produce any new human metabolites. Significant off-target pharmacokinetic interactions are unlikely given that the target organ profiles are different.

Administration of the combination product is unlikely to exacerbate known toxicities of the individual agents. Although NRTIs carry a class labeling for mitochondrial toxicity, FTC and TDF have been shown to have a low potential for mitochondrial toxicity in *in vitro* studies and long-term toxicity studies. Since EVG is not anticipated to significantly increase the exposure of FTC or TDF, the potential for exacerbating mitochondrial toxicity is low.

Nonclinical studies with EVG have not identified any specific target organ toxicities or cause for concern. EVG related changes in the cecum and upper small intestine in rats and dogs were due to high local concentrations and were not considered adverse. Potential toxicities related to COBI observed in nonclinical toxicology studies (hematology, clinical chemistry, and urinalysis changes; lower IgG antibody titers; and adaptive liver and thyroid changes) have not been observed in clinical studies with E/C/F/T. The only toxicity observed in chronic animal studies with FTC was mild, reversible anemia at large multiples of clinical exposure; therefore, these hematological findings are not considered relevant to clinical use.

Emtricitabine (FTC) has an established clinical safety profile with slight toxicological effects (mild anemia) noted only at exposure levels above anticipated human clinical exposures. The principal target organs of toxicity in animals following oral administration of TDF were the kidney (karyomegaly, tubular degeneration), bone, and GI tract (in rodents). These findings correlate with the known clinical toxicities for TDF (renal and bone toxicity).
Combination toxicity studies with EVG+COBI or FTC+TDF did not reveal any new or additive toxicities.

COBI alone was associated with nonadverse urinalysis and urine chemistry changes (diuretic effects) at high doses in rats and dogs, which were reversible. COBI also caused serum creatinine and BUN changes, but without morphological evidence of kidney damage. A potential COBI+TDF interaction was suggested by a slight increase in probable cases of renal damage in the COBI+TDF clinical trial arms compared to the RTV+TDF clinical trial arms. COBI may increase the local exposures of TDF in the kidney and exacerbate TDF renal toxicity. (b) (4)

The mild hematological changes with FTC are unlikely to cause an overlapping toxicity with COBI which was associated with minimal decreases in red blood cell parameters in rats at exposures 5- to 8-fold higher than clinical exposures.

Gastrointestinal toxicity is dose limiting in rodents for TDF, and was due to high local concentrations of TDF. The changes in the cecum and upper small intestine in rats and dogs by EVG are not considered adverse or relevant for humans and should not result in overlapping toxicity.

COBI, EVG, and FTC have not shown any potential for bone toxicity. Exacerbation of any TDF effects on bone is not expected.

Of the E/C/F/T products, only TDF had positive findings in genotoxicity studies. Although EVG was equivocal in one *in vitro* study, it was negative in 2 *in vivo* studies. Therefore, EVG is unlikely to induce chromosome aberrations *in vivo*. The combination of FTC and TDF in a mouse lymphoma cell assay did not exacerbate the genotoxic potential of TDF. The E/C/F/T combination is not anticipated to alter the genotoxicity profiles of the individual agents.

EVG, FTC, and TDF demonstrated low carcinogenic potential in the 2-year bioassays. Although carcinogenicity studies with COBI are ongoing, it is considered unlikely that combination dosing would change these profiles. No exacerbation of carcinogenic potential is expected.

EVG, COBI, FTC, and TDF alone did not have significant adverse effects in reproductive and developmental toxicity studies. The E/C/F/T combination is not expected to have an altered reproductive toxicity profile compared with that of the individual agents.

The majority of the identified impurities and degradants have been assessed as part of the routine toxicology or qualification studies with the individual agents, with the FTC/TDF combination, and with the EVG/COBI layer of the bilayer EVG/COBI/FTC/TDF tablet. All impurities or degradants of concern will be controlled at acceptable levels.

The lack of overlapping toxicity in animals, along with clinical data with EVG and COBI, and the E/C/F/T tablet support the overall risk/benefit of E/C/F/T tablet.

Based on findings in the nonclinical studies, we agree with the Sponsor that the key safety points for consideration that are related to EVG, COBI, FTC, or TDF include: (1) potential for bone loss upon chronic dosing due to TDF, (2) potential for renal toxicity due to TDF, especially related to use with other drugs that have been shown to cause renal toxicity and in patients with

renal impairment (3) decreases in estimated creatinine clearance due to COBI (with no clinical correlates), (4) use in patients with hepatic impairment, (5) the potential for CYP3A associated drug interactions, (6) use during pregnancy and lactation, (7) potential for mitochondrial toxicity, (8) potential for carcinogenicity and , (9) potential for PR interval prolongation and decreased left ventricular function due to COBI. An additional concern (10) is the potential for COBI+ TDF drug/drug interactions which might exacerbate TDF renal exposure and renal toxicity

12. Appendix/Attachments:

Appendix A: EVG Review

Appendix B: COBI Review

Appendix C: EVG, COBI, FTC and TDF Impurities Review

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	203-100 (Appendix A)
Supporting document/s:	1
Applicant's letter date:	October 26, 2011
CDER stamp date:	October 27, 2011
Product:	Elvitegravir 150 mg/Cobicistat 150 mg/Emtricitabine 200 mg/Tenofovir DF 300 mg Tablet (E/C/F/T) Appendix A: Elvitegravir 150 mg
Indication:	Treatment of HIV-1 infection
Applicant:	Gilead Sciences, Inc.
	333 Lakeside Drive
	Foster City, CA 94404
Review Division:	DAVP
Reviewer:	Pritam Verma, Ph.D.
Supervisor/Team Leader:	Hanan Ghantous, Ph.D., DABT
Division Director:	Debra Birnkrant, M.D.
Project Manager:	Stacey Min, Pharm.D.

Disclaimer

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1 Executive Summary

1.1 Introduction: Elvitegravir (EVG; GS-9137; JTK-303) is a new chemical entity that belongs to the class of human immunodeficiency virus-1 (HIV-1) integrase strand-transfer inhibitors. EVG prevents integration of HIV-1 genetic material into the host-cell genome. The integration of the viral genome into the host cell genome is an essential step of the HIV-1 replication cycle. This NDA is submitted for a single tablet regimen that contains a fixed-dose combination of EVG 150 mg, cobicistat 150 mg (COBI), Emtriva 200 mg (FTC) and Viread 300 mg (TDF), for the treatment of HIV-1 infection. Thus, EVG 150 mg as one of the components of the E/C/F/T, being evaluated for the treatment of HIV-1 infection.

1.2 Brief Discussion of Nonclinical Findings: The safety pharmacology, pharmacokinetics, general toxicology (single- and repeat-dose), carcinogenicity, reproductive and developmental toxicology, and special toxicology were characterized in a variety of animal models. Genotoxicity studies were conducted in *in vitro* and *in vivo* assays. Combination toxicology studies of EVG with COBI or ritonavir (increases the systemic exposures of co-administered agents) were also conducted. The rat and dog were the appropriate animal models for the toxicology studies because of the similar disposition of EVG in humans.

Elvitegravir showed modest bioavailability in rats (30% to 35%) and dogs (27% to 33%). In rats, EVG was rapidly absorbed and widely distributed. Binding to human plasma and purified human albumin was high (≥ 99.3%). Elimination from tissues paralleled that from plasma and was complete by 96 hours after dosing. EVG was extensively metabolized by oxidation, glucuronidation and combinations of the two. The most abundant metabolites were common between mouse, rat, rabbit, dog and human. The predominant metabolite was GS-9202 followed by GS-9200 and JTP-74488 (a glucuronide of GS-9202). However, the parent EVG accounted for majority of the radioactivity in plasma.

Elvitegravir had a low potential for drug interactions through inhibition of human cytochromes P450 or P-glycoprotein. Metabolism of EVG by human hepatic microsomal fraction was reduced by CYP3A inhibitors, such as COBI and ritonavir.

In single oral dose toxicology studies in rats and dogs, no lethality was seen at dose levels of 2000 mg/kg or 1000 mg/kg, respectively. A series of GLP oral repeat-dose toxicology studies were conducted with EVG in mice (13-week), rats (4-, 13- and 26-week), and dogs (4- and 39-week). In the repeat-dose toxicology studies, two treatment-related effects included (1) the presence of lipid vacuoles in the lamina propria of the upper small intestines, and (2) changes in cecum weights and dilation of the cecum in rats and dogs.

In rats, cecal weights and/or its contents were increased at doses ≥ 300 mg/kg/day, with dilatation of the cecum observed at ≥ 1000 mg/kg/day (3-, 6-month studies). In dogs, dilatation of the cecum was observed in males at 100 mg/kg/day only in the 4-week repeat-dose study. These observations were not accompanied by any histological changes in the cecum or GI adverse events. Similar changes in the cecum have been reported with antibacterial quinolones which affect the GI microflora. Elvitegravir has a quinolone moiety and was confirmed to have antibacterial activity in a reverse mutation assay (23.4 μg/plate) as part of the genotoxicology

studies. Although the activity was much weaker than that of the antibacterial quinolones, the changes in the cecum were considered to be due to the antibacterial activity of high local concentrations of EVG in the GI tract.

Lipid-like vacuoles were observed in the lamina propria in the upper small intestine (duodenum and/or jejunum) in rats, with increased incidence and severity at doses ≥ 1000 mg/kg/day. The incidence and severity did not increase with long term dosing, and there was no evidence of toxicity or any adverse tissue reactions associated with these vacuoles. The cause of the vacuolization was considered to be related to the high local EVG concentrations to which the GI epithelium was exposed. In a series of mechanistic studies (2-week repeated oral dose toxicity study in rats with 1-, 2-, and 8-week recovery period), the vacuoles were shown to contain mainly triglycerides, tended to disappear slowly after withdrawal of the treatment with EVG. The vacuoles formation may be related to the lipid absorption process in the GI tract, although there were no changes in plasma lipid parameters or adverse clinical observations. In dogs, lipid vacuoles containing mainly triglycerides were observed in the upper small intestinal lamina propria in both sexes at doses of 30 (mid) and 100 mg/kg/day (high) in the 39-week study. Similar to rats, these observations were also not accompanied by any GI adverse events or histological changes in the cecum or the small intestines, and they were not considered adverse. Furthermore, in the carcinogenicity study in rats, there were no notable findings in the upper small intestine, suggesting that the presence of the vacuoles was not adverse.

The NOAELs for EVG were determined to be 2000 mg/kg/day in mice and rats, and 100 mg/kg/day for dogs. The exposures based on plasma AUC values at the NOAELs in the animals were approximately 2- to 3-fold (mice), 20- to 36-fold (rats), and 2- to 3-fold (dogs) higher than the AUC in patients treated once daily with EVG150 mg as part of the E/C/F/T.

There were no significant adverse effects observed in a 13-week combination toxicity study conducted in rats with EVG alone, COBI alone, or the combination of EVG and COBI. The combination of 1000 mg/kg/day EVG with 30 mg/kg/day COBI or with 10 mg/kg/day ritonavir (90-day study in rats) did not result in new or additive toxicity.

Elvitegravir induced a slight increase in the number of cells with chromosomal structural aberrations at levels greater than or equal to 55 μg/mL in Chinese Hamster Lung cells when tested in the 6-hour treatment without metabolic (S9) activation. However, no evidence of chromosomal aberrations was observed after 24-hour treatment without S9 up to 45 μg/mL, or in the presence of S9 up to 175 μg/mL. EVG may have a weak potential to induce chromosomal aberrations. Elvitegravir was negative for mutagenic potential in a bacterial reverse mutation test (Ames). In a micronucleus test in rats, single oral administration of EVG up to a dose of 2000 mg/kg (Cmax 43.5 μg/mL in males and 68.3 μg/mL in females) did not show any evidence of genotoxic activity for induction of chromosome damage.

Long-term carcinogenicity studies in mice (2-year) and rats (88-90 weeks) with EVG showed no carcinogenic potential at exposures 2- to 4-fold (mice) and 12- to 27-fold (rat) greater than the exposure observed in humans at the EVG 150 mg as part of the E/C/F/T. In the mouse study, high-dose EVG (2000 mg/kg/day) was also dosed in combination with ritonavir (25 mg/kg/day).

No drug-related increases in tumor incidence were noted in these animals at exposures approximately 14-fold the human systemic exposure at the therapeutic EVG dose.

There were no EVG-related significant adverse effects observed in fertility studies in male and female. The NOAEL for reproductive parameters in the fertility studies was 2000 mg/kg/day in male and female rats at exposures approximately 16.5- to 30-fold higher than human therapeutic exposure. In embryo-fetal developmental studies in rats, there were no effects on Caesarean-sectioning, litter parameters at dose levels up to 2000 mg/kg/day. The maternal and fetal NOAEL for EVG was 2000 mg/kg/day at exposures 23-fold higher than human therapeutic exposure. In a combination study with EVG and ritonavir in rats, the maternal and fetal NOAELs were 10 mg/kg/day ritonavir and 1000 mg/kg/day EVG when administered separately or in combination at exposures approximately 8-fold higher EVG than therapeutic exposure. In rabbits, the maternal NOAEL of EVG was 50 mg/kg/day. The 150 and 450 mg/kg/day dosages were associated with reduced body weight gains and feed consumption during the post-dosage period. There were no adverse effects on embryo-fetal development and the developmental NOAEL was 450 mg/kg/day at exposures 0.2-fold higher than human therapeutic exposure.

In rats, in the perinatal/postnatal reproduction toxicity study, including the postnatal behavioral/functional evaluation, there were no adverse effects at dosages up to 2000 mg/kg/day. The maternal NOAEL for general toxicity of EVG and the NOAEL for reproduction in the dams and viability and growth in the offspring were 2000 mg/kg/day at exposures 18-fold higher than human therapeutic exposures. In the juvenile toxicity evaluation portion of the study, the only drug-related observation was increased cecum weights at 2000 mg/kg/day (high) for male rats and at 1000 (mid) and 2000 mg/kg/day (high) for female rats. There were no histopathological correlates for this finding in the rat. The NOAEL for toxicity of EVG was 2000 mg/kg/day for juvenile animals at exposures 7-fold higher than human therapeutic exposures.

The overall nonclinical program of EVG, including the data from the combination of COBI and EVG studies were considered adequate to support the safety of EVG as part of the E/C/F/T.

1.3 Recommendations

1.3.1 Approvability: There are no nonclinical pharmacology and toxicology issues which would preclude the approval of EVG 150 mg as part of the E/C/F/T.

1.3.2 Additional Non Clinical Recommendations: None

1.3.3 Labeling: See E/C/F/T NDA

2 Drug Information

2.1 Drug

CAS Registry Number: 697761-98-1

Generic Name: Elvitegravir

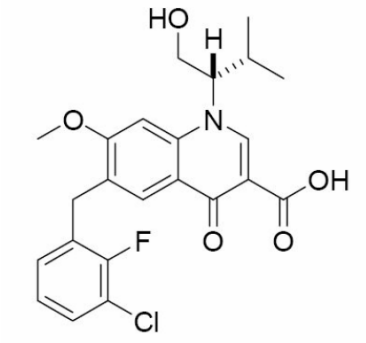
Code Name: GS-9137; JTK-303; EVG

Chemical Name: 3-Quinolinecarboxylic acid, 6-[(3-chloro-2-fluorophenyl)methyl]-1,4-dihydro-1-[(1S)-1-(hydroxymethyl)-2-methylpropyl]-7-methoxy-4-oxo-

Molecular Formula/Molecular Weight: $C_{23}H_{23}ClFNO_5$/447.88

Structure or Biochemical Description:

Figure 5. Structure of EVG



Pharmacologic Class: HIV-integrase inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs:
Elvitegravir: IND (b) (4), DMF 025187; Cobicistat: IND (b) (4), DMF 025188; Emtricitabine: IND 53,971, NDA 21-500, NDA 21-896; Tenofovir DF: IND 52,849, NDA 21-356, NDA 22-577; Emtricitabine/Tenofovir DF: IND 67,671 and NDA 21-752.

2.3 Drug Formulation: E/C/F/T tablets contain 150 mg of EVG, 150 mg of COBI, 200 mg of FTC, and 300 mg of TDF. Each tablet contains the following inactive ingredients: croscarmellose sodium, hydroxypropyl cellulose, indigo carmine (FD&C Blue #2) aluminum lake (b) (4), lactose monohydrate, magnesium stearate, microcrystalline cellulose, polyvinyl alcohol, polyethylene glycol, silicon dioxide, sodium lauryl sulfate, talc (b) (4), titanium dioxide (b) (4), and yellow iron oxide (b) (4).

2.4 Comments on Novel Excipients: None

2.5 Comments on Impurities/Degradants of Concern: Over 18 impurities and degradation products related to EVG were identified in batches of the active pharmaceutical ingredient or drug product. The multiple GLP batches of EVG tested in the toxicology program were considered to be representative of the GMP material. The proposed specifications for impurities in the EVG drug substance were deemed acceptable based on repeat dose general toxicology

studies, in silico evaluation of mutagenic potential, and the serious nature of the clinical indication. The proposed specifications for residual solvents were also deemed acceptable based on calculated PDE values or those listed in ICH Q3C(R5). A compound specific risk assessment was made for (b) (4) a potentially genotoxic impurity. Using carcinogenicity data for this impurity, a TTC (b) (4) was calculated. The impurities have been reviewed in Appendix C.

2.6 Proposed Clinical Population and Dosing Regimen: This NDA is for a single-tablet regimen that contains a fixed-dose combination of EVG, COBI, FTC and TDF (150/150/200/300 mg) tablet. The proposed indication for the E/C/F/T tablet is for use once daily as a complete regimen for the treatment HIV-1 infection in adults.

2.7 Regulatory Background: EVG and COBI components are new chemical entities. Emtricitabine and Tenofovir DF are approved products. Only EVG is discussed in Appendix A.

3 Studies Submitted:

Primary Pharmacology: See microbiology review.

Secondary Pharmacodynamics
In vitro evaluation of the effect of GS-9137 on mitochondrial DNA content in HepG2 liver cells (TX-183-2009)
Activity of GS-9137 against Cellular Homologs (PH-004)
In Vitro Receptor Binding Potencies of GS-9137 (PH-008)
Cytotoxicity of GS-9137 in Human Cells (PH-010)

Safety Pharmacology
Effects of JTK-303 on the Central Nervous System in Rats (JTK303-SP-001)
Effects of JTK-303 on hERG Current (JTK303-SP-003)
Effects of JTK-303 on Action Potential Parameters in Isolated Guinea Pig Papillary Muscle (JTK303-SP-004
Effects of JTK-303 on the Cardiovascular and Respiratory Systems in Conscious Dogs (JTK303-SP-002)
Effect of GS-9137 on Gastrointestinal system-Effect of JTK-303 on Acetylcholine-Histamine- and BaCl2-induced Contraction in Isolated Guinea Pig Ileum (JTK303-SP-005)
Effect of JTK-303 on Gastrointestinal Transport of Charcoal Meal in Rats (JTK303-SP-006)
Effect of GS-9137 on Renal/urinary System-Effect of JTK-303 on Urine Volume and Urinary Electrolytes Excretion in Saline-loaded Rats (JTK303-SP-007)

Pharmacokinetics/ADME/Toxicokinetics
PK/ADME
Absorption
Pharmacokinetics in Rats after Single Administration of ^{14}C-JTK-303 (JTK303-AD-005)
Pharmacokinetics in Dogs after Single Administration of ^{14}C-JTK-303 (JTK303-AD-006)
Calculation of Pharmacokinetic Parameters of Plasma Radioactivity Concentration in Rats after Oral or Intravenous Administration of ^{14}C-JTK-303 (JTK303-AD-007)

Calculation of Pharmacokinetic Parameters of Plasma Radioactivity Concentration in Dogs after Oral or Intravenous Administration of ^{14}C-JTK-303 (JTK303-AD-008)
Collection of Plasma from Rats and Determination of concentration of JTK-303 in Plasma (JTK303-AD-009)
Collection of Plasma from Dogs and Determination of concentration of JTK-303 in Plasma (JTK303-AD-010)
Pharmacokinetic Analysis of the Plasma Parent Drug Concentration in Rats after Oral or Intravenous Administration of JTK-303-AD-009 andJTK303-AD-011)
Pharmacokinetic Analysis of the Plasma Parent Drug Concentration in Dogs after Oral or Intravenous Administration of JTK-303 (JTK-303-AD-012)
Pharmacokinetics study of JTK-303 in rats after repeated oral administration of ^{14}C-JTK-303 (AD-022)
Analysis of Pharmacokinetic Parameters of the Plasma Radioactivity Concentration in Rats Treated with Repeated Oral Administration of ^{14}C-JTK-303 (AD-028)
Comparative Studies on Dissolution and Oral Absorption between JTK-303 Tablets, 50 mg and JTK-303 Tablets, 50 mg (SD) (JTKJ03-P2- 102)

Distribution

Protein Binding of JTK-303 in Vitro (JTK303-AD-014)
Ex Vivo plasma protein binding determination of GS-9137 in CD-1 mice receiving once daily oral administration of GS-9137 for seven days ((b) (4) Study No: 60N-0630).
Distribution of JTK-303 into Blood Cells in Vitro (JTK303-AD-013)
GS-9137: Quantitative Tissue Distribution of Drug-Related Material Using Whole- Body Autoradiography Following a Single Oral Dose of [^{14}C]GS-9137 With or Without Ritonavir to Male and Female Sprague Dawley Rats ((b) (4) Study No. 60N-0518)

Metabolism

Metabolite Profiling and Identification of [^{14}C]-GS-9137/GS-9137 in pooled female new Zealand Rabbit Liver Microsomes (Report No.: 60N-0508)
In Vitro Metabolism of **^{14}**C-JTK-303: Oxidative Reaction in Liver Microsomes (JTK303-AD-015)
In Vitro Metabolism of ^{14}C-JTK-303 (Glucuronide Conjugation in Liver Microsomes) (AD-016)
Metabolite Profiling of Biological Samples from Rats after Administration of ^{14}C-JTK-303 (JTK303-AD-019)
Metabolite Profiling of Biological Samples from Dogs after Administration of ^{14}C-JTK-303 (AD-020)
Identification and Characterization of Metabolites of **^{14}**C-JTK-303 *In Vivo* and *In Vitro* Samples (JTK-303-AD-021)
Determination of in vitro metabolic stability of [^{14}C]-GS-9137 in mouse liver microsomes (ad-183-2019)
Determination of activities of NADPH-Cytochrome P450 Reductase and cytochrome P450 isozymes in mouse liver microsomes from commercial source and mouse treated with GS-9137 (ad-183-2021)
In Vitro Study of JTK-303 [II] Determination of Km and Vmax Using Human Liver Microsomes (AD-024)

Excretion

Pharmacokinetics study of JTK-303 in rats after repeated oral administration of 14C-JTK-303 (AD-022)

Pharmacokinetics in Rats after Single Administration of ^{14}C-JTK-303 (JTK303-AD-005)
Pharmacokinetics in Dogs after Single Administration of ^{14}C-JTK-303 (JTK303-AD-006)
Please see review of Excretion studies in the Absorption section.

Pharmacokinetic drug interactions
UDP-Glucuronosyl Transferase Phenotyping of Elvitegravir (AD-183-2034)
In Vitro Assessment of Inhibition of Human Elvitegravir Glucuronidation by Ketoconazole (AD-183-2028)
In Vitro Metabolism Study of JTK-303 Enzyme Inhibition Study Using Human Liver Microsomes: Determination of IC_{50} (AD-027)
In Vitro Study of JTK-303: Interaction Study of JTK-303 with Coadministered Drugs (AD-025)
Determination of activities of NADPH-Cytochrome P450 Reductase and cytochrome P450 isozymes in mouse liver microsomes from commercial source and mouse treated with GS-9137 (ad-183-2021) see Metabolism section

General Toxicology
Single dose toxicity
Single Oral Dose Toxicity Study of JTK-303 in Rats (TX-001)
Single Oral Dose Toxicity Study of JTK-303 in Dogs (TX-002)
Repeat-dose toxicity
GS-9137: A 13-Week Oral Gavage Toxicity and Toxicokinetic Study in Mice (TOX-183-2004)
One month oral toxicity study of JTK-303 in rats (TOX-003)
Three-Month Oral Dose Toxicity Study of JTK-303 in Rats with a Three-Month Recovery Period (TX-021)
Six month oral dose toxicity study of JTK-303 in rats (TX-022)
Ninety-day Oral Gavage Bridging Study with GS-9137 and Ritonavir in Rats (TOX-183-2007)
Thirteen-Week Oral Gavage Bridging Study with GS-9350 and GS-9137 in Rats with a 1-Month Recovery Period (TOX-236-2001)
One month oral toxicity study of JTK-303 in dogs (TOX-004)
Nine-month oral dose toxicity study in dogs with a 3 month recovery period (TOX-023)
Genetic Toxicology
Bacterial reverse mutation test of JTK-303 (Study No. 03913)
Chromosomal aberration test of JTK-303 in cultured mammalian cells (Study No. 03912)
Micronucleus test of JTK-303 in rats (TOX-007)
Carcinogenicity
A 104-Week oral gavage carcinogenicity study of GS-9137 with or without ritonavir in the Albino mouse (TX183-2011)
A 104-Week oral gavage carcinogenicity study of GS-9137 in rats (TX183-2012)

Reproductive and developmental toxicology
Fertility and Early Embryonic Development
Oral fertility and early embryonic development study of JTK-303 in female rats (TX-019)
Oral fertility and early embryonic development study of JTK-303 in male rats (TX-183-2003)
Embryonic Fetal Development
Study for Effects of JTK-303 on Embryo-Fetal Development in Rats (TX-020)

Oral Gavage Study for Effects on Embryo-fetal Development and Toxicokinetics with GS-9137 and Ritonavir in Rats (TX-183-2008)
Oral (stomach tube) development study of JTK-303 in rabbits (TOX-183-2002)

Prenatal and Postnatal Development

Oral (Gavage) Developmental and Perinatal/Postnatal Reproduction Toxicity Study of GS-9137 in Rats, Including a Postnatal Behavioral/Functional Evaluation and a 28-Day Juvenile Toxicity Evaluation (TX-183-2006)

Special Toxicology Studies

Two week oral dose toxicity study in rats with 1-, 2- and 8-week recovery period (TX-018)
Two-Week Repeated Oral Dose Study with Low Dose Levels of JTK-303 in Rats with Two-, Eight- and Sixteen-Week Recovery Period (TX-024)
Four-Week Repeated Oral Dose Study of JTK-303 in Rats-Sequential Evaluation of JTK-303-Induced Histopathological Findings in the Small Intestine (TX-025)
Two-Week Repeated Oral Dose Study of JTK-303 in Rats - Sequential Evaluation of JTK-303-Induced Histopathological Findings in the Small Intestine (TX-026)
Three-Day Oral Dose Study of JTK-303 in Spaced-Fed Rats (JTK303-TX-027)
Two-Week Dietary Administration Study of JTK-303 in Rats (TX-028)
Ultrastructural Analyses of JTK-303-Induced Histopathological Findings in the Small Intestine in Rats after a Single Oral Dose of JTK-303 (JTK303-TX-029)

Impurities Toxicology

28-day Oral Gavage Bridging Study with GS-9137 in Female Rats (TOX-183-2010)
28-Day Oral Gavage Qualification Study with GS-9137 in Rats (TX-183-2023).
In Silico Evaluation of Potential Genotoxicity and Carcinogenicity for Intermediates and Impurities of GS-9137 (TX-183-2024).
28-Day Oral Gavage Qualification Toxicity and Toxicokinetic Study with GS-9350/GS-9137 Tablets in Rats (TX-236-2002).

Local Tolerance Studies

GS-9137: The bovine corneal opacity and permeability assay (TX-183-2021)
GS-9137: Skin Irritation to the Rabbit (TX-183-2020)

Immunotoxicity

Four week oral gavage immunotoxicity study in rats (Tox-183-011)

Other Toxicity Studies

Single Intravenous Dose Toxicity Study of JTK-303 in Dogs (TX-008)
Study of Synergistic Effects of JTK-303 and Nonsteroidal Anti-Inflammatory Drugs (NSAID) in Mice (JTK303-TX-009)
GS-9137: Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (Individual animal approach) (TX-183-2022)
Single Oral Dose Phototoxicity Study of JTK-303 in Mice (JTK-303-TX-010)

3.1 Studies Reviewed

Above listed studies were reviewed

3.2 Studies Not Reviewed
Exploratory studies were not reviewed

3.3 Previous Reviews Referenced
None

4 Pharmacology

4.1 Primary Pharmacology: See Microbiology review

4.2 Secondary Pharmacology

In vitro evaluation of the effect of GS-9137 on mitochondrial DNA content in HepG2 liver cells (TX-183-2009). In an *in vitro* experiment, HepG2 human hepatoma cells were incubated with GS-9137 at concentrations ranging from 10 to 300 μM for 14 days for its effect on mitochondrial DNA levels in the cells. Mitochondrial DNA was analyzed by a dot-blot hybridization method. Following a 14 day treatment, GS-9137 induced no measurable changes in the content of mitochondrial DNA.

Activity of GS-9137 against Cellular Homologs (PH-004). There is no functional equivalent to integrase (IN) activity in host cells. However, IN and topoisomerases display the analogous activities of deoxyribonucleic acid (DNA) binding, DNA cleavage, and transesterification reactions. The effects of EVG on human topoisomerase I and II activities were examined by measuring the conversion of supercoiled DNA to the relaxed form. Elvitegravir did not inhibit the activity of human topoisomerase I and II enzymes at up to 50 and 150 μM, respectively (Table 2). The control compounds camptothecin and amsacrine inhibited topoisomerase I and II, with EC_{50} values of 9.8 and 105 μM, respectively.

Table 2. Effect of EVG Against Human Topoisomerase Activities in DNA Relaxation Assays

Enzyme	IC_{50} (μM)[a]		
	EVG	Camptothecin	Amsacrine
Human topoisomerase I	> 50 (–3.2 ± 2.1%)[b]	9.8 ± 2.2	ND
Human topoisomerase II	> 150 (2.4 ± 2.5%)[c]	ND	105.1 ± 8.0

EVG = elvitegravir; ND = not done

a Mean ± standard deviation from 3 separate experiments

b % inhibition at 50 μM, Mean ± standard deviation

c % inhibition at 150 μM, Mean ± standard deviation

Source: Report JTK303-PH-004

***In Vitro* Receptor Binding Potencies of GS-9137 (PH-008).** The effects of EVG on 22 receptors, 7 enzymes, and 3 cell-based assay systems, including the immune cell functions of cell adhesion (ICAM-1/VCAM-1 mediated), IL-2 secretion, and mixed lymphocyte reaction (splenic

lymphocytes) were evaluated. IC_{50} values were not determined because EVG did not show greater than 50% inhibition at 10 μM in any of these systems.

Cytotoxicity of GS-9137 in Human Cells (PH-010). The cytotoxicity of EVG was evaluated in a number of human cell lines and primary cells. Elvitegravir showed weak cytotoxicity to primary peripheral blood mononuclear cells (PBMC), primary T-lymphocytes primary monocytes/macrophages, and macrophages. The results are presented in Table 3.

Table 3. Cytotoxicity of EVG on Human Cells

Cell Type	Study Duration (Days)	CC_{50} (μM)[a]
Primary Human PBMC[b]	7	> 100
Primary Human T-lymphocytes[c]	5	40
Primary Human Monocytes/macrophages[c]	7	> 500
Human Macrophages[c]	5	25.6

CC_{50} = concentration at which 50% maximum cytotoxicity was achieved

a In the absence of human serum

b MTS-based assay

c XTT-based assay

In a separate study, EVG showed cytotoxicity in a dose-dependent manner after 7 days of culture with PBMCs using a [^{3}H]thymidine incorporation assay, with a CC_{50} value of 9.7 mM (selectivity index [SI] of > 48,000) in the absence of HS and 170 μM in the presence of 50% HS (SI of > 100,000. No difference in the cytotoxicity of EVG was detected in unstimulated versus stimulated PBMCs in the absence of HS (mean CC_{50} values of 10.8 and 16.6 μM in stimulated and unstimulated PBMCs, respectively. In HepG2 and Huh7 cells containing hepatitis B virus (HBV) and hepatitis C virus (HCV), respectively, the EVG CC_{50} values were 16 and >6 μM, respectively

4.3 Safety Pharmacology

Effects of JTK-303 on the Central Nervous System in Rats (JTK303-SP-001). The effects of GS-9137 on the central nervous system, general appearance and behavior in rats were evaluated using the Irwin's multiple observation method. When given orally to SD rats (6 rats/group) at doses of 100, 300, and 2000 mg/kg, GS-9137 did not show any effects on general appearance and behavior of rats up to 24 hours after dosing at any dose level. The study showed that GS-9137 had no effect on the central nervous system of rats at doses up to and including 2000 mg/kg.

Effects of JTK-303 on hERG Current (JTK303-SP-003). The effect of GS-9137 on hERG current was investigated in HEK293 cells, stably expressing the hERG channel, by a whole cell patch clamp method. The relative currents (% of the currents just before treatment) after treatment with GS-9137 at 0 (vehicle control, 0.1% DMSO), 0.1, 1, and 10 μM were 93.1%, 93.0%, 89.9%, and 70.5%, respectively, with a statistically significance being observed at 10

μM. At the maximum concentration tested, GS-9137 inhibited the hERG current by 24.3% compared to the vehicle control. The positive control (E-4031 at 0.1 μM) decreased the hERG current to 8.0% of the current just before treatment. The study showed that GS-9137 slightly decreased the hERG tail current at 10 μM.

Effects of JTK-303 on Action Potential Parameters in Isolated Guinea Pig Papillary Muscle (JTK303-SP-004). The effects of GS-9137 on action potential parameters in isolated guinea pig papillary muscles were investigated. GS-9137 was applied at 0.1, 1, and 3 μM. The following parameters were examined: resting membrane potential (RMP), action potential amplitude (APA), action potential duration at 50% (APD_{50}) and 90% (APD_{90}) of repolarization, and maximal upstroke velocity (Vmax). In addition, (±)-sotalol hydrochloride (30 μM) was applied as a positive control. GS-9137 at all concentrations showed no effect on the RMP, APA, APD50, APD_{90}, or Vmax in isolated guinea pig papillary muscles, while (±)-sotalol hydrochloride (30 μM) as a positive control prolonged APD_{50} and APD_{90} by approximately 20% of the initial values. The study showed that GS-9137 had no effect on action potential parameters in isolated papillary muscle at concentrations up to and including 3 μM.

Effects of JTK-303 on the Cardiovascular and Respiratory Systems in Conscious Dogs (JTK303-SP-002). The effects of JTK-303 on the cardiovascular and respiratory systems, the effects of JTK-303 on blood pressure, heart rate, ECG, respiratory rate, and oxygen saturation in conscious dogs were examined using a telemetry method. JTK-303 was given orally to dogs (4/group) at doses of 10, 30, and 100 mg/kg in ascending order at an interval of 7 days between each of the doses.

JTK-303 did not show any effects on blood pressure, heart rate, ECG (PR interval, QRS duration, QT interval, and QTC), respiratory rate, or oxygen saturation up to 24 hours after administration at any dose. The study showed that JTK-303 had no effect on the cardiovascular and respiratory systems at doses as high as 100 mg/kg.

Effect of JTK-303 on Gastrointestinal system-Effect of JTK-303 on Acetylcholine-Histamine-, and $BaCl_2$-induced Contraction in Isolated Guinea Pig Ileum (JTK303-SP-005). Guinea pigs fasted overnight were sacrificed by exsanguination and the ileum was harvested. The effect of EVG on single contractions induced by acetylcholine (1 μM), histamine (1 μM), or barium chloride (3 mM) were measured, and the effect on resting tone was examined. Elvitegravir at 1.0 and 10 μM had no effect on single contractions by any of the contraction inducers. At 30 μM, inhibition of contractions induced by acetylcholine (9.0%) and barium chloride (19.6%) was observed. Slight inhibition (14.7%) was observed with contractions induced by histamine, but the difference was not significant. For resting tone, EVG showed no differences in the single contraction rate when compared with the addition of vehicle. EVG at a high concentration (30 μM) inhibited single contractions by each of the contraction inducers, but the inhibition was slight, indicating that EVG has no definite effect on the autonomic nervous system or smooth muscle.

Effect of JTK-303 on Gastrointestinal Transport of Charcoal Meal in Rats (JTK303-SP-006). JTK-303 was given orally to SD rats (10/group) at doses of 100, 300, and 2000 mg/kg. Atropine sulfate as a positive control was given orally at a dose of 100 mg/kg. Sixty minutes after the administration of the test article or vehicle and 30 minutes after the administration of the

positive control, animals received a 5% (w/v) charcoal suspension by oral administration. Thirty minutes after charcoal administration the animals were sacrificed, the gastrointestinal tract was dissected and the distance that the charcoal had moved was measured. The charcoal transport rate showed no significant differences between the JTK-303 100, 300 and 2000 mg/kg groups and the vehicle group. Atropine, the positive control, significantly inhibited the transport rate as compared with the vehicle control. The study showed that JTK-303 had no effect on gastrointestinal transport in rats at doses up to and including of 2000 mg/kg.

Effect of JTK-303 on Renal/urinary System-Effect of JTK-303 on Urine Volume and Urinary Electrolytes Excretion in Saline-loaded Rats (JTK303-SP-007). The effects of JTK-303 on urine volume and the urinary excretion of electrolytes (Na, K, Cl) in rats were investigated. JTK-303 was given orally to SD rats (6/group) at doses of 100, 300, and 2000 mg/kg. Furosemide as a positive control was given at a dose of 30 mg/kg. Immediately following dosing, the animals were given an oral loading dose of physiological saline, 25 mL/kg. Urine was collected for 5 hr. No effects on urine volume or urinary electrolytes excretion (Na, K, Cl) were observed in animals treated with JTK-303. In animals receiving the positive control drug, there was increased urine volume and urinary excretion of electrolytes 2-3 times higher than the those in the vehicle control group. The study showed that JTK-303 had no effects on urine volume and urinary excretion of electrolytes (Na, K, Cl) in rats at doses up to and including 2000 mg/kg.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Pharmacokinetics

Single dose pharmacokinetic parameters for JTK-303 were assessed in rats and dogs under fed and fasted conditions. In rats, but not dogs, results suggest that the C_{max} is lowered by a meal, whereas systemic exposures ($AUC_{0\text{-}inf}$) was unaffected (see tables below, excerpted from sponsor).

Table 4. Mean Pharmacokinetic Parameters Following Oral Administration of EVG to Male Rats (Mean ± SD, n = 3)

Dose	1 mg/kg	3 mg/kg	3 mg/kg	10 mg/kg
Feeding Status	Nonfasted	Nonfasted	Fasted	Nonfasted
t_{max} (h)	0.42 ± 0.14	0.25 ± 0.0	0.5 ± 0.0	0.83 ± 0.29
C_{max} (ng/mL)	251 ± 51	755 ± 311	1536 ± 240	1947 ± 971
MRT (h)	3.4 ± 0.7	3.4 ± 0.4	1.6 ± 0.3	4.9 ± 1.6
$AUC_{0\text{-}\infty}$ (ng•h/mL)	643 ± 285	1999 ± 675	1762 ± 215	6825 ± 2455
F (%)	32.9 ± 14.6	34.1 ± 11.5	30.0 ± 3.7	34.9 ± 12.5

Table 5. Effect of Diet on Absorption of JTK-303 in Dogs after Single Oral Administration

Feeding condition	Postprandial	Fasting	P-value
t_{max} (hr)	1.00 ± 0.87	0.83 ± 0.29	NE
C_{max} (ng/mL)	136 ± 61 (1.0)	312 ± 158 (2.3)	0.258
$t_{1/2}$ [1)] (hr)	5.2 ± 2.6	1.7 ± 0.6	NE
$AUC_{0\text{-inf}}$ (ng•hr/mL)	843 ± 73 (1.0)	923 ± 320 (1.1)	0.734

Mean ± SD (n = 3), Dose: 3 mg/kg
The values in parentheses represent a ratio relative to the values of postprandial dogs
Statistical test: Paired t-test
1): Postprandial; t_{max}-24 hr, Fasting; t_{max}-12 hr
NE: Not Examined

Repeat dose pharmacokinetic parameters of JTK-303 were assessed in rats, mice and dogs. In rats, plasma radioactivity concentrations reached steady state approximately on Day 3 of of ^{14}C-JTK-303 administration (see sponsor's table below). The $AUC_{0\text{-inf}}$ ratio (the ratio of first and 7th dosing) was 1.16. Therefore, it was considered that repetitive administration of JTK-303 is unlikely to cause accumulation.

Pharmacokinetics of EVG in mice and dogs were assessed as part of repeat-dose toxicology studies. In general, there were no significant differences in pharmacokinetics following single and multiple dosing. Systemic exposures did not change during repeat dosing, indicating that induction of metabolic elimination pathways did not occur.

Table 6. Pharmacokinetic Parameters of the Plasma Radioactivity Concentration in Rats Treated with Repeated Oral Administration of 14C-JTK-303 Once Daily for 7 Days

Parameter	1st Administration Mean ± SD	After the 7th Administration Mean ± SD
Tmax (hr)	0.7 ± 0.3	0.5 ± 0.0
Cmax (ng eq./mL)	996 ± 236	1411 ± 178
$T_{1/2\alpha}$ (hr)	1.3 ± 0.1	1.7 ± 0.2
$T_{1/2\beta}$ (hr)	5.2 ± 1.0	20.6 ± 0.7
$AUC_{0\text{-inf}}$ (ng eq.·hr/mL)	2897 ± 98	3284 ± 679
$MRT_{0\text{-inf}}$ (hr)	4.6 ± 0.2	15.3 ± 1.6
CL/F (L/hr/kg)	1.0 ± 0.1	0.7 ± 0.1
Vz/F (L/kg)	7.8 ± 1.5	21.7 ± 3.3

Absorption

In rats and dogs, absorption of JTK-303 after oral dosing was rapid and bioavailability was moderate (30%−35% and 26%–33% in rats and dogs, respectively).

In dogs, the oral absorption after dosing of JTK-303 Tablets, 50 mg (SD) was higher than that of the JTK-303 Tablets, 50 mg in both the fasting and post-prandial conditions (see sponsor's table below).

Table 7. Pharmacokinetic Parameters of Parent drug in Plasma after Oral Administrations of JTK-303 Tablets, 50 mg and JTK-303 Tablets, 50 mg (SD) (50 mg/dog) to fasting dogs

Parameter	JTK-303 Tablets, 50 mg	JTK-303 Tablets, 50 mg (SD)
t_{max} (hr)	1.38±0.75	1.25±0.50
C_{max} (ng/mL)	162±81 (1.00)	580±0.05 (3.58)
$t_{1/2}$ *1 (hr)	2.1±0.3	1.7±0.1
$AUC_{0\text{-}12hr}$ (ng·hr/mL)	691±321 (1.00)	1827±702 (2.64)

Each value represents the mean ± SD (n=4).
Values in parentheses show the ratio to the JTK-303 Tablets, 50 mg values.
*1: t_{max}-12 hr.

Distribution

In male rats, following a single oral dose of ^{14}C-JTK-303 (3 mg/kg), the radioactivity in the plasma was respectively 44% at 2 hr, 16% at 4 hr, 3% at 12 hr, and 1.2% or less after 24 hr post administration as compared with that at 30 min. The radioactivity concentrations in most of the tissues peaked at 30 min post-dose. At this time point, the radioactivity concentration in the liver and digestive tract were higher than that in the plasma. In other tissues, especially the prostate gland, skin, thymus, epididymis, seminal vesicle, fat, testis, eyeball, and brain, radioactivity concentrations were low. The radioactivity distributed in most of the tissues was eliminated in parallel with that in the plasma up to 24 hr after administration.

When EVG was co-administered with RTV, tissue concentrations at 1 h post-dose in the rats administered ritonavir prior to [^{14}C]EVG tended to be lower than the concentrations in the rats administered [^{14}C]EVG alone.

In dogs, after a single intravenous administration of ^{14}C-JTK-303 at a dose of 1 mg/kg, the distribution of radioactivity into blood cells ranged from 0.0% to 40.9% from 5 min to 168 hr after administration, and was minimal (< 0.05%) in other tissues. After a single oral administration of ^{14}C-JTK-303 at a dose of 3 mg/kg, the distribution of radioactivity into blood cells ranged 11.3% to 34.4% from 15 min to 168 hr after administration.

Protein binding of JTK-303 was high, and concentration-independent in plasma from rats, dogs, monkeys and humans. The fraction unbound varied from 0.1% in rats to 1.2% in monkey. The fraction unbound in human plasma, or in a physiological concentration of HSA, averaged 0.7%. Binding to human AAG was low and addition of AAG to a solution of HSA did not affect the fraction of EVG unbound. The mean values of the rate of protein binding are shown in Table 8.

JTK-303 does not distribute well into the cellular fraction of blood from rat, dog, monkey, or human in vitro. The whole blood/plasma ratio for human blood was 0.7 and this value was confirmed in an in vivo study.

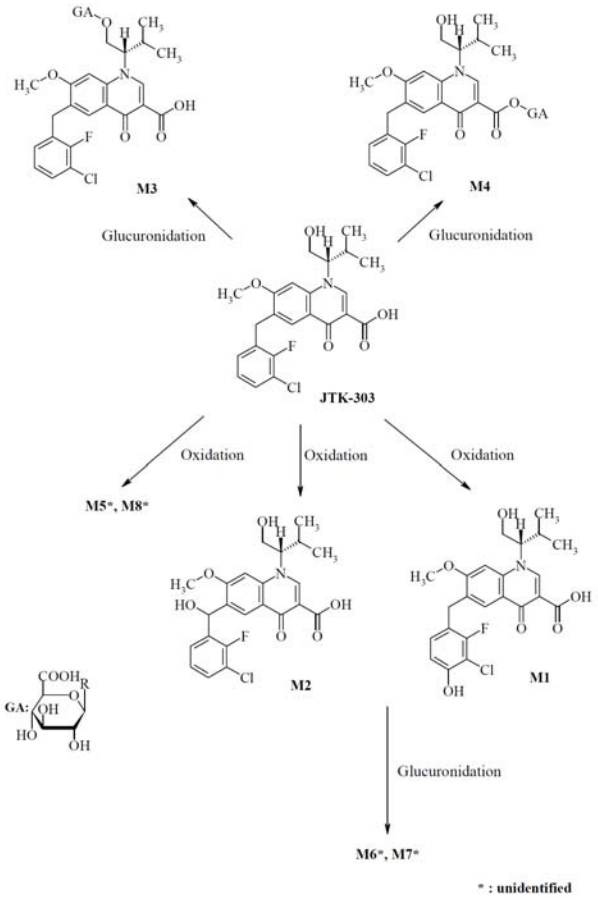
Table 8. Rate of the Protein Binding of 14C-JTK-303 in Rats, Dogs, Monkeys and Humans

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% α1 acidic glycoprotein	0.1	39.25 ± 1.04
		1	39.05 ± 0.93
		10	40.68 ± 1.99
	0.05% α1 acidic glycoprotein/HSA	0.1	99.45 ± 0.01
		1	99.39 ± 0.01
		10	99.36 ± 0.03
	0.1% α1 acidic glycoprotein/HAS	0.1	99.06 ± 0.63
		1	99.331)
		10	99.44 ± 0.01
	0.2% α1 acidic glycoprotein/HAS	0.1	99.44 ± 0.02
		1	99.43 ± 0.01
		10	99.41 ± 0.01

Metabolism

The metabolic pathway of JTK-303 has been postulated based on in vivo and in vitro studies. It is proposed that JTK-303 undergoes oxidation to become metabolites M1, M2, M5 and M8, and undergoes glucuronide conjugation to become metabolites M3 and M4. In addition, metabolites M1 and M2 undergo further glucuronide conjugation to become metabolites M6 and M7 (Figure 6).

Figure 6. Postulated Metabolic Pathway of JTK-303



In vitro

Metabolism of ^{14}C-JTK-303 was assessed using rabbit, monkey, rat, human and dog liver microsomes in the presence of NADPH. ^{14}C-JTK-303 was primarily metabolized to M1, a chlorofluorophenyl group hydroxide of JTK-303, by microsomes from all animal species, and was also metabolized to small amounts of M5 (rabbit, human, dog and monkey), M6 (rabbit; a glucuronide of M1 with glucuronidation on the hydroxyl group of fluoro-chlorophenyl ring) and M8 (human, rat and monkey). This oxidative reaction did not proceed in the absence of NADPH.

In the presence of uridine diphospho glucuronic acid (UDPGA), the metabolism of JTK-303 was most rapid by rat microsomes, followed by monkey and dog, and then human. In each of the animal species studies, acyl-glucuronide conjugate (M4) was formed, and ether-glucuronide conjugate (M3) was also formed in rat and dog.

In vivo

The radioactivity in rat plasma after oral administration of ^{14}C-JTK-303 was due mostly to the parent drug. In plasma and liver, M4 was the most prominent metabolite. M7 was also observed in the plasma samples collected 4 hours after administration. In dogs, after oral administration of ^{14}C-JTK-303, parent drug, a trace amount of M6, and M4 were detected in plasma. After intravenous administration, radioactivity in plasma was mainly associated with the parent drug. Traces of metabolites M1, M2 and M4 were also observed.

Excretion

As noted above, in dogs, after a single intravenous administration of ^{14}C-JTK-303 at a dose of 1 mg/kg, the excretion of radioactivity in the urine and feces was almost completed by 48 hr after administration, and 1.0% and 98.8% of the radioactivity administered were excreted in the urine and feces, respectively, up to 168 hr after administration.

Similarly, in rats, following an oral or iv dose, the radioactivity derived from ^{14}C-JTK- 303 was mainly excreted in the feces (up to 98%). Results of studies of intraduodenal injection suggest that approximately 6% of the radioactivity derived from ^{14}C-JTK-303 in the bile is reabsorbed.

Table 9. Cumulative excretion of radioactivity in urine, feces and cage washing after single oral administration of 14C-JTK-303 to non-fasting male rats (dose: 3/kg mg)

	Excretion of radioactivity (% of dose)			
Time (hr)	Urine	Feces	Cage washing	Total
0-24	0.1±0.1	79.6±14.2	0.0±0.0	79.7±14.2
48	0.1±0.1	96.5±2.0	0.0±0.0	96.7±1.9
72	0.2±0.1	97.5±0.9	0.0±0.0	97.7±0.9
96	0.2±0.1	97.6±0.9	0.0±0.0	97.7±0.8

Data are expressed as the mean values ± S.D. of three animals.

Table 10. Cumulative excretion of radioactivity in urine, feces and cage washing after single IV administration of 14C-JTK-303 to non-fasting male rats (dose: 1mg/kg)

	Excretion of radioactivity (% of dose)			
Time (hr)	Urine	Feces	Cage washing	Total
0-24	0.4±0.1	80.5±14.1	0.0±0.0	80.9±14.1
48	0.4±0.1	97.5±0.2	0.0±0.0	97.9±0.2
72	0.4±0.1	98.2±0.7	0.0±0.0	98.6±0.7
96	0.4±0.1	98.2±0.8	0.0±0.0	98.6±0.7

Data are expressed as the mean values ± S.D. of three animals.

In a repeat dose study in rats (7 days of dosing), the excretion of radioactivity in the urine and feces up to 24 hr after daily oral administration was almost constant after the 2nd dose. The excretion of radioactivity in the urine and feces up to 24 hr after the 7th dose was 0.1 % and

93.3% to 94.9% of the cumulative dose, respectively. The excretion of radioactivity in the urine and feces up to 168 hr after the 7th dose was 0.1% and 95.3% of the cumulative dose, respectively.

Pharmacokinetic Drug Interactions

EVG was not a substrate for recombinant human UGT1A4, 1A6, 1A7, 1A8, 1A10, 2B4, 2B7 or 2B17. There was slow turnover by UGT1A9 and 2B15, but this is unlikely to be relevant in vivo. The most efficient catalysts of acyl glucuronide (GS-9200) formation were UGT1A1 and 1A3. Inhibitors or inducers of UGT1A1 or 1A3 may thus affect the pharmacokinetics of EVG under conditions where oxidative metabolism of EVG is inhibited.

Ketoconazole was a modest inhibitor of the hepatic microsomal glucuronidation of EVG with a potency 6-fold less than ATV. Clinical regimens for EVG can include co-dosed ritonavir (RTV). A representative clinical Cmax for ketoconazole is 5311 ng/mL (10.0 μM) when dosed 200 mg twice daily and this increased to 10501 ng/mL (19.8 μM) when the regimen included RTV (100 mg twice daily). Under these conditions inhibition of EVG glucuronidation would be expected.

6 General Toxicology

6.1 Single-Dose Toxicity

Single Oral Dose Toxicity Study of JTK-303 in Rats (TX-001). Groups of 5 male and 5 female Sprague-Dawley rats were given a single oral dose of 2000 mg/kg of JTK-303. There was no mortality nor any clinical signs of toxicity in the animals: findings noted were whitish loose stools and soiled ano-genital region 8 hours after administration, and whitish stools on the day after administration. There were no effects of JTK-303 on body weights or food consumption nor any treatment-related changes at necropsy. Therefore, the approximate lethal dose of JTK-303 was concluded to be greater than 2000 mg/kg for rats.

Single Oral Dose Toxicity Study of JTK-303 in Dogs (TX-002). Three female beagle dogs were given JTK-303 at a dose of 1000 mg/kg by single oral administration. No deaths were observed. Vomiting was observed up to 8 hours after administration. In a preliminary toxicokinetic study in dogs (dose levels: 30, 100, 300 mg/kg), vomiting was notable at 100 mg/kg and above. To investigate the cause of vomiting, JTK-303 was administered intravenously to dogs at a dose of 20 mg/kg. Vomiting was not observed after dosing even though systemic exposure after the intravenous dose at 20 mg/kg was comparable to that after oral dosing at 100 mg/kg. These results suggested that the vomiting results from a direct local effect of JTK-303 on the digestive tract.

6.2 Repeat-Dose Toxicity

GS-9137: A 13-Week Oral Gavage Toxicity and Toxicokinetic Study in Mice.

Key study findings: groups of male and female CD1 mice were administered GS-9137 for at least 13 weeks. Male and female mice were assigned to four groups (15/sex/group) and received

either the vehicle (0.5% (w/v) Methylcellulose in water), or GS-9137 at 100 (low), 500 (mid) and 2000 (high) mg/kg/day respectively at a dose volume of 10 mL/kg. A satellite group of animals (36 animals/sex/dose) were assigned to the toxicokinetic (TK) groups and were bled at various time points postdose on Days 1 and 91. One 500 mg/kg/day female and one 2000 mg/kg/day male were found dead on Day 63 and Day 79, respectively. The death of the 500 mg/kg/day female was attributed to a dosing error. The death of the 2000 mg/kg/day male was not determined. Possible test article-related findings included unkempt appearance in the males at 500 mg/kg/day and females at 2000 mg/kg/day. Female animals at 2000 mg/kg/day gained slightly less weight, though not statistically significant over the course of the study when compared to control females (10% decrease). During Week 1, male animals administered 2000 mg/kg/day and female animals administered 500 and 2000 mg/kg/day had statistically significantly decreased food consumption relative to the control groups. There were no test article-related ophthalmic, hematology, serum chemistry, bone marrow cytology, macroscopic or microscopic findings. The toxicokinetic portion of this study demonstrated overall dose-related exposure for GS-9137 and its metabolites, GS-9200 and GS-9202 on study Days 1 and 91 for male and female mice. Based on the results of this study, the NOAEL for 13 week oral administration was considered to be 2000 mg/kg/day.

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Conducting laboratory and location: (b) (4)

Date of study initiation: April 4, 2006

Date of study completion: July 31, 2007

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: GS-9137: 061240; 99.1% pure and Ritonavir: 39098AW21; 100% pure

Methods

Doses: male and female mice were assigned to four groups (15/sex/group) and received either the vehicle (0.5% (w/v) Methylcellulose in water), or GS-9137 at 100 (low), 500 (mid) and 2000 (high) mg/kg/day respectively at a dose volume of 10 mL/kg. A satellite group of animals (36 animals/sex/dose) were assigned to toxicokinetics.

Species/strain: male and female Crl: CD1(ICR) mice

Number/sex/group or time point (main study): 15/sex/group)

Route, formulation, volume, and infusion rate: oral gavage, 10 ml/kg/day
Satellite groups used for toxicokinetics: 36 animals/sex/dose

Age: & Weights: at initiation of treatment, the animals were approximately 8 weeks old, and their body weights ranged from 27.9 to 34.0 g for males and 20.9 to 26.2 g for females.

Sampling times: blood samples were collected for clinical pathology immediately prior to necropsy. Samples were collected from TK animals on Days 1 and 91 of the dosing phase.

Observations and Results

Mortality: a single 2000 mg/kg/day male (animal number 1049) was found dead on Day 79. Body weight and food consumption for this animal were comparable to controls throughout the study with no abnormal clinical signs and no postmortem observations evident. As a result, the cause of death for this animal could not be determined. A single 500 mg/kg/day female (animal number 1222) was found dead on Day 63. This death was considered to be due to a dosing error based on moderate hemorrhage noted in the thoracic cavity at the time of necropsy. All other main study animals survived until study termination.

In the toxicokinetic groups, a single 100 mg/kg/day male was found dead on Day 48 (animal number 1106), a single 100 mg/kg/day toxicokinetic female was found dead on Day 89 (animal number 1286), and a single 2000 mg/kg/day male was euthanized *in extremis* on Day 32 (animal number 1169). There were no postmortem observations indicative of dosing error in these animals and no in-life findings indicative of compound-related effects. Therefore, the cause of these deaths could not be determined.

Clinical signs: possible test article related clinical findings were limited to unkempt appearance in one male, and yellow discolored hair in two males in the 500 mg/kg/day group, and unkempt appearance and yellow discolored hair in one female in the 2000 mg/kg/day group. These findings were not associated with reduced body weight or food consumption or effects on clinical or anatomic pathology parameters in these animals and are not considered to be adverse effects of GS-9137 administration.

Body weights: female animals administered 2000 mg/kg/day gained slightly less weight (10% decrease) over the course of the study when compared to control females, with differences reaching statistical significance on Days 14 and 77. Though the differences in body weight were transient and sporadic, they were present in conjunction with test article-related decreases in food consumption. Therefore, the decreased body weights and body weight gain in 2000 mg/kg/day females is considered to be test article related. Because the body weight among animals that received GS-9137 did not differ significantly from control at the end of the study, body weight gain differences are not considered to be an adverse effect of the test article.

There were no clear effects of test article on body weights for TK animals.

Food consumption: statistically significant decreases in food consumption were noted during Week 1 in males administered 2000 mg/kg/day and females administered 500 and 2000

mg/kg/day; thereafter, food consumption was similar between GS-9137-treated and control animals. This transient effect on food consumption, evident only during the first week of dosing, was considered to be test article related, but not adverse.

Ophthalmology: there were no test article related ocular changes.

Hematology: there were no test article-related effects on hematology parameters.

Chemistry: there was the suggestion of dose dependent, mild increases in cholesterol in females which reached statistical significance at 2000 mg/kg/day; the values remaining well within normal ranges. In addition, sporadic increases in AST and ALT in individual females within all groups including controls were often within, or just outside of, normal ranges.

Bone Marrow Cytology: there were no test article-related effects on bone marrow cytologic parameters at termination compared to controls. There was complete maturation and expected proportionality of all cell lineages with very similar M:E ratios and no evidence of any cellular atypia.

Organ weights: the mean cecum relative to brain weight was statistically increased in males, but the mean absolute and relative to body weight were not statistically different. The only statistically significant difference in the females at 2000 mg/kg/day was a 30% decrement in mean absolute salivary gland weights. No microscopic differences were noted in the salivary gland between controls and this group, and only three animals had salivary gland weights that were lower than the typical control animal weight.

Histopathology: adequate Battery: yes; Peer review: yes

Gross pathology: there were no macroscopic findings interpreted to be related to the administration of test article.

Histopathology: no test article-related microscopic observations were noted in either sex.

Unscheduled Deaths: two animals died prior to the termination of the study. One male at 2000 mg/kg/day (animal number R1049) was found dead on Day 79. The cause of death was undetermined. One female animal at 500 mg/kg/day (animal number 1222) was found dead on Day 63. The cause of death was determined to be secondary to dosing error as evidenced by hemorrhage in the thoracic cavity.

Toxicokinetics: data are shown in Tables 11 (GS-9137, parent), Table 12 (GS-9200, metabolite) and Table 13 (GS-9202, metabolite). For GS-9137 and GS-9200, there were no consistent substantial differences in Cmax, Tmax, or T1/2 values between male and female animals. However, AUC0-t values in female animals were generally higher (~1.5-fold) than male animals.

On Day 1, exposure increased with dose, and generally in an approximately dose-proportional manner for both GS-9137 and GS-9200 for all animals. On Day 91, GS-9137 exposure increased

in a dose proportional manner up to 500 mg/kg/day in the females while in the males, exposure generally increased in dose but in a less than dose proportional manner.
Exposure of GS-9200 in females generally increased with dose in a less than dose proportional manner, and in males, increases in Cmax was less than dose proportional but AUC_{0-t} increased in a dose proportional manner. Based on Day 1 and Day 91 exposures, Cmax and AUC_{0-t} values for GS-9137 and GS-9200 decreased after multiple dosing and the decrease was more pronounced as the dose increased. For GS-9202, Only limited plasma concentration data were available for this metabolite; of the six dosage groups yielding toxicokinetic data, two showed measurable concentrations at only one time point and three showed measurable concentrations at only two time points.

Table 11. Mean Toxicokinetic Parameters of EVG in Mice Following Daily Oral Gavage Administration of EVG for 13 Weeks

EVG (mg/kg/day)	Sex	AUC_{0-t} (µg·h/mL)		C_{max} (µg/mL)	
		Day 1	Day 91	Day 1	Day 91
100	Male	5.37	3.44	3.17	3.02
	Female	10.1	4.56	2.71	2.74
500	Male	29.1	17.1	17.3	8.9
	Female	40.3	33.4	13.4	13.3
2000	Male	128	44.4	36.3	13.5
	Female	192	58.6	52.5	19.1

Table 12. Mean Toxicokinetic Parameters of GS-9200 in Mice Following Daily Oral Gavage Administration of EVG for 13 Weeks

EVG (mg/kg/day)	Sex	AUC_{0-t} (µg·h/mL)		C_{max} (µg/mL)	
		Day 1	Day 91	Day 1	Day 91
100	Male	1.31	0.632	0.636	0.607
	Female	0.927	2.12	0.266	0.668
500	Male	5.70	3.85	2.76	2.40
	Female	6.51	4.85	2.96	2.05
2000	Male	36.1	12.0	14.1	3.00
	Female	44.0	17.8	17.2	4.00

Table 13. Mean Toxicokinetic Parameters of GS-9202 in Mice Following Daily Oral Gavage Administration of EVG for 13 Weeks

EVG (mg/kg/day)	Sex	AUC_{0-t} (µg·h/mL)		C_{max} (µg/mL)	
		Day 1	Day 91	Day 1	Day 91
100	Male	NC	NC	NC	NC
	Female	NC	NC	NC	NC
500	Male	NC	NC	NC	NC
	Female	0.154	0.0322	0.123	0.129
2000	Male	0.367	0.0336	0.314	0.135
	Female	1.21	0.518	0.584	0.247

GS-9137 administration at doses of 100, 500, or 2000 mg/kg/day for 13 weeks to CD1 mice had no effect on mortality, clinical signs, body weight, or clinical and anatomic pathology parameters. Based on the results of this study, the NOAEL for 13 week oral administration was considered to be at least 2000 mg/kg/day, the highest dose administered in this study.

One month oral toxicity study of JTK-303 in rats

Key study findings: groups of male and female rats (12 animals/sex/group) were administered JTK-303 orally via gavage at dose levels of 0 (vehicle), 100, 300, 1000 and 2000 mg/kg/day for three months. Satellite groups of five males and five females at each dose level were provided for determination of plasma concentrations of the parent drug. Dilatation of the cecum with whitish loose contents was noted in females at 1000 and 2000 mg/kg/day. The changes were associated with increased cecal weights. Cecal weights were increased in males at 300 mg/kg/day and above and in females at 2000 mg/kg/day. The weight of cecal contents as well as the weight of whole cecum including its contents was increased in males at 2000 mg/kg/day and in females at 300 mg/kg/day and above. No compound-related histopathology, however, was found in the cecum in all groups including control group. Lipid-like vacuoles of medium to large size were observed in the lamina propria in the small intestine in all treated groups except for the female 100 mg/kg/day. The incidence and severity of the finding were higher in the 1000 and 2000 mg/kg/day than the lower dose groups. The mainly affected regions were the upper small intestine (duodenum to jejunum), but the lipid-like vacuoles were also noted in the lower small intestine (ileum) and mesenteric lymph nodes in a small number of animals at 2000 mg/kg/day. The change, however, was unaccompanied by any other pathological events (cell/tissue injury or reactive responses) or clinical signs including diarrhea. There was no histological evidence of toxicity nor any tissue reactive changes to the intestinal vacuoles. Focal retinal atrophy was seen in 2/12 females at 100 mg/kg/day; 1/12 female at 300 mg/kg/day; 1/12 male at 1000 mg/kg/day; 4/12 males and 2/12 females at 2000 mg/kg/day. Degree ranged from very slight to moderate and the lesion occurred unilaterally except for 1 HD male. Under the conditions of this study, a dose level of 2000 mg/kg/day could be considered the NOAEL.

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Conducting laboratory and location: (b) (4)

Date of study initiation: 9/2003
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity: JTK-303; Lot K; 99.4% pure

Methods

Doses: 100, 300, 1000, 2000 mg/kg/day
Species/strain: Sprague-Dawley rats
Number/sex/group or time point (main study): 12/sex/group
Route, formulation, volume, and infusion rate: oral gavage; solution in 0.5% methylcellulose; 10 ml/kg
Satellite groups used for toxicokinetics or recovery: 5/sex/group
Age: 4 wks
Weight: 145-186 females; 200-242 males
Sampling times:
Unique study design or methodology (if any):

Observations and Results:

Mortality: none

Clinical signs: whitish stools were seen in 1000 and 2000 mg/kg/day animals. Salivation was seen in 1000 and 2000 mg/kg/day from 2 weeks onward. Salivation occurred immediately before and after dosing and also occurred in one 300 mg/kg/day male one time.

Body weights: no effects

Food consumption: no effects

Ophthalmoscopy: no effects

EKG: not done

Hematology: increased platelet counts (13% over controls) in HD males.

Clinical chemistry: no drug related changes

Urinalysis: slight decrease in pH in HD males. Occult blood positive urine detected in 1/6 LD, 2/6 1000 mg/kg/day, and 1/6 HD female and 1/6 HD male.

Gross pathology: dilation of the cecum with whitish loose contents in 6/12 and 12/12 1000 and 2000 mg/kg/day females.

Organ weights Cecum weights were increased in males at 300 mg/kg/day and above and in females at 2000 mg/kg/day. Weight of cecum contents was also increased in HD males and in females at 300 mg/kg/day and above.

Liver weight was increased in females at 300 mg/kg/day only.

Histopathology: adequate Battery: yes , Peer review: yes

Lipid like vacuoles of medium to large size were seen in the lamina propria in the upper small intestine in males at 100 mg/kg/day and above and in females at 300 mg/kg/day and above. Degree ranged from very slight to moderate but the occurrence of moderate was restricted to the two high doses.

Table 14. Incidence of lipid like vacuoles (n=12)

Dose (mg/kg/day)	Duodenum		Jejunum	
	Males	females	males	females
0	0	0	0	0
100	2	0	3	0
300	2	4	7	1
1000	7	9	7	7
2000	8	9	7	6

A small number of HD animals had the same lipid like vacuoles in the ileum (1/12 male) and mesenteric lymph nodes (3/12 males; 1/12 female).

Focal retinal atrophy was seen in 2/12 females at 100 mg/kg/day; 1/12 female at 300 mg/kg/day; 1/12 male at 1000 mg/kg/day; 4/12 males and 2/12 females at 2000 mg/kg/day. Degree ranged from very slight to moderate and the lesion occurred unilaterally except for 1 HD male. This same male also had another unilateral retinal lesion consisting of focal thinness of the inner part of retina (the external plexiform layer to the nerve fiber layer). No retinal lesions were found in the control group. Although sponsor suggests that the incidence and severity were within normal range, retinal lesions should be considered possibly drug related.

The increased cecal weight and dilatation with whitish loose contents were not accompanied by histopathologic changes or adverse GI events. There was no histological evidence of toxicity nor any tissue reactive changes to the intestinal vacuoles.

Toxicokinetics: data are shown in Table 15. Plasma concentrations of the parent drug (the group mean Cmax and $AUC_{0\text{-}24hr}$ values) showed a dose-dependent increase up to 2000 mg/kg/day. Systemic exposure to the parent drug (Cmax and $AUC_{0\text{-}24}$ hr) was similar on days 1 and 27 for males and females, and higher in females than in males at all dose levels.

Table 15. Mean toxicokinetic parameters of JTK-303 (5 animals/sex)

Dose (mg/kg/day)	Sex	Day	$AUC_{0\text{-}24h}$ (µg h/ml)	Cmax (µg/ml)
100	M	1	57	21
		27	63	16
	F	1	89	33
		27	85	26
300	M	1	157	29
		27	123	17
	F	1	235	58
		27	230	38
1000	M	1	419	37
		27	304	25
	F	1	532	59
		27	415	46
2000	M	1	490	44
		27	379	31
	F	1	724	68
		27	695	50

The sponsor considers 2000 mg/kg/day to be the no adverse effect dose. I agree that 2000 mg/kg/day could be considered the NOAEL, at least until data from longer term studies are submitted.

Three-Month Oral Dose Toxicity Study of JTK-303 in Rats with a Three-Month Recovery Period

Key study findings: groups of male and female rats (15 animals/sex/group) were administered JTK-303 orally via gavage at dose levels of 0 (vehicle), 100, 300, 1000 and 2000 mg/kg/day for three months. Satellite groups of five males and five females at each dose level were provided for determination of plasma concentrations of the parent drug. The reversibility of findings was investigated by adding groups of six males and six females to the vehicle control group and the 1000 and 2000 mg/kg/day and establishing a withdrawal period of three months after the dosing period. Whitish stools, which appeared to contain unabsorbed test article, and salivation were noted in males and females at 1000 and 2000 mg/kg/day throughout the dosing period. The salivation occurred immediately before and after dosing. These findings disappeared soon after withdrawal of the drug. Minimal to moderate lipid vacuoles, which seemed to be related to changes in the lipid absorption process, were observed in the upper small intestinal lamina propria (duodenum and jejunum) in both sexes at 1000 and 2000 mg/kg/day after the dosing period. The lipid vacuoles were also observed in the jejunum in both sexes at 100 and 300 mg/kg/day groups. Minimal to slight lipid vacuoles were still observed in the upper small intestinal lamina propria in both sexes at 1000 and 2000 mg/kg/day after the recovery period. In conclusion, no findings indicative of systemic or organ toxicity were observed at up to the dose level of 2000 mg/kg/day and therefore the dose level of 2000 mg/kg/day was considered to be the NOAEL.

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Conducting laboratory and location: (b) (4)
Date of study initiation: June, 2004
Date of completion: April, 2005
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity: JTK-303, Lot S, 99.1% pure

Methods

Doses: 100, 300, 1000, 2000 mg/kg/day
Species/strain: rats; Sprague-Dawley (Crj:CD(SD) IGS)
Number/sex/group or time point (main study): 15/sex/group
Route, formulation, volume, and infusion rate: oral gavage, 0.5% methylcellulose aqueous solution; 10 ml/kg
Satellite groups used for toxicokinetics or recovery: 5/sex/group for TK and recovery but only 1000 and 2000 mg/kg/day had recovery animals.
Age: 5 wks
Weight: 168-197 g males; 139-171 g females

Observations and Results:

Mortality: no treatment related mortality

Clinical signs: whitish stools and salivation in both sexes at the two highest doses throughout the dosing period but stopped during recovery.

Body weights: no changes

Food consumption: no changes

Ophthalmoscopy: no changes (there were retinal changes in the 1-month rat study)

EKG: not done

Hematology: no changes

Clinical chemistry: an increase in sodium was observed in females at 100, 300 and 2000 mg/kg/day after the dosing period (an increase by 1% as compared with the control mean value). A similar finding was not observed in females at 1000 or 2000 mg/kg/day after the recovery period. An increase in inorganic phosphorus was noted in males at 1000 mg/kg/day after the recovery period, but a similar finding was not noted in males at 1000 or 2000 mg/kg/day after the dosing period or in males at 2000 mg/kg/day after the recovery period.

Urinalysis: trend toward decreased urinary pH was observed in females at 300 mg/kg/day and above in the dosing period. A similar trend was not observed in females at 1000 or 2000 mg/kg/day during the recovery period.

Gross pathology: cecal dilation with whitish loose contents was observed in both sexes at 1000 and 2000 mg/kg/day. Cecum was normal after recovery.

Organ weights: cecal weights were increased in the 1000 and 2000 mg/kg/day males and 2000 mg/kg/day females. Decreases in weights of the submandibular gland and brain were noted in females at 1000 and 2000 mg/kg/day after the recovery period but not after the dosing period.

Histopathology: Adequate Battery: yes Peer review: yes

Minimal to moderate lipid vacuoles were observed in the lamina propria in the duodenum and jejunum in both sexes at 1000 and 2000 mg/kg/day after the dosing period. The lipid vacuoles were also observed in the jejunum in both sexes at 100 and 300 mg/kg/day, but the degree of the lipid vacuoles in the jejunum was minimal to slight in each animal. The incidence of lipid vacuoles was lower at 100 mg/kg/day than that at the two highest doses in both sexes. The lipid vacuoles were rarely observed in the duodenum in both sexes at 100 and 300 mg/kg/day.

Minimal to slight lipid vacuoles were still observed in the lamina propria in the upper small intestine in both sexes at 1000 and 2000 mg/kg/day after the recovery period. Moderate lipid vacuoles almost disappeared.

Toxicokinetics: data are shown in Table 16. The plasma concentrations of the parent drug (the mean Cmax and AUC_{0-24}hr values) showed dose-dependent increases at dose levels up to 2000 mg/kg/day. The systemic exposure to the parent drug (Cmax and AUC_{0-24}hr) was higher in females than in males at all dose levels. There were no marked differences in the mean Cmax values in males at 300 mg/kg/day and above. The systemic exposure to the parent drug varied but was mostly similar at each dose level on days 1 and 90, and it was considered that systemic exposure to the parent drug was not affected by repeated dosing. Levels of GS-9137 metabolites were not measured in this study.

Table 16. Mean toxicokinetics parameters for JTK-303 in the 3-month rat study

Dose (mg/kg/day)	Study Day	AUC_{0-24} JTK-303 (μg•hr/mL)	
		Males	Females
100	Day 1	35.95	75.35
	Day 27	45.05	84.63
	Day 55	32.33	43.34
	Day 90	55.17	91.90
300	Day 1	138.60	204.56
	Day 27	102.39	145.74
	Day 55	121.61	151.73
	Day 90	147.44	141.10
1000	Day 1	173.32	178.57
	Day 27	173.95	232.75
	Day 55	224.38	316.28
	Day 90	194.34	288.03
2000	Day 1	217.49	244.53
	Day 27	274.21	452.97
	Day 55	295.63	437.05
	Day 90	264.91	368.89

No findings indicative of systemic or organ toxicity were observed at dose levels up to 2000 mg/kg/day under the conditions of this study. The lipid vacuoles in the lamina propria in the upper small intestine tended to disappear, and the other findings observed at the end of the dosing period disappeared after the recovery period. The NOAEL was considered to be 2000 mg/kg/day in this study.

Six month oral dose toxicity study of JTK-303 in rats

Key study findings: groups of male and female rats (18 animals/sex/group) were orally (via gavage) administered JTK-303 at dose levels of 0 (0.5% methylcellulose aqueous solution, vehicle control), 100, 300 and 2000 mg/kg/day for six months. Satellite groups of five males and five females at each dose level were provided for determination of plasma concentrations of the parent drug. Cecal dilatation with whitish loose contents was mainly observed in both sexes at 2000 mg/kg/day. Cecal weights were also increased in males at all dose levels and in females at 2000 mg/kg/day. However, treatment-related histopathological findings were not observed in the cecum in either sex at 2000 mg/kg/day. These (dilatation and increased cecal weights) were considered to be due to the weak antibacterial activity of JTK-303. Minimal to moderate lipid droplets (vacuoles) were seen in the upper small intestinal (duodenal and jejunal) lamina propria in both sexes at all dose levels. Slight or moderate erosion was seen in the glandular stomach in 1 male at 300 mg/kg/day, 3 males at 2000 mg/kg/day and 2 females at 2000 mg/kg/day. Slight fibrosis was also observed in the glandular stomach in 6 HD males. Slight sinus erythrocytosis (blood absorption; the presence of an increased number of RBC's) was seen in the mesenteric lymph nodes in 5 males and 7 females at 2000 mg/kg/day. Similar finding was also seen in the mesenteric lymph nodes in 1 male at the 100 and 300 mg/kg/day and 1 female each in the control and 300 mg/kg/day. Slight basophilic altered hepatocellular foci of tigroid pattern were observed

in the liver of 7 females at 300 mg/kg/day and in 8 females at 2000 mg/kg/day. Similar findings were also observed in the liver in 1-4 animals in most groups including controls in both sexes.

Under the conditions of this study, a dose level of 100 mg/kg/day may be considered the NOAEL. Note: with the availability of long-term toxicology data in rat (carcinogenicity studies), changes seen in the GI track may be considered not dose-related. Thus, a dose level of 2000 mg/kg/day may be considered the NOAEL.

Study no.: JTK303-TX-022
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Conducting laboratory and location: (b) (4)
Date of study initiation: Aug, 2005
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity: JTK-303, lot no. AC, 99.3% pure

Methods

Doses: 100, 300, 2000 mg/kg/day
Species/strain: Sprague-Dawley rats
Number/sex/group or time point (main study): 18/sex/group
Route, formulation, volume, and infusion rate: oral gavage, 0.5% methyl cellulose, 10 ml/kg.
Satellite groups used for toxicokinetics or recovery: 5/sex/group
Age: 6 wks
Weight: 133-196 g

Observations and Results:

Mortality: none treatment related

Clinical signs: whitish stools and salivation in both sexes at HD throughout the dosing period.

Body weights: no effects

Food consumption: no effects

Ophthalmoscopy: no effects

EKG: not done

Hematology: PT and APTT were prolonged by 23% and 22%, in males at 2000 mg/kg/day. In females, APTT was slightly prolonged by 7% at the HD.

Clinical chemistry: plasma ALT and CPK were increased by 24% and 46% in HD males.

Urinalysis: urinary Na concentration and osmotic pressure were decreased by 34% and 23%, respectively in HD males.

Gross pathology: cecal dilatation with whitish loose contents were seen in all HD males and 11/18 HD females.

Organ weights : cecal weights were increased in all dosed males and HD females.

Histopathology: adequate Battery: yes Peer review: yes

Minimal to moderate lipid droplets (vacuoles) were seen in the upper small intestinal (duodenal and jejunal) lamina propria in both sexes at all dose levels. Slight or moderate erosion was seen in the glandular stomach in 1 male at 300 mg/kg/day, 3 males at 2000 mg/kg/day and 2 females at 2000 mg/kg/day. Slight fibrosis was also observed in the glandular stomach in 6 HD males.

Slight sinus erythrocytosis (blood absorption; the presence of an increased number of RBC's) was seen in the mesenteric lymph nodes in 5 males and 7 females at 2000 mg/kg/day. Similar finding was also seen in the mesenteric lymph nodes in 1 male at the 100 and 300 mg/kg/day and 1 female each in the control and 300 mg/kg/day.

Slight basophilic altered hepatocellular foci of tigroid pattern were observed in the liver of 7 females at 300 mg/kg/day and in 8 females at 2000 mg/kg/day. Similar findings were also observed in the liver in 1-4 animals in most groups including controls in both sexes.

Toxicokinetics: parameters are shown in Table 17. The plasma concentrations of the parent drug (the group mean Cmax and $AUC_{(0\text{-}24hr)}$) showed dose-dependent increases at dose levels up to 2000 mg/kg/day on all sampling days. The systemic exposure to the parent drug (Cmax and $AUC_{0\text{-}24hr}$ was approximately 2-fold higher in females than in males at each dose level. The systemic exposure to the parent drug was mostly similar at each dose level on days 1, 27, 90 and 181 and was not affected by repeated dosing.

Table 17. Mean toxicokinetic parameters of JTK-303 in rats

Dose (mg/kg/day)	Day	Cmax (µg/ml)		$AUC_{0\text{-}24h}$ (µg.h/ml)	
		Males	Females	Males	Females
100	1	19.4	40.0	66.8	123.9
	27	15.1	35.7	90.9	111.1
	90	15.6	34.7	72.0	171.0
	181	14.7	49.4	64.3	180.3
300	1	33.7	47.1	169.6	267.1
	27	23.1	44.3	200.9	268.7
	90	25.7	58.8	152.6	307.5
	181	23.6	65.2	153.2	376.5
2000	1	39.7	68.8	478.0	807.1
	27	34.1	67.8	408.4	866.5
	90	30.6	71.0	427.7	795.6
	181	32.0	75.9	459.7	836.2

90-day Oral Gavage Bridging Study with GS-9137 and Ritonavir in Rats

Key study findings: This study evaluated the toxicity of two test articles (GS-9137 and ritonavir) when administered once daily, either alone or in combination, via oral gavage to rats for at least 90 days. Male and female Crl:CD(SD) rats (5-10 animals/sex/group) were assigned to seven main study groups (toxicity animals) and received the test articles at dose levels of 0 (vehicle control I, 0.5% methylcellulose), 0 (vehicle control II, ethanol:water:propylene glycol; 43:15:42), 0 (vehicle controls I & II), GS-9137 (1000), ritonavir alone (10), GS-9137/ritonavir (100/10 and 1000/10) mg/kg/day. Clinical observations or ophthalmic examinations did not yield any test article-related findings. There were increased incidence of subepithelial vacuolation in the duodenum and ileum of male, and jejunum and cecum of female rats treated with 1000 mg/kg/day GS-9137 compared to their (Group 1) vehicle control. Increased incidence of subepithelial vacuolation was also noted in the duodenum and ileum of male rats (in Group 7) treated with ritonavir and high dose GS-9137 when compared to the (Group 3) controls. No vacuolation of the intestinal mucosal epithelium itself was seen. The NOAEL following 13 weeks of once daily oral gavage dosing of ritonavir and GS-9137, either alone or in combination, was considered to be 10 mg/kg/day ritonavir and 1000 mg/kg/day GS-9137.

Study no.: TOX-183-2007

Volume # and page #: electronic

Conducting laboratory and location: (b) (4)

Date of study initiation: June 6, 2006

Date of study completion: July 27, 2007

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: GS-9137: 061240; 99.1% pure and Ritonavir: 39098AW21; 100% pure

Methods

Group	Number of Animals		GS-9137 (mg/kg/ day)	Ritonavir (mg/kg/day)	GS-9137 (mg/ml)	Ritonavir (mg/ml)	Dose volume (ml/kg)
	♂	♀					
	Toxicity Animals						
1. Vehicle Control I only	5	5	0	0	0	0	5
2. Vehicle Control II only	5	5	0	0	0	0	1
3. Vehicle Controls I & II a	10	10	0	0	0	0	5, 1b
4. GS-9137 alone	10	10	1000	0	200	0	5
5.Ritonavir alone	10	10	0	10	0	10	1
6.Ritonavir & GS-9137	10	10	100	10	20	10	5, 1c
7.Ritonavir & GS-9137	10	10	1000	10	200	10	5, 1c
	Toxicokinetic Animals						
8. Vehicle Control I only	3	3	0	0	0	0	5
9.Vehicle Control II only	3	3	0	0	0	0	1
10.Vehicle Controls I & II a	9	9	0	0	0	0	5,1b
11.GS-9137 alone	9	9	1000	0	200	0	5
12. Ritonavir alone	9	9	0	10	0	10	1
13. Ritonavir & GS-9137	9	9	100	10	20	10	5, 1c
14.Ritonavir & GS-9137	9	9	1000	10	200	10	5, 1c

a Formulated separately and dosed sequentially.
b Vehicle I was dosed at a volume of 5 mL/kg, while Vehicle II was dosed at a volume of 1 mL/kg.
c GS-9137 was dosed at a volume of 5 mL/kg, while ritonavir was dosed at a volume of 1mL/kg.

Doses: male and female Crl:CD(SD) rats (5-10 animals/sex/group) were assigned to seven main study groups (toxicity animals) and received the test articles at dose levels of 0 (vehicle control I, 0.5% methylcellulose), 0 (vehicle control II, ethanol:water:propylene glycol; 43:15:42), 0 (vehicle controls I & II), GS-9137 (1000), ritonavir alone (10), GS-9137/ritonavir (100/10 and 1000/10) mg/kg/day.

Species/strain: male and female rats; Crl: CD (SD)

Number/sex/group or time point (main study): see methods

Route, formulation, volume, and infusion rate: oral gavage, 1-5 ml/kg/day

Satellite groups used for toxicokinetics: see methods

Age: & Weights: at initiation of treatment, the animals were approximately 7.5 weeks old, and their body weights ranged from 186 to 233 g for the males and 133 to 196 g for the females.

Sampling times: blood samples for clinical pathology immediately prior to necropsy. Samples were collected from TK animals on Days 1 and 90 of the dosing phase.

Observations and Results

Mortality: two animals died prior to terminal sacrifice. One Group 6 male (B05609) was sacrificed in moribund condition on Day 16. The last clinical signs for this animal included swollen right front digits, hypoactive behavior, audible and labored respiration, and cold to touch. The clinical pathology for this male indicated there were no abnormalities in the parameters that could be considered an effect from the combination of the two test articles. Necropsy findings for this animal were limited to firm material in the cecum and brown mucosa. One Group 5 female (B05712) was found dead on Day 87. The last clinical signs for this animal included malocclusion, and alopecia. Its necropsy findings were limited to discolored lung and mesenteric lymph node. Both of these unscheduled deaths were attributed to gavage accidents. All other animals survived to the scheduled sacrifice on Day 95 or 96

Clinical signs: the most common clinical sign, alopecia, began on Dosing Day 22; however, it did not follow a dose-response pattern. Other clinical signs included limited use of a paw or digit, swollen paw, malocclusion, liquid feces, rough haircoat, red eye discharge, and red haircoat or skin. All of these clinical signs were sporadic.

Body weights: there were no drug related effects on body weights and body weight gain in this study.

Food consumption: no statistically significant changes in mean food consumption were noted in males. In females, Group 2 (Vehicle Control II alone) generally and sometimes significantly had lower food consumption versus Group1 (Vehicle Control I alone) and Group 3 (Vehicle Controls I and II).

Ophthalmology: there were no drug related ocular changes.

Clinical pathology: the clinical pathology data (hematology, coagulation, serum chemistry and urinalysis) were generally unremarkable and similar among the groups.

Organ weights: no organ weight changes were attributed to test article effects.

Histopathology: adequate Battery: yes; Peer review: yes

Gross pathology: there were no macroscopic findings interpreted to be related to the administration of drug.

Histopathology: microscopic findings in animals receiving the vehicle controls were compared to each other while test article treated animals were compared to the control animals receiving the same vehicle or combination of vehicles. No toxicologically significant microscopic differences were observed between the control groups (1,2,3) employed in this study.

Rats treated with 1000 mg/kg/day GS-9137 (Group 4) had an increased incidence of subepithelial vacuolation in the duodenum and ileum in males, and the jejunum and

cecum in females, compared to their (Group 1) vehicle controls. Male rats treated with ritonavir and 1000 mg/kg/day GS-9137 (Group 7) had an increased incidence of subepithelial vacuolation in the duodenum and ileum over their (Group 3) controls. No vacuolation of the intestinal mucosal epithelium itself was seen.

Subepithelial vacuolation was characterized by the presence of clear vacuoles of somewhat variable size, located in the lamina propria of the intestine immediately adjacent to the intestinal epithelium. Because of artifactual separation of the epithelium from the lamina propria at this location in a number of animals, sometimes resulting in a frayed or vacuolated appearance.

Toxicokinetics: summary of mean toxicokinetic parameters is shown in Table 18. Systemic exposure of GS-9137, and metabolites GS-9200 and GS-9202, was generally similar on Day 1 and Day 90. Ritonavir co-administration with GS-9137 increased AUC values ≤ 2-fold on Day 1 and Day 90 in male and female animals. Co-administration of 1000 mg/kg/day GS-9137 decreased exposure to ritonavir in male animals on Days 1 and 90 and in female animals on Day 90.

Table 18. Day 1 and Day 90 Toxicokinetic Parameters for GS-9137 Following Once Daily Oral Gavage Administration of GS-9137 With or Without Ritonavir to Rats for 90 Days

Dose GS9137/ Ritonavir (mg/kg/day)	Sex	Day	Tmax (hr)	Cmax (ng/mL)	AUC_{0-t} (ng·hr/mL)	$T_{½}$ (hr)
1000/0	Female	1	0.500	40800	179000	3.95
	Male	1	1.00	24400	75800	125
100/10	Female	1	0.500	13000	65300	1.83
	Male	1	2.00	10200	51000	1.61
1000/10	Female	1	1.00	29700	180000	3.66
	Male	1	1.00	23700	111000	3.61
1000/0	Female	90	0.500	47700	133000	3.78
	Male	90	1.00	21600	72800	11.4
100/10	Female	90	1.00	24400	81300	2.68
	Male	90	1.00	14100	51600	2.07
1000/10	Female	90	0.500	50000	167000	20.3
	Male	90	1.00	31200	140000	2.11

Table 19. Day 1 and Day 90 Toxicokinetic Parameters for GS-9200 Following Once Daily Oral Gavage Administration of GS-9137 With or Without Ritonavir to Rats for 90 Days

Dose GS-9137/ Ritonavir (mg/kg/day)	Sex	Day	Tmax (hr)	Cmax (ng/mL)	AUC_{0-t} (ng·hr/mL)	$T_{½}$ (hr)
1000/0	Female	1	1.00	4600	16300	6.61
	Male	1	1.00	3670	13700	NC
100/10	Female	1	0.500	1290	4000	3.33
	Male	1	2.00	1720	5030	8.35
1000/10	Female	1	1.00	3150	17400	1.58
	Male	1	1.00	3210	11100	1.97
1000/0	Female	90	1.00	3880	10700	12.0
	Male	90	1.00	2960	11300	7.59
100/10	Female	90	1.00	1340	4690	5.05
	Male	90	1.00	1390	4640	1.52
1000/10	Female	90	1.00	3130	14000	6.43
	Male	90	1.00	2540	12500	3.86

Table 20. Day 1 and Day 90 Toxicokinetic Parameters for GS-9202 Following Once Daily Oral Gavage Administration of GS-9137 With or Without Ritonavir to Rats for 90 Days

Dose GS9137/ Ritonavir (mg/kg/day)	Sex	Day	Tmax (hr)	Cmax (ng/mL)	AUC_{0-t} (ng·hr/mL)
1000/0	Female	1	2.00	45.7	77.3
	Male	1	0.500	94.3	66.6
	Female	90	0.500	38.0	9.50
	Male	90	1.00	91.7	45.1
1000/10	Female	90	1.00	82.7	20.7

Table 21. Day 1 and Day 90 Toxicokinetic Parameters for Ritonavir Following Once Daily Oral Gavage Administration of Ritonavir With or Without GS-9137 to Rats for 90 Days

Dose GS9137/ Ritonavir (mg/kg/day)	Sex	Day	Tmax (hr)	Cmax (ng/mL)	AUC_{0-t} (ng·hr/mL)	$T_{½}$ (hr)
0/10	Female	1	2.00	1030	4790	1.37
	Male	1	0.500	362	1270	1.37
100/10	Female	1	4.00	458	3200	1.14
	Male	1	2.00	261	1260	1.21
1000/10	Female	1	2.00	896	3590	1.44
	Male	1	0.500	233	716	1.10
0/10	Female	90	2.00	1960	8380	1.52
	Male	90	2.00	823	4340	1.44
100/10	Female	90	1.00	1750	8690	1.42
	Male	90	2.00	816	3680	1.38
1000/10	Female	90	4.00	1120	5530	1.29
	Male	90	1.00	351	1670	1.18

Although potential test article related vacuolation was observed at the highest dose of GS-9137 with or without ritonavir, the NOAEL following 13 weeks of once daily oral gavage dosing of ritonavir and GS-9137, either alone or in combination, was considered to be 10 mg/kg/day ritonavir and 1000 mg/kg/day GS-9137, the highest dose levels in this study.

Thirteen Week Oral Gavage Bridging Study with GS-9350 and GS-9137 in Rats with a 1-Month Recovery Period

Key study findings: this study evaluated the toxicity and determined the toxicokinetics of GS-9350 and GS-9137 when administered daily either alone, or in combination, via oral gavage to rats for at least 90 days and assessed the reversibility, persistence, or delayed occurrence of any effects after at least a 28-day recovery phase. Terminal body weights were unaffected by treatment, and macroscopic and microscopic findings were unremarkable. Significantly increased mean absolute and relative liver weights (up to 1.5-fold of control means) were observed in males and females given GS-9350 alone, and in combination with GS-9137 at both dose levels. Liver weights remained slightly elevated at the end of the recovery period in 30 mg/kg/day GS-9350 animals. There were no microscopic correlates to the increased liver weights, and they were considered adaptive changes secondary to microsomal enzyme induction. In conclusion, the NOAEL for male and female rats following at least 90 days of once daily oral gavage dosing of GS-9350 and GS-9137, either alone or in combination, is considered to be 30 mg/kg/day GS-9350 and 1000 mg/kg/day GS-9137. At the 30/1000 mg/kg/day GS-9350/GS-9137 dose, exposures on Day 90 were: GS-9350 Cmax: 1743 and 4023 ng/mL, AUC_{0-t}: 5184 and 7548 ng•hr/mL in males and females, respectively; GS-9137 Cmax: 48367 and 56633 ng/mL, AUC_{0-t}: 183023 and 201287 ng•hr/mL in males and females, respectively.

Study no.: TOX-236-2001

Volume # and page #: electronic

Conducting laboratory and location: (b) (4)

Date of study initiation: September 23, 2008

Date of study completion: 11 Dec 2009

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: GS-9350: 3168-157-8 (97.5% pure) and GS-9137: 081147 (99.6% pure)

Methods

Species/strain: male and female Hsd:Sprague Dawley SD rats

Number/sex/group or time point (main study): male and female rats were assigned to study groups as follows:

Group	No. of Animals Male	Female	Dose Level (mg/kg/day) GS-9350	GS-9137	Concentration (mg/mL) GS-9350	GS-9137	Dose Volume (mL/kg/day)
Toxicity Animals							
1 (Vehicle Control I – PG/EtOH 95/5)[a]	10	10	0	0	-	-	1
2 (Vehicle Control II – 0.5% Methylcellulose)[b]	10	10	0	0	-	-	5
3 (Vehicle Control I/II only)[a,b,c]	15	15	0	0	-	-	1, 5
4 (GS-9350)[a,c]	15	15	30	-	30	-	1
5 (GS-9137)[b,c]	15	15	-	1000	0	200	5
6 (GS-9350 and GS-9137 – Low)[a,b]	10	10	30	100	30	20	1, 5
7 (GS-9350 and GS-9137 – High)[a,b,c]	15	15	30	1000	30	200	1, 5
Toxicokinetic Animals							
8 (Vehicle Control I – PG/EtOH 95/5)[a]	3	3	0	0	-	-	1
9 (Vehicle Control II – 0.5% Methylcellulose)[b]	3	3	0	0	-	-	5
10 (Vehicle Control I/II only)[a,b]	3	3	0	0	-	-	1, 5
11 (GS-9350)[a]	9	9	30	-	30	-	1
12 (GS-9137)[b]	9	9	-	1000	-	200	5
13 (GS-9350 and GS-9137 – Low)[a,b]	9	9	30	100	30	20	1, 5
14 (GS-9350 and GS-9137 – High)[a,b]	9	9	30	1000	30	200	1, 5

a Animals in Groups 1, 3, 4, 6-8, 10-11, 13-14 were administered Vehicle Control I (PG/EtOH 95/5) or GS-9350 at a dose volume of 1 mL/kg.
b Animals in Groups 2, 3, 5-7, 9-10, 12-14 were administered Vehicle Control II (0.5% methylcellulose) or GS-9137 at a dose volume of 5 mL/kg.
c Five animals/sex/group in Groups 3, 4, 5, and 7, designated as recovery-sacrifice animals, underwent at least 28 days of recovery following dose administration.

Route, formulation, volume, and infusion rate: oral gavage, 1-5 ml/kg/day

Satellite groups used for toxicokinetics: see above

Age: & Weights: at initiation of treatment, the animals were 46 days old. Body weights ranged from 188 to 225 g and 142 to 176 g for males and females, respectively.

Sampling times: blood samples for clinical pathology immediately prior to necropsy. Samples were collected from TK animals on Days 1 and 90 of the dosing phase.

Gross and Histopathology: tissues weighed, preserved and examined.

Observations and Results

Mortality: all animals survived to the scheduled dosing and recovery phase sacrifices.

Clinical signs: there were no clinical signs related to treatment with GS-9350, GS-9137, or GS-9350/GS-9137 in combination.

Body weights: there were no effects on body weight or body weight gain related to treatment with GS-9350, GS-9137, or GS-9350/GS-9137 in combination. Mean body weights and body weight gains in treated groups were similar to controls at the end of the dosing and recovery phases.

Food consumption: there were no changes in mean food consumption related to treatment with GS-9350, GS-9137, or GS-9350/GS-9137 in combination; all treated groups were similar to controls.

Ophthalmology: there were no drug related ocular changes.

Clinical pathology: there were no adverse hematological alterations associated with treatment of GS-9350 or GS-9137, or the combination. Slight, generally statistically significant GS-9350-related changes in the hematology data included slightly lower mean values for erythrocyte count, hemoglobin and hematocrit in females given 30 mg/kg/day GS-9350, and 30/100 and 30/1000 mg/kg/day GS-9350/GS-9137 in combination. Slight increases in mean platelets counts were noted in females given 30 mg/kg/day GS-9350 and in males and females given 30/100 mg/kg/day GS-9350/GS-9137 in combination. Slight decreases in prothrombin time were observed in females given 30 mg/kg/day GS-9350 or 1000 mg/kg/day GS-9137, but not in animals administered both compounds in combination. Changes in mean APTT were variable, with slight increases noted in males given 30 mg/kg/day GS-9350, and in males and females given 30/100 mg/kg/day GS-9350/GS-9137, with decreases noted in females given 1000 mg/kg/day GS-9137. All these changes were of small magnitude, of no biologic importance (not adverse), and had no microscopic correlate. After the 13-week recovery period, the values of the aforementioned parameters were generally unremarkable and comparable between control, indicating the reversibility of the changes.

Statistically significant changes in clinical chemistry parameters included minimal increases (1.2-fold) in ALT in females given 30/100 and 30/1000 mg/kg/day GS-9350/GS-9137 in combination, as well as minimal increases (up to 1.2-fold) in urea nitrogen in males given 30 mg/kg/day GS-9350 alone and in combination with 100 or 1000 mg/kg/day of GS-9137. All of these changes were of small magnitude, not clearly dose-related and/or inconsistent between the sexes, were not associated with microscopic correlates, and were not considered toxicologically relevant.

Organ weights: significantly increased mean absolute and relative liver weights (up to 1.5-fold control means) were observed in male and female rats given 30 mg/kg/day GS-9350 alone, and in combination with GS-9137 at both dose levels. Increased liver weights were considered related to GS-9350 treatment, and were not observed in animals administered GS-9137 alone. At the end of the 28-day recovery phase, absolute and relative mean liver weights in males and females administered 30 mg/kg/day GS-9350 remained slightly increased versus controls. There were no histopathological correlates to the increased liver weights, although they are likely adaptive changes secondary to the observed microsomal enzyme induction in GS-9350-treated animals. Significant increases in absolute and/or relative cecum weights were noted in females administered 30 mg/kg/day GS-9350 alone, and in combination with GS-9137 at both dose levels. In male rats there was considerable variability in absolute and relative cecum weights

between control groups, which precluded a definite assessment of cecum weight changes, although absolute and relative weights were significantly increased in males given 30/1000 mg/kg/day GS-9350/GS-9137 compared to Group 3 control animals. There were no microscopic correlates to these weight changes, they were not considered adverse, and the relationship to treatment is unclear. There were no remarkable changes in cecum weights in recovery males and females showing the reversibility of these changes.

Histopathology: adequate Battery: yes; Peer review: yes

Gross pathology: there were no macroscopic findings interpreted to be related to the administration of drug.

Histopathology: there were no treatment-related microscopic changes in dosing or recovery phase animals. A similar frequency and severity of commonly seen spontaneous and incidental findings were noted in animals from control and treated groups.

Toxicokinetics: summary of mean toxicokinetic parameters is shown in Tables 22 and 23. Exposure to GS-9350, when administered alone or in combination with GS-9137, was generally lower in males than females. After multiple dosing, GS-9350 exposures were similar indicating no marked (> 2-fold) accumulation. Co-administration of GS-9350 with GS-9137 generally resulted in similar GS-9350 exposures. Exposure to GS-9137 increased with the increase in dose level from 100 to 1000 mg/kg/day. When administered alone, exposures were generally higher in females than males. Following co-administration with GS-9350, GS-9137 exposures were similar in females and slightly increased in males compared to when administered alone. After multiple dosing in combination with GS-9350, exposures to GS-9137 generally decreased compared to Day 1 values.

Table 22. Toxicokinetic Parameters for GS-9350 in Rat Plasma

Dose	Dose Level (mg/kg/day)			C_{max}	T_{max}	AUC_{0-t}	C_{last}	T_{last}	Dose Group Ratio 13/11		14/11	
Group	GS-9137	GS-9350	Sex	(ng/mL)	(hr)	(ng•hr/mL)	(ng/mL)	(hr)	C_{max}	AUC_{0-t}	C_{max}	AUC_{0-t}
						Day 1						
11	-	30	M	2727	1.00	11189	8.93	16.0				
			F	2143	2.00	13930	215	16.0				
13	100	30	M	1447	1.00	5451	73.4	12.0	0.5	0.5		
			F	1963	0.500	10962	88.1	16.0	0.9	0.8		
14	1000	30	M	691	4.00	3231	6.33	16.0			0.3	0.3
			F	1927	1.00	11266	45.9	24.0			0.9	0.8
						Day 90						
11	-	30	M	1660	2.00	7406	5.92	12.0				
			F	3745	1.00	15435	33.3	24.0				
13	100	30	M	2217	1.00	8950	46.8	8.00	1.3	1.2		
			F	3336	2.00	16387	12.4	24.0	0.9	1.1		
14	1000	30	M	1743	1.00	5184	9.11	8.00			1.1	0.7
			F	4023	1.00	7548	39.5	24.0			1.1	0.5

Table 23. Toxicokinetic Parameters for GS-9137 in Rat Plasma

Dose Group	Dose Level (mg/kg/day) GS-9137	GS-9350	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	C_{last} (ng/mL)	T_{last} (hr)	Dose Group Ratio 14/12 C_{max}	AUC_{0-t}
					Day 1					
12	1000	-	M	43367	1.00	138575	336	24.0		
			F	56933	1.00	248348	564	24.0		
13	100	30	M	33033	1.00	135539	37.0	24.0		
			F	29500	0.500	118091	132	24.0		
14	1000	30	M	57200	1.00	420686	233	24.0	1.3	3.0
			F	65233	1.00	390468	5380	24.0	1.1	1.6
					Day 90					
12	1000	-	M	27300	0.500	108570	317	24.0		
			F	59433	0.500	228628	942	24.0		
13	100	30	M	20933	1.00	50908	322	24.0		
			F	17613	2.00	86007	426	24.0		
14	1000	30	M	48367	1.00	183023	3055	24.0	1.8	1.7
			F	56633	1.00	201287	2208	24.0	1.0	0.9

One month oral toxicity study of JTK-303 in dogs

Key study findings: GS-9137 suspended in corn oil was dosed orally once daily for 1 month to beagle dogs (3 animals/sex/group) at dose levels of 10, 30 and 100 mg/kg. The control group received the vehicle (corn oil) alone. Dilatation of the cecum was seen in males dosed at 100 mg/kg. No histopathological findings were observed in the cecum of males dosed at 100 mg/kg. Any pathology findings noted were considered incidental or spontaneous. In conclusion, systemic or target organ toxicity was not observed in males or females at doses up to 100 mg/kg which may be considered the NOAEL.

Study no: JTK303-TOX-004
Volume #, and page #: vol 9; pg 48
Conducting laboratory and location: (b) (4)
Date of study initiation: 3/2003
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: JTK-303; lot K; purity 99.4%

Methods

Doses: 10, 30, 100 mg/kg
Species/strain: Beagle dogs
Number/sex/group or time point (main study): 3/sex/group
Route, formulation, volume, and infusion rate: oral gavage; suspension in corn oil; 2.5 mL/kg
Age: 6 mo.
Weight: 7.7-9.9 kg females; 8.4-10.5 kg males

Observations and Results:

Mortality: none

Clinical signs: vomiting and loose stools in all groups due possibly to drug and to corn oil (little difference in incidence among groups including controls).

Body weights: no effects

Food consumption: no effects

Ophthalmoscopy: no effects

EKG: no effects

Hematology: no effects

Clinical chemistry: statistically significant decrease in plasma K levels in HD males.

Urinalysis: no effects

Gross pathology: dilation of cecum in 2 HD males; whitish focus in lung in 1 HD male; reddish focus in the lung of another HD male; and reddish focus in jejunum in 2 MD males (one with slight hemorrhage) were seen. Whitish focus in lung of 1 MD female and reddish focus in the adrenal in 1 LD female were observed.

Organ weights: no changes.

Histopathology: adequate Battery: yes Peer review: yes

No compound related changes.

Toxicokinetics: parameters are shown in Table 24. Plasma Concentrations of the Parent Drug: Systemic exposure to the parent drug, both the Cmax and AUC_{0-24}, increased dose-dependently in both sexes without any gender differences on Day 1 or in Week 4. The plasma concentrations of the parent drug in week 4 were comparable to those on Day 1.

Table 24. Mean toxicokinetic parameters in dogs (n=3)

Dose (mg/kg)	Sex	Day	$AUC_{0\text{-}24h}$ (µg*h/ml)	Cmax (µg/ml)
10	M	1	8	1.3
		23	9	1.3
	F	1	7	1.1
		23	8	1.2
30	M	1	21	3.9
		23	19	3.9
	F	1	29	4
		23	30	3.4
100	M	1	66	8
		23	55	8
	F	1	83	11
		23	71	11

Nine-month oral dose toxicity study in dogs with a 3 month recovery period.

Key study findings: groups of male and female beagle dogs (4 animals/sex/group) were administered orally once daily at dose levels of 0 (vehicle controls, corn oil), 10, 30 and 100 mg/kg/day for nine months. The reversibility of any findings was investigated by adding groups of two males and two females to the vehicle control group and the 100 mg/kg group and setting a withdrawal period of three months after the dosing period. A water-loaded group of four males and four females was provided to investigate the effects of the vehicle during the dosing period. Minimal or slight lipid vacuoles containing mainly triglycerides were observed in the upper small intestinal lamina propria in both sexes mainly at 30 and 100 mg/kg after the dosing period. The incidence and degree of the lipid vacuoles tended to be decreased in both sexes at 100 mg/kg after the recovery period when compared with those at the end of the dosing period. Thus the lipid vacuoles appeared to resolve slowly after withdrawal of the compound. Systemic or target organ toxicity was not observed in males or females at doses up to 100 mg/kg/day which may be considered the NOAEL.

Study no.: JTK303-TOX-023
Volume #, and page #: vol 04; pg 1
Conducting laboratory and location: (b) (4)
Date of study initiation: July, 2004
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity: JTK-303, lot no. R, 98.1% pure

Methods

Doses: 10, 30, 100 mg/kg/day
Species/strain: Beagle dogs
Number/sex/group or time point (main study): 4/sex/group
Route, formulation, volume, and infusion rate: oral gavage, suspension in corn oil, 2.5 ml/kg.

Satellite groups used for toxicokinetics or recovery: 2/sex/in vehicle control and HD groups.
Age: 5-6 months
Weight: 5.9-10.0 kg

Observations and Results:

Mortality: none

Clinical signs: none

Body weights: no effects

Food consumption: no effects

Ophthalmoscopy: no effects

EKG: no effects

Hematology: lymphocyte ratio was increased by 31% in HD males at wk 39. Normal at recovery. Decrease in APTT in HD males at wks 13 and 39.

Clinical chemistry: serum A/G ratio and ALB levels were increased by 23% and 11% in HD females at wk 39 when compared to controls. No effects at recovery.

Urinalysis: decrease in urinary excretion of K, Cl, Na and urine volume in HD females at wk 39.

Gross pathology: no findings

Organ weights : absolute liver weight was decreased and relative adrenal and submandibular gland weights were increased in HD males at the end of dosing. Relative ovarian weights were increased in HD females. All were normal at recovery.

Histopathology: adequate Battery: yes Peer review: yes

Minimal or slight lipid vacuoles were seen in the upper small intestinal (duodenal and jejunal) lamina propria in all animals of both sexes at 30 and 100 mg/kg dose levels. Subtle lipid vacuoles were also observed in the duodenal lamina propria in 1 LD female. Vacuoles contained primarily triglycerides.

Vacuoles remained in 2 males and 2 females of the HD after the recovery period.

Effects of the Vehicle (Corn Oil): body weights were slightly higher in males in the vehicle control group than in the water-loaded group. The number of animals with left over food was increased in males in the vehicle control group. Accordingly food consumption was sporadically lower in males in the vehicle control group than in the water-loaded group. These changes were

limited to males and were likely related to an increased calorie intake due to corn oil. Taking in account the magnitude of the higher body weights and lower food consumption, corn oil did not affect the toxicological evaluation of the test article.

Toxicokinetics: parameters are shown in Table 25. The plasma concentrations of the parent drug (Cmax and AUC_{0-24} values) showed a dose-dependent increase at dose levels up to 100 mg/kg on all sampling days. Systemic exposure to the parent drug (Cmax and AUC_{0-24} values) was comparable between males and females at each dose level.
There were no marked differences in systemic exposure for males at 10 and 30 mg/kg and females at all dose levels between the sampling days. Taking these results, systemic exposure to the parent drug was not affected by repeated dosing.

Table 25. Mean toxicokinetic parameters of JTK-303 in dogs

Dose (mg/kg/day)	Day	Cmax (µg/ml)		AUC_{0-24h} (µg*hr/ml)	
		Males	Females	Males	Females
10	1	1.1	1.1	6.1	5.9
	91	0.9	1.3	7.0	7.7
	273	1.0	0.8	7.6	7.8
30	1	3.2	2.2	18.1	14.5
	91	3.5	3.3	22.0	23.8
	273	3.7	2.9	25.2	23.9
100	1	3.7	6.3	26.0	40.6
	91	9.6	9.1	60.5	63.7
	273	8.7	7.5	53.9	66.0

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Bacterial reverse mutation test of JTK-303

Key findings: the mutagenic potential of JTK-303 (concentrations ranging from 1.5 – 750 µg/plate) was evaluated in a bacterial reverse mutation test using 4 strains of *Salmonella typhimurium* (TA98, TA1537, TA100 and TA1535) and 1 strain of *Escherichia coli* (WP2*uvrA*). Under the conditions of this study, JTK-303 did not induce any bacterial reverse mutations either with or without S9 mix.

Study no.: 03913
Volume #, and page #: vol 9, pg 270
Conducting laboratory and location: (b) (4)
Date of study initiation: 11/03
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity: JTK-303, lot K, 99.4% pure

Methods

Strains/species/cell line: Salmonella typhimurium (TA98, TA1537, TA100, TA1535) and E. Coli (WP2uvrA).

Doses used in definitive study: up to 750 µg/plate

Basis of dose selection: inhibitory effect on growth occurred at 750, 46.9, 93.8, 93.8, and 750 µg/plate for TA98, 1537, 100, 1535 and WP2uvrA, respectively with S9 mix. It occurred at 375, 23.4, 46.9, 46.9 and 750 µg/plate for TA 98, 1537, 100, 1535 and WP2uvrA, respectively, without S9.

Negative controls: 0.1 µg/plate DMSO

Positive controls: 2-aminoanthracene, 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide, sodium azide, 9-aminoacridine.

Incubation times: 20 min preincubation and 48 hr or longer incubation in agar plates.

Results

No increase occurred in the number of revertants per plate with any of the bacterial strains at any dose level either with or without S9 mix.

Study validity: study was valid.

Study outcome: negative with or without S9.

7.2 *In Vitro* Assays in Mammalian Cells

Chromosomal aberration test of JTK-303 in cultured mammalian cells

Key findings: the potential for JTK-303 to induce chromosomal aberrations in cultured mammalian cells was evaluated using Chinese Hamster Lung Cells, either with or without rat liver metabolic activation (S9 mix). Cytotoxicity levels (percent cell growth inhibition) at the dose levels chosen for chromosomal analysis were 26.1, 35.6 and 47.5% for the 6-hour treatment with S9 mix, 20.9, 30.9 and 48.1% for the 6-hour treatment without S9 mix and 23.7, 44.4 and 46.5% for the 24-hour treatments without S9 mix, respectively.

In the chromosomal examinations, the frequency of cells with structural chromosomal aberrations was 8.0, 7.5 and 9.0% at 55, 65 and 75 µg/mL for the 6-hour treatment without S9 mix, respectively. No structurally aberrant cells were observed at any dose level for the 6-hour treatment with S9 mix or for the 24-hour treatment without S9 mix. In a confirmatory study (dose levels: 35, 45, 55, 65, 75, 85 µg/mL), the frequency of cells with structural chromosomal aberrations was 7.5, 8.5 and 7.0% at 55, 65 and 75 µg/mL for the 6-hour treatment without S9 mix, respectively, and this was reproducible. Cytotoxicity levels were 23.1, 30.7 and 45.1% at 55, 65 and 75 µg/mL, respectively.

No numerical chromosomal aberrations were induced by any treatment with JTK-303. The incidences rate of chromosomal aberrations in the negative control group was 1.5% or less, with a high frequency of structural chromosomal aberrations being observed in the positive control group.

Study no.: 03912
Volume #, and page #: vol. 9, pg 312
Conducting laboratory and location: (b) (4)
Date of study initiation: 10/03
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity: JTK-303, lot K, 99.4% pure

Methods

Strains/species/cell line: Chinese Hamster Lung Cells (CHL)

Doses used in definitive study:

S9 Mix	Duration of Exposure (hr)	Dose Levels of JTK-303 (µg/mL)
+	6	145, 155, 165, 175, 185
-	6	55, 65, 75, 85
	24	25, 35, 45, 55

From these, following dose selection by evaluating the cytotoxicity levels at each dose, chromosomal examinations were performed at 155, 165 and 175 µg/mL for the 6-hour treatment with S9 mix, 55, 65, 75 µg/mL for the 6-hour treatment without S9 mix, and at 25, 35 and 45 µg/mL for the 24-hour treatment without S9 mix.

Basis of dose selection: dose range finding study

Negative controls: DMSO

Positive controls: mitomycin C, Cyclophosphamide.

Incubation and sampling times: 6 hr with S9, 6 and 24 hr without S9.

Results:

Cytotoxicity levels (percent cell growth inhibition) in the cultures at the highest dose levels of JTK-303 were 72.2% (185 µg/mL) for the 6-hour treatment with S9 mix, 69.6% (85 µg/mL) and 55.6% (55 µg/mL) for 6- and 24-hour treatments without S9 mix, respectively. Based on these findings, chromosomal aberrations were observed at 155, 165 and 175 µg/mL for the 6-hour

treatment with S9 mix, at 55, 65 and 75 µg/mL for the 6-hour treatment without S9 mix, and at 25, 35 and 45 µg/mL for the 24-hour treatment without S9 mix.
Cytotoxicity levels at the dose levels chosen for chromosomal analysis were 26.1, 35.6 and 47.5% for the 6-hour treatment with S9 mix, 20.9, 30.9 and 48.1% for the 6-hour treatment without S9 mix and 23.7, 44.4 and 46.5% for the 24-hour treatments without S9 mix, respectively.

Chromosomal aberration results are shown in Tables 26, 27 and 28. In the chromosomal examinations, the frequency of cells with structural chromosomal aberrations was 8.0, 7.5 and 9.0% at 55, 65 and 75 µg/mL for the 6-hour treatment without S9 mix, respectively. No structurally aberrant cells were observed at any dose level for the 6-hour treatment with S9 mix or the 24-hour treatment without S9 mix.

In the confirmatory study, dose levels were 35, 45, 55, 65, 75 and 85 µg/mL. In the confirmatory test, cytotoxicity levels were 12.3, 20.8, 23.1, 30.7, 45.1 and 61.9% at 35, 45, 55, 65, 75 and 85 µg/mL, respectively. Based on these results, chromosomal examinations were performed at five dose groups except for 85 µg/mL. The frequency of cells with structural chromosomal aberrations was 7.5, 8.5 and 7.0% at 55, 65 and 75 µg/mL for the 6-hour treatment without S9 mix, respectively, and it was effectively reproducible.

Study validity: study was valid

Study outcome: weak potential to induce chromosomal aberrations.

Table 26. Chromosomal aberration test on CHL cells treated with JTK-303 for 6 hours with or without metabolic activation

Test article	Dose (µ g/mL)	S9mix	No. of cells observed	Frequency of aberrant cells (%)						Judge-ment	gap (%)	Polyploid (%)	Polyploid Judgement	Cytotoxicity (%)
				ctb	cte	csb	cse	others	TA					
DMSO	(1%)	+	200	0	0.5	0	0	0	0.5	-	0	0.5	-	0
JTK-303	155	+	200	1.0	1.5	0	0	0	2.0	-	0.5	1.5	-	26.1
	165	+	200	0.5	3.0	0	0	0	3.5	-	0.5	1.0	-	35.6
	175	+	200	1.0	2.0	0	0	0	3.0	-	0.5	1.5	-	47.5
CP	10	+	200	13.0	21.0	0	0	0	29.5	+	1.5	0	-	NC
DMSO	(1%)	-	200	0.5	0.5	0	0.5	0	1.5	-	1.0	0.5	-	0
JTK-303	55	-	200	3.0	5.0	0.5	1.0	0	8.0	±	0.5	3.0	-	20.9
	65	-	200	2.0	7.0	0	0	0	7.5	±	1.5	1.0	-	30.9
	75	-	200	2.5	7.0	0	0.5	0	9.0	±	1.0	1.0	-	48.1
MMC	0.1	-	200	20.5	36.0	0	0.5	0	46.5	+	6.0	0.5	-	NC

ctb: Chromatid break, cte: Chromatid exchange, csb: Chromosome break,
cse: Chromosome exchange, others: Fragmentation or multiple aberrations
TA: Total cells with structural aberrations
gap: Chromatid gap and chromosome gap, gap(%): Frequency of cells with gaps
DMSO: Dimethylsulfoxide
CP: Cyclophosphamide
MMC: Mitomycin C
Cytotoxicity: Percent cell growth inhibition, NC: Not counted
Judgement: -Negative(TA or polyploid <5%); ±Equivocal(5%≤TA or polyploid <10%); +Positive(10%≤TA or polyploid)

Table 27. Chromosomal aberration test on CHL cells treated with JTK-303 for 24 hours without metabolic activation

Test article	Dose (μg/mL)	S9mix	No. of cells observed	Frequency of aberrant cells (%)						Judge-ment	gap (%)	Polyploid (%)	Polyploid Judgement	Cytotoxicity (%)
				ctb	cte	csb	cse	others	TA					
DMSO	(1%)	-	200	0	0.5	0	0	0	0.5	-	0.5	0	-	0
JTK-303	25	-	200	2.5	1.0	0	0	0	3.5	-	2.5	0	-	23.7
	35	-	200	3.0	0.5	0	0	0	3.5	-	4.0	0	-	44.4
	45	-	200	2.5	1.0	0	0	0	3.0	-	3.0	0	-	46.5
MMC	0.05	-	200	20.5	24.0	0	1.0	0	39.0	+	6.0	0.5	-	NC

ctb: Chromatid break, cte: Chromatid exchange, csb: Chromosome break,
cse: Chromosome exchange, others: Fragmentation or multiple aberrations
TA: Total cells with structural aberrations
gap: Chromatid gap and chromosome gap, gap(%): Frequency of cells with gaps
DMSO: Dimethylsulfoxide
MMC: Mitomycin C
Cytotoxicity: Percent cell growth inhibition, NC: Not counted
Judgement: -Negative(TA or polyploid <5%); ±Equivocal(5%≤TA or polyploid <10%); +Positive(10%≤TA or polyploid)

Table 28. Chromosomal aberration test on CHL cells treated with JTK-303 for 6 hours without metabolic activation (confirmatory test)

Test article	Dose (μg/mL)	S9mix	No. of cells observed	Frequency of aberrant cells (%)						Judge-ment	gap (%)	Polyploid (%)	Polyploid Judgement	Cytotoxicity (%)
				ctb	cte	csb	cse	others	TA					
DMSO	(1%)	-	200	1.0	0	0	0	0	1.0	-	1.0	0.5	-	0
JTK-303	35	-	200	0.5	0	0	0.5	0	1.0	-	2.5	1.0	-	12.3
	45	-	200	1.0	2.0	0	0	0	2.5	-	1.0	1.0	-	20.8
	55	-	200	3.0	7.5	0	0	0	7.5	±	0.5	1.0	-	23.1
	65	-	200	5.5	6.0	0	0	0	8.5	±	2.0	0.5	-	30.7
	75	-	200	3.5	5.0	0	0	0	7.0	±	2.5	0.5	-	45.1
MMC	0.1	-	200	17.0	19.5	0	0	0	33.5	+	5.0	0.5	-	NC

ctb: Chromatid break, cte: Chromatid exchange, csb: Chromosome break,
cse: Chromosome exchange, others: Fragmentation or multiple aberrations
TA: Total cells with structural aberrations
gap: Chromatid gap and chromosome gap, gap(%): Frequency of cells with gaps
DMSO: Dimethylsulfoxide
MMC: Mitomycin C
Cytotoxicity: Percent cell growth inhibition, NC: Not counted
Judgement: -Negative(TA or polyploid <5%); ±Equivocal(5%≤TA or polyploid <10%); +Positive(10%≤TA or polyploid)

The sponsor considers JTK-303 to have weak or equivocal potential to induce chromosomal aberrations with a 6 hr treatment but not with 24 h treatment without S9 and no potential for chromosomal damage in the presence of S9.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Micronucleus test of JTK-303 in rats

Key findings: Groups of male and female Sprague-Dawley rats were given JTK-303 orally as single doses of 0, 500, 1000 and 2000 mg/kg. From the treated animals, then the bone marrow, including polychromatic erythrocytes, was harvested at 24 or 48 hours post dosing and examined for micronuclei under fluorescence microscopy. Cyclophosphamide was used as a positive control. In the micronucleus assay, JTK-303 caused no increase in micronuclei in bone marrow polychromatic erythrocytes at any dose, whereas cyclophosphamide produced a marked and significant increase in micronuclei. JTK-303 was considered to have no potential to induce micronuclei in vivo.

Study no.: 03914
Volume #, and page #: vol. 9, pg 360
Conducting laboratory and location: (b) (4)
Date of study initiation: 11/03
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity: JTK-303; lot K, 99.4% pure

Methods

Strains/species/cell line: Sprague-Dawley rats

Doses used in definitive study: single oral doses of 0, 500, 1000, 2000 mg/kg

Basis of dose selection: MFD

Negative controls: 0.5% methylcellulose

Positive controls: 50 mg/kg cyclophosphamide

Incubation and sampling times: 24 and 48 hrs after drug administration

Results

Clinical signs: whitish stools were observed in both sexes at 2000 mg/kg on the day after dosing.

No changes in the incidences of reticulocytes, which could be indicative of bone marrow toxicity, were noted in any of the JTK-303 groups, when compared with the vehicle control group. In the positive control group, the incidence of reticulocytes was significantly higher than that in the vehicle control group, at a probability of $p<0.05$.

From the results of the one month oral toxicity study of this compound in rats, the plasma

concentrations of the parent drug were 43.52 ± 9.74 µg/mL (C_{max}) and 490.02 ± 98.55 µg•hr/mL (AUC_{0-24h}) in males, and 68.26 ± 7.57 µg/mL (C_{max}) and 723.52 ± 183.84 µg•hr/mL (AUC_{0-24h}) in females at 2000 mg/kg.

JTK-303 did not cause any increase in micronuclei in bone marrow polychromatic erythrocytes at 500, 1000 and 2000 mg/kg. In addition no compound-related changes suggesting toxicity of JTK-303 on the bone marrow were noted. In conclusion, the test article is considered not to have any potential to induce micronuclei in vivo.

Study validity: valid

Study outcome: negative

8 Carcinogenicity

A 104-Week Oral Gavage Carcinogenicity Study of GS-9137 with or without Ritonavir in the Albino Mouse

Study no.:	TX-183-2011
Study report location:	electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 8, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	EVG: 071090, 081147, and 081244; 100% pure Ritonavir: 18T1066703/78022/01N03CA; 100% pure
CAC concurrence:	Yes

Key Study Findings: daily oral administration of GS-9137 alone or in combination with ritonavir at doses of 0, 200, 600, 2000 mg/kg/day GS-9137 and 2000/25 mg/kg/day GS-9137/ritonavir for a minimum of 104 consecutive weeks produced no carcinogenic effect in male or female mice.

The most probable causes of death that can be associated with the administration of the test articles were dilatation of various segments of the gastrointestinal tract (stomach, duodenum, jejunum, colon and/or cecum) and chronic inflammation of the trachea. Dilatation of the gastrointestinal tract was noted macroscopically in both genders of all groups, with an increased incidence in animals given ritonavir vehicle, ritonavir alone, GS-9137 at 2000 mg/kg/day and 2000/25 mg/kg/day GS-9137/ritonavir.

Adequacy of Carcinogenicity Study: The dose levels of 0 (vehicle control for GS-9137), 200, 600 and 2000 mg/kg/day GS-9137 alone, and 0 (vehicle control for ritonavir), 25 mg/kg/day ritonavir alone and 2000 mg/kg/day GS-9137 in combination with 25 mg/kg/day ritonavir were

approved by the Exec CAC. The high dose of 2000 mg/kg/day was proposed, based on the maximal feasible dose.

Percentage survival in the high dose group (GS-9137 2000mg) was 77.1% (male) and 91.3% (female), and 55.7% (male) and 82.9 % (female) at the end of Weeks 52 and 79 respectively.

The carcinogenicity study in mice was considered to be acceptable.

Appropriateness of Test Models: mice historically have been used in safety evaluation studies and are recommended by appropriate regulatory agencies. In general GS-9137 was metabolized via a combination of oxidation and glucuronidation in all species tested including mouse and humans. In the in vivo studies, GS-9137 was the predominant circulating chemical species. Overall, the metabolic species produced appear to be comparable across species.

Evaluation of Tumor Findings: the oncogenicity potential of GS-9137 alone and in combination of ritonavir was investigated in male and female Crl:CD1 (ICR) mice with oral gavage dosages of 0 (vehicle control for GS-9137), 200, 600 and 2000 mg/kg/day GS-9137 alone, and 0 (vehicle control for ritonavir), 25 mg/kg/day ritonavir alone and 2000 mg/kg/day GS-9137 in combination with 25 mg/kg/day ritonavir. The protocol was approved by the Exec CAC. The systemic exposures were 2.4 and 3.8 times that in humans (150 mg once daily tablet AUCss = 23,000 ng*hr/ml) in male and female mice at the high dose level, respectively.
No significant increase in neoplasms was noted in male or female mice related to ritonavir alone or GS-9137 with or without ritonavir in this study.

Methods

Doses:	0 (vehicle control for GS-9137), 200, 600 and 2000 mg/kg/day GS-9137 alone, and 0 (vehicle control for ritonavir), 25 mg/kg/day ritonavir alone and 2000 mg/kg/day GS-9137 in combination with 25 mg/kg/day ritonavir
Frequency of dosing:	Once daily
Dose volume:	10 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Vehicle for GS-9137 (0.5% methylcellulose); vehicles for GS-9137 and ritonavir (0.5% methylcellulose and 43:15:42 ethanol: water: propylene glycol)
Basis of dose selection:	13-week oral gavage toxicology study and a 7-day PK study of GS-9137 in mice.
Species/Strain:	Male and female Crl:CD1 (ICR) mice
Number/Sex/Group:	70/sex/group
Age:	6 weeks
Animal housing:	2 per cage in stainless steel wire mesh floor cages equipped with an automatic watering valve and water bottle

Paradigm for dietary restriction:	All animals had free access to a standard certified pelleted commercial laboratory diet (PMI Certified Rodent 5002: PMI Nutrition International, Inc.),
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	20/sex/group toxicokinetics
Deviation from study protocol:	No

The study design

Group Number/ Identification	Dose (mg/kg/day)	Animal Numbers			
		Main Study		Toxicokinetic Study[c]	
		Males	Females	Males	Females
1/ Control Article[a]	0	70	70	6	6
2/ Vehicle Articles[b]	0/0	70	70	6	6
3/ ritonavir	25	70	70	21	21
4/ GS-9137	200	70	70	21	21
5/ GS-9137	600	70	70	21	21
6/ GS-9137	2000	70	70	21	21
7/ GS-9137/ ritonavir	2000/25	70	70	21	21
8 Health Screen	-	10	10	-	-

a The control article was 0.5% methylcellulose
b The vehicle articles were 43:15:42 ethanol water:PG and 0.5% methylcellulose. They were formulated separately and dosed as 2 sequential gavages
c The toxicokinetic population included 3 additional dosed animals per sex per group to be used as possible replacements

Control Article for GS-9137: 0.5% methylcellulose, aqueous
Control Article for Ritonavir: ethanol:water:propylene glycol (43:15:42)

Administration of Test/Control Article: each mouse in Groups 1, 3, 4, 5 or 6 was given a single daily oral dose of control or test article using a flexible gavage tube. Mice in Groups 2 and 7 were given two gavages per day. The ritonavir vehicle or ritonavir was given first followed immediately by the GS-9137 vehicle or GS-9137. Animals were treated at approximately the same time each day, 7 days a week for a minimum of 104 weeks. The dosing volume was 10 mL/kg for GS-9137 formulations and 1 mL/kg for ritonavir formulations. Each actual volume administered was based on the most recent practical body weight of each animal.

Observations and Results

Mortality: between 33 and 51 mice per sex/group died or were euthanized prior to the end of the study between Day 45 and Day 735. The percent survival in each group ranged from 29% to 47%. There was at least 20 animals/sex/group alive at the end of the treatment period.

Table 29. Percentage Survival at the end of the Treatment Period

Group Number/ Identification	Dose level (mg/kg/day)	Survival %			
		Males	%	Females[a]	%
1/ Control Article	0	37/70	53	33/70	47
2/ Vehicle Articles	0	32/70	46	20/70	29
3/ ritonavir	25	26/70	37	23/70	33
4/ GS-9137	200	38/70	54	32/70	46
5/ GS-9137	600	31/70	44	27/70	39
6/ GS-9137	2000	26/70	37	26/70	37
7/ GS-9137/ ritonavir	2000/25	21/70	30	22/70	31

a Week 103 (females only); Table does not include animals sent to necropsy after end of the minimum 103;females/104;males week dosing period.

Mortality Rates in GS-9137 alone treated animals at the End of the Experiment

	Cont.	**200 mg**	**600 mg**	**2000 mg**
Male	47.1%	45.7%	55.7%	62.9%
Female	51.4%	51.4%	61.4%	62.3%

This shows that the morality rate of in the high dose group in males is 14.8% higher than the control, while in females it is about 10.9% higher in high dose group compared to the control.

Most frequent cause of death/euthanasia of pre-terminal decedent animals are outlined in Tables 30 and 31.

Table 30. Most Common Causes of Death/Euthanasia of Pre-terminal Decedent Male* Mice

Tissue/Finding	Sex	Males						
	Dose (mg/kg/day)	0[a]	0[b]	25[c]	200[d]	600[d]	2000[d]	2000/25[e]
Neoplastic								
Lung								
Carcinoma: alveolar/bronchiolar		5	1	4	6	—	2	4
Liver								
Hemangiosarcoma		1	1	1	3	1	—	2
Hemolymphatic tissue								
Lymphoma		1	—	—	1	4	2	1
Non-neoplastic								
Kidney/Urogenital System								
Chronic Progressive Nephropathy		4	5	4	1	1	3	1
Obstructive Nephropathy		4	6	7	1	6	6	6
Urogenital Disease		1	4	2	2	5	8	1
Heart								
Thrombosis		2	1	2	1	1	3	—
Trachea								
Chronic inflammation		—	4	5	—	—	—	3
Digestive system								
Dilatation gastrointestinal (GI) tract		—	5	2	—	—	3	7
Systemic								
Amyloidosis		6	4	7	4	9	6	5
Septicemia		4	1	2	4	4	2	2
Gavage Accident		—	2	—	—	—	1	—
Undetermined		—	1	3	2	3	2	8
Total pre-terminal deaths		33	39	44	33	41	46	49

a control article (0.5% methylcellulose)

b vehicle articles (43:15:42 ethanol:water:propylene glycol and 0.5% methylcellulose)

c ritonavir

d GS-9137

e GS-9137 + ritonavir

*: Including animals found dead or euthanized between the end of the treatment period and the scheduled necropsy.

Table 31. Most Common Causes of Death/Euthanasia of Pre-terminal Decedent Female* Mice

Tissue/Finding	Sex	Female						
	Dose (mg/kg/day)	0[a]	0[b]	25[c]	200[d]	600[d]	2000[d]	2000/25[e]
Neoplastic								
Lung								
Carcinoma: alveolar/bronchiolar		1	—	1	1	3	1	2
Ovary								
Hemangiosarcoma		1	2	—	—	1	3	—
Hemolymphatic tissue								
Lymphoma		8	5	7	12	8	8	8
Histiocytic Sarcoma		7	1	5	4	4	—	—
Non-neoplastic								
Kidney								
Chronic Progressive Nephropathy		5	4	8	5	6	4	4
Uterus								
Thrombosis		1	1	2	—	—	3	1
Ovary								
Inflammation		3	1	—	1	1	1	3
Cyst		2	1	1	3	3	2	—
Trachea								
Chronic inflammation		—	7	5	—	—	—	3
Digestive system								
Dilatation gastrointestinal (GI) tract		—	4	3	—	—	3	9
Systemic								
Amyloidosis		—	3	1	—	—	—	—
Septicemia		2	6	1	2	3	1	3
Gavage Accident		-	4	2	1	1	-	1
Undetermined		3	2	2	-	3	5	8
Total pre-terminal deaths		40	51	49	39	43	44	49

a control article (0.5% methylcellulose)

b vehicle articles (43:15:42 ethanol:water:propylene glycol and 0.5% methylcellulose)

c: ritonavir

d: GS-9137

e: GS-9137+ ritonavir

*: Including animals found dead or euthanized between the end of the treatment period and the scheduled necropsy.

The most probable causes of death that can be associated with the administration of the vehicles and/or test articles were dilatation of various segments of the gastrointestinal tract (stomach, duodenum, jejunum, colon and/or cecum) and chronic inflammation of the trachea. Dilatation of the GI tract was observed macroscopically in animals (both genders) given both vehicles, ritonavir alone, 2000 mg/kg/day GS-9137 alone, and 2000/25 mg/kg/day GS-9137/ritonavir, with an increased incidence in mice administered GS-9137 with ritonavir. The dilatation was likely related to vehicle for ritonavir (ethanol:water:propylene glycol) and GS-9137 at 2000 mg/kg/day. Chronic inflammation (minimal to marked squamous metaplasia and/or moderate degeneration and/or necrosis) of the trachea, likely related to the ritonavir vehicle occurred with a comparable incidence in both genders given both vehicles, ritonavir alone, and GS-9137 in combination with ritonavir. In the absence of any other significant lesion, the pathology findings in the gastrointestinal tract and trachea were consistent with the act of gasping for breath secondary to the reflux of gastric fluid with gavage material into and obstructing the nasal cavities.

An increased incidence of deaths of undetermined cause was observed in the GS-9137/ritonavir group. It is possible that some of these undetermined deaths are related to the gastric reflux with nasal obstruction in the absence of an associated pathology.

Percentage of survival in the high dose group (GS-9137 2000mg) at the end of Weeks 52 and 79

	Percentage of survival	
	End of 52 wk	End of 79 wk
Male	77.1%	55.7%
Female	91.3%	82.9%

Clinical Signs: there were no GS-9137 related clinical signs noted during the study. Suspected dehydration together with an associated decreased fecal output was noted in a higher incidence in animals receiving 2000/25 mg/kg/day GS-9137/ritonavir when compared to all control groups.

Masses: the time to mass appearance and incidence of clinically observed masses was similar in all groups and there was no evidence of any treatment related effect. The incidence of clinically in observed masses is presented in Table 32. Please note that there were 2 masses only in the 200 mg GS-9137 dose group (32 is a typing mistake).

Table 32. Incidence of Clinically Observed Masses at Necropsy

Group Number Identification	Dose Level (mg/kg/day)	Masses			
		Males	%	Females	%
1/ Control Article[a]	0	5/70	7	4/70	6
2/ Vehicle Articles[b]	0	4/70	6	3/70	4
3/ ritonavir	25	3/70	4	4/70	6
4/ GS-9137	200	4/70	6	32/70	3
5/ GS-9137	600	6/70	9	2/70	3
6/ GS-9137	2000	1/70	1	3/70	4
7/ GS-9137/ ritonavir	2000/25	2/70	3	7/70	10

a control article (0.5% methylcellulose)

b vehicle articles (43:15:42 ethanol:water: propylene glycol and 0.5% methylcellulose)

Body Weights: reduced group mean body weights were noted in female mice receiving 2000/25 mg/kg/day GS-9137/ritonavir when compared to all control groups. Occasional differences were also noted in males at the same dose level. Any changes in the body weight data, including those attaining statistical significance were considered unrelated to the administration of GS-9137.

Figure 7. Group Mean Body Weights – Males

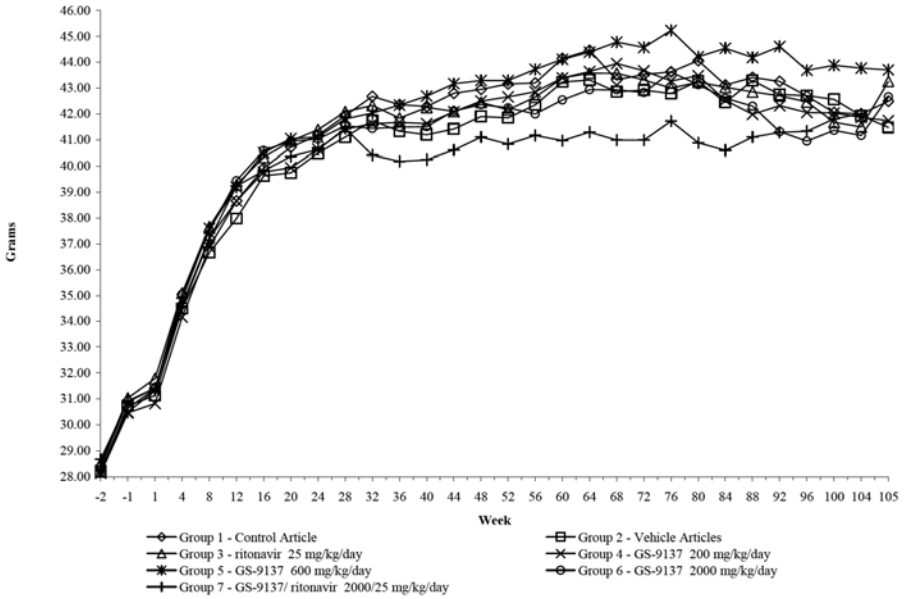
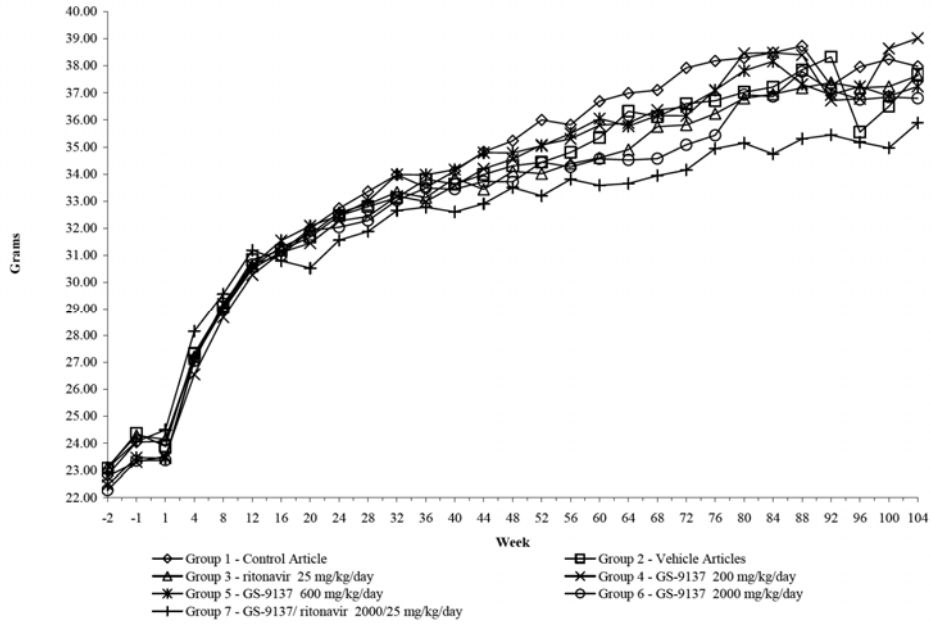


Figure 8. Group Mean Body Weights – Females



Percent (%) Difference in Mean body weights relative to the vehicle control in GS-9137 treated animals at end of the experiment

Male			Female		
200 mg	600 mg	2000 mg	200 mg	600 mg	2000 mg
-0.3	+4.2	-2.0	+2.7	-2.0	-3.0

Feed Consumption: there were no GS-9137-related effects on food consumption. Occasional differences between GS-9137/ritonavir and the ritonavir group or control noted in both sexes were minimal and biologically not significant.

Gross Pathology: macroscopic observations likely related to ritonavir vehicle (ethanol: water: propylene glycol) and GS-9137 at 2000 mg/kg/day occurred in pre-terminal decedent mice and consisted of dilatation and pale material in the various segments of the gastrointestinal tract. The incidence of these macroscopic findings was summarized in Table 33.

Table 33. Macroscopic Findings related to Administered Compounds in Pre-terminal Decedent* Mice

Tissue/Finding	Sex	Male/Female						
	Dose (mg/kg/day)	0[a]	0[b]	25[c]	200[d]	600[d]	2000[d]	2000/25[e]
	Number of animals examined	33/39	39/51	44/49	33/39	41/43	46/44	49/49
Non-neoplastic								
Stomach								
Dilatation		1/1	4/6	3/4	-/-	-/-	5/1	10/10
Material pale		-/-	-/-	1/-	1/-	2/1	4/1	7/4
Duodenum								
Dilatation		-/2	5/7	2/5	2/-	-/1	4/1	7/6
Material pale		-/1	-/-	-/-	-/-	1/1	1/-	5/3
Jejunum								
Dilatation		-/1	6/9	2/7	1/-	1/2	5/3	11/10
Material pale		-/-	-/-	-/-	-/-	1/-	5/-	4/4
Ileum								
Dilatation		-/1	6/7	2/6	-/-	-/-	3/2	9/10
Material pale		-/-	-/-	-/-	-/-	-/-	-/1	2/-
Colon								
Dilatation		-/-	3/3	-/-	-/-	-/1	1/-	1/5
Material pale		-/-	-/-	-/-	-/-	1/-	2/-	1/-
Cecum								
Dilatation		-/1	6/5	-/3	-/-	-/-	3/1	7/7
Material pale		-/-	-/-	-/-	-/-	-/-	3/-	3/-

a control article (0.5% methylcellulose)

b vehicle articles (43:15:42 ethanol:water:propylene glycol and 0.5% methylcellulose)

c: ritonavir

d: GS-9137

e: GS-9137 + ritonavir

*: Including animals found dead or euthanized between the end of the treatment period and the scheduled necropsy.

Dilatation of various segments of the gastrointestinal tract occurred in mice from all groups with an increased incidence in male and female pre-terminal mice given ritonavir vehicle, ritonavir, 2000 mg/kg/day GS-9137, and particularly, 2000/25 GS-9137/ritonavir. Based on the increased incidence and/or number of affected segments when compared to mice given GS-9137 vehicle (0.5% methylcellulose) and the low and mid doses of GS-9137, the dilatation was considered an

effect of the ritonavir vehicle and GS-9137 at 2000 mg/kg/day. Gastrointestinal tract dilatation, in the absence of any other significant lesions, was consistent with the act of gasping for breath secondary to the reflux of gastric fluid with or without gavage material into and obstructing the nasal cavities. Similar macroscopic changes in the gastrointestinal tract did not occur in terminal euthanized mice. Dilatation of the gastrointestinal tract had no microscopic correlates.

Pale material adhering to the mucosa of various segments of the gastrointestinal tract occurred in at least one segment in most of the treatment groups. It was likely related to GS-9137 at 2000 mg/kg/day and 2000/25 mg/kg/day GS-9137/ritonavir based on the increased incidence compared to control groups and other groups of mice given GS-9137 or ritonavir. Pale material in the gastrointestinal tract had no microscopic correlates and did not occur in terminal euthanized mice.

For all remaining macroscopic changes including masses in all examined organs and tissues, the spectrum and distribution were usually not different between the pre-terminal and terminal euthanized animals. They were not considered related to ritonavir vehicle, GS-9137 and/or ritonavir because they were consistent with spontaneously occurring findings described in the literature for mice of this strain and age range. These findings were randomly distributed among the groups, were of low incidence, and/or their appearance was similar to findings found in controls from this and previous studies.

Histopathology:

Neoplastic Changes: the study period for the males and the females was defined as lasting 104 weeks and 103 weeks respectively. To verify sponsor's analyses and to perform the additional analysis suggested by the reviewing pharmacologist, this reviewer independently performed survival and tumor data analyses. Since there are seven groups with two testing drugs: GS-9137 with or without Ritonavir, the reviewing pharmacologist suggested to do three sets of analyses:

Groups 1, 4, 5, 6 (GS-9137 alone: 0, low, mid and high). The classical carcinogenicity portion of the study of GS-9137 in mouse.
Groups 2 vs 3 (vehicle for ritonavir vs 25 mg/kg/day ritonavir alone)
Group 2 vs 7 (vehicle for ritonavir + GS-9137 vs GS-9137 high dose with 25 mg/kg/day ritonavir)
Groups 6 vs 7 (GS-9137 high dose vs GS-9137 high dose with 25 mg/kg/day ritonavir)

Analysis of the combinations of all organ/tumors as the following:

- Hemangioma, Hemangiosarcoma and combined hemangioma and hemangiosarcoma from all sites;
- Adenoma and carcinoma from liver; adenoma and carcinoma from lung; For female mice:
- leiomyoma and leioma from uterus.

Following tumor types showed p-values less than or equal to 0.05 either tests for dose response relationship or pair-wise comparisons between control and each of individual treated groups.

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pair-wise Comparisons

Incidence of tumors in the GS-9137 treated male and female mouse

Organ	Tumor type	Control	Low 200 mg	Mid 600 mg	High 2000 mg	p-value dose resp	p-value C vs H
Male Testes	Adenoma: interstitial cell	0	0	1	2	0.042	0.193

Comparison of the two high doses: GS-9137 High dose alone vs GS-9137 High dose with 25 mg Ritonavir

Sex	Organ	Tumor type	GS-9137 2000 mg	GS-9137 2000 mg + 25 mg ritonavir	P value
Male	Liver	Hepatocellular: Carcinoma	0	4	0.047
		Hepatocellular: adenoma	9	14	0.093
		Hepatocellular adenoma + carcinoma	9	18	0.015

Based on the criteria of adjustment for multiple testing of trends by Lin and Rahman, the incidence of none of the above or any other tested tumor types in either sex was considered to have a statistically significant positive dose response relationship. Also based on the criteria of Haseman, none of the pair-wise comparisons of treated groups with the control was considered to be statistically significant in either sex for increased tumor incidence in the treated group.

Tests showed no statistically significant positive dose response relationship and the statistically significant difference in pair-wise comparisons in tumor incidence when compared to the control group in both females and males for all four sets of analysis (groups 1, 4, 5, 6; groups 2, 3; groups 6, 7 and groups 2, 7).

Peer Review: a peer review was performed.

Non Neoplastic: microscopic changes likely related to the ritonavir vehicle (ethanol: water: propylene glycol) was observed in the trachea of pre-terminal decedent mice and were summarized in Table 34.

Table 34. Non-Neoplastic Microscopic Findings Related to Vehicle Article (ethanol:water:propylene glycol) in Pre-terminal Decedent* Mice

Tissue/Finding	Sex	Male/Female						
	Dose (mg/kg/day)	0[a]	0[b]	25[c]	200[d]	600[d]	2000[d]	2000/25[e]
Trachea	Number examined	33/39	39/51	44/49	33/39	41/43	46/44	49/49
Inflammation: chronic								
	Total Number affected	-/-	5/10	5/5	-/-	-/-	-/-	3/3
	Minimal	-/-	1/2	1/1	-/-	-/-	-/-	1/1
	Slight	-/-	3/4	1/3	-/-	-/-	-/-	-/-
	Moderate	-/-	1/4	3/-	-/-	-/-	-/-	2/2
	Marked	-/-	-/-	-/1	-/-	-/-	-/-	-/-
Metaplasia: squamous								
	Total Number affected	-/-	3/6	4/3	-/-	-/-	-/-	2/3
	Minimal	-/-	-/-	-/-	-/-	-/-	-/-	-/1
	Slight	-/-	1/3	3/-	-/-	-/-	-/-	2/-
	Moderate	-/-	2/2	1/3	-/-	-/-	-/-	-/2
	Marked	-/-	-/1	-/-	-/-	-/-	-/-	-/-
Degeneration and/or necrosis								
	Total Number affected	-/-	-/-	1/2	-/-	-/-	-/-	-/-
	Moderate	-/-	-/-	1/2	-/-	-/-	-/-	-/-

a control article (0.5% methylcellulose)

b vehicle articles (43:15:42 ethanol:water:propylene glycol and 0.5% methylcellulose)

c: ritonavir

d: GS-9137

e: GS-9137 + ritonavir

*: Including animals found dead or euthanized between the end of the treatment period and the scheduled necropsy.

Minimal to marked chronic inflammation, squamous metaplasia and/or moderate degeneration and/or necrosis were found in the trachea of mice administered the ritonavir vehicle, ritonavir, and GS-9137 with ritonavir. These tracheal lesions, in the absence of any other significant lesions, were consistent with tracheal aspiration secondary to the reflux of gastric fluid with or without gavage material into and obstructing the nasal cavities. They were considered probable effects of the ritonavir vehicle (ethanol: water: propylene glycol) based on the absence of changes in mice given GS-9137 vehicle (0.5% methylcellulose) and GS-9137, and the comparable incidence among groups of affected mice. Similar microscopic changes in the trachea were not observed in terminal euthanized animals.

For all remaining non-neoplastic microscopic changes, the spectrum and distribution of microscopic findings were usually not different between the pre-terminal and terminal euthanized animals. They were not considered related to the vehicle articles, GS-9137 and/or ritonavir because they were consistent with spontaneously occurring findings described in the literature. These non-neoplastic changes were distributed randomly among groups, and their appearance was similar to findings found in controls from this study and control CD1 mice as published in literature. They were considered to be spontaneous or agonal in origin, therefore, they were not considered to be of any toxicological importance. Differences in the incidence of microscopic changes were considered to be due to natural variability and attributed to chance variation.

Toxicokinetics: was evaluated up to Week 26 in male and female CD1 mice when administered alone or in combination with ritonavir (25 mg/kg/day) by daily oral gavage administration for

104 consecutive weeks at GS-9137 dose levels of 200, 600 and 2000 mg/kg/day. When administered alone, the absorption of GS-9137 was rapid, while, co-administration of ritonavir generally slightly delayed the time to maximum plasma concentrations. The estimated terminal elimination half-lives of GS-9137 and ritonavir were short, ranging between 1.15 and 3.92 hours and 0.711 and 1.19 hours, respectively. The concurrent administration of ritonavir and GS-9137 resulted in a 3.85- to 6.96-fold increase in the total systemic exposure ($AUC_{0\text{-}tlast}$ of GS-9137. However, there was no apparent influence of ritonavir co-administration on the shape of plasma profiles or on the terminal elimination half-lives. The systemic exposure of GS-9137 was approximately proportional to the dose in the dose range of 200 to 2000 mg/kg/day in both genders. Repeated daily administration of GS-9137 alone, or in combination with ritonavir for 26 weeks, resulted in moderate increases in the systemic exposure in both male and female mice. There was slightly higher systemic exposure of GS-9137 in the female mice relative to the male mice in Week 26, when administered alone. However, co-administration of GS-9137 with ritonavir was observed to generally result in slightly lower exposure of GS-9137 in the females compared to the males.

Table 35. Toxicokinetic Parameters of GS-9137 in Albino Mouse Plasma Following Oral Gavage Administration of Ritonavir or GS-9137 Alone or GS-9137 in Combination with Ritonavir

Week 1 (Males)

Group No.	Dose Level (mg/kg/day)	T_{max} (h)	T_{last} (h)	T½ (h)	C_{max} (ng/mL)	$AUC_{(0\text{-}tlast)}$ (ng•h/mL)
6 (GS-9137)	2000	0.50	8.00	2.32	13033	31297
7 (GS-9137/Ritonavir)	2000/25	2.00	8.00	1.32	54300	217885

Week 1 (Females)

Group No.	Dose Level (mg/kg/day)	T_{max} (h)	T_{last} (h)	T½ (h)	C_{max} (ng/mL)	$AUC_{(0\text{-}tlast)}$ (ng•h/mL)
6 (GS-9137)	2000	0.50	8.00	1.51	16867	43145
7 (GS-9137/Ritonavir)	2000/25	2.00	8.00	1.98	41900	166672

Week 26 (Males)

Group No.	Dose Level (mg/kg/day)	T_{max} (h)	T_{last} (h)	T½ (h)	C_{max} (ng/mL)	$AUC_{(0\text{-}tlast)}$ (ng•h/mL)
3 (Ritonavir)	25	a	a	a	a	a
4 (GS-9137)	200	0.50	8.00	3.40	4117	7508
5 (GS-9137)	600	0.50	8.00	3.92	12567	21430
6 (GS-9137)	2000	0.50	8.00	1.86	27467	54207
7 (GS-9137/Ritonavir)	2000/25	2.00	8.00	1.39	91633	318165

Week 26 (Females)

Group No.	Dose Level (mg/kg/day)	T_{max} (h)	T_{last} (h)	T½ (h)	C_{max} (ng/mL)	$AUC_{(0\text{-}tlast)}$ (ng•h/mL)
3 (Ritonavir)	25	a	a	a	a	a
4 (GS-9137)	200	0.50	8.00	1.32	7630	13437
5 (GS-9137)	600	0.50	8.00	1.15	24767	36310
6 (GS-9137)	2000	0.50	8.00	1.54	43033	86563
7 (GS-9137/Ritonavir)	2000/25	0.50	24.0	2.57	69967	333377

a TK parameters not estimated due to samples being < LLOQ.

Table 36. Ratios of GS-9137 Exposure in Albino Mouse Plasma Following Oral

Gender	Occasion	C_{max} (ng/mL) Group 6 (GS-9137) 2000 mg/kg/day	C_{max} (ng/mL) Group 7 (GS-9137/Ritonavir) 2000/25 mg/kg/day	Ratio
Males	Week 1	13033	54300	4.17
	Week 26	27467	91633	3.34
Females	Week 1	16867	41900	2.48
	Week 26	43033	69967	1.63

Gender	Occasion	$AUC_{(0\text{-}tlast)}$ (ng•h/mL) Group 6 (GS-9137) 2000 mg/kg/day	$AUC_{(0\text{-}tlast)}$ (ng•h/mL) Group 7 (GS-9137/Ritonavir) 2000/25 mg/kg/day	Ratio
Males	Week 1	31297	217885	6.96
	Week 26	54207	318165	5.87
Females	Week 1	43145	166672	3.86
	Week 26	86563	333377	3.85

Dosing Solution Analysis: GS-9137: all study samples analyzed were within the acceptance criteria of ± 15% (individual values within ± 20%)

Ritonavir: all study samples analyzed were within the acceptance criteria of ± 10% (individual values within ± 15%) of their nominal concentrations.

A 104-week Oral Gavage Carcinogenicity Study of GS-9137 in the Albino Rat

Study no.: TX-183-2012
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: December 27, 2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: 071090, 081147, and 081244; 100%
CAC concurrence: Yes

Key Study Findings: GS-9137 given to Sprague Dawley rats by oral gavage for 88 (males) or 90 (females) weeks was not considered carcinogenic at 100, 300 or 2000 mg/kg/day. The administration of GS-9137 did not result in any changes in the toxicology parameters tested. There were no macroscopic or microscopic changes related to the administration of GS-9137. At 2000 mg/kg/day (high dose) at end of the experiment, the mean C_{max} and $AUC_{(0\text{-}t)}$ were 34367

ng/mL and 72167 ng/mL, and 284121 ng·h/mL and 632562 ng·h/mL, in males and females, respectively.

Adequacy of Carcinogenicity Study: the dose levels of 0, 100, 300 and 2000 mg/kg/day of GS-9137 were approved by the Exec CAC. The high dose of 2000 mg/kg/day was proposed, based on the maximal feasible dose.

Percentage survival in the high dose group was 85% (male) and 98.3% (female), and 60% (male) and 66.7 % (female) at the end of Weeks 52 and 79, respectively.

The carcinogenicity study in rats was considered to be acceptable.

Appropriateness of Test Models: rats historically have been used in safety evaluation studies and are recommended by appropriate regulatory agencies. In general GS-9137 was metabolized via a combination of oxidation and glucuronidation in all species tested including rat and humans. In the in vivo studies, GS-9137 was the predominant circulating chemical species. Overall, the metabolic species produced appear to be comparable across species.

Evaluation of Tumor Findings: the oncogenicity potential of GS-9137 was investigated in male and female Crl:CD(SD) rats with oral gavage dosages of 0, 100, 300 and 2000 mg/kg/day of GS-9137. The protocol was approved by the Exec CAC. No significant increase in neoplasms was noted in male or female rats related to GS-9137 in this study. The systemic exposures were 12.4 and 26.8 times that in humans (150 mg once daily tablet AUCss = 23,000 ng*hr/ml) in male and female rats at the high dose level, respectively.

Methods

Doses:	0 (vehicle control), 100, 300 and 2000 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% methylcellulose, aqueous in deionized Water
Basis of dose selection:	3-month dose range study of GS-9137 in rats
Species/Strain:	Male and female Crl:CD(SD)
Number/Sex/Group:	60/sex/group
Age:	6 weeks
Animal housing:	Animals were housed individually in stainless steel wire mesh bottomed cages equipped with an Automatic watering valve and/or water bottle.
Paradigm for dietary restriction:	All animals had free access to a standard certified pelleted commercial laboratory diet.
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	9/sex/group toxicokinetics
Deviation from study protocol:	No

Observations and Results

Mortality: because the number of surviving animals in the control groups reached 20 animals earlier than expected, males were dosed for a minimum of 88 weeks and females were dosed for a minimum of 90 weeks.

Between 31 and 40 main study animals per group died or were euthanized prior to the end of the study between Day 50 and Day 625 and were examined at necropsy and histopathologically. The incidences of the lesions observed are discussed in macroscopic and microscopic sections of the report. For each decedent animal, the major factor considered to be contributory to its death was determined. The cause of death or other contributory factors to death could not be determined for a small number of animals.

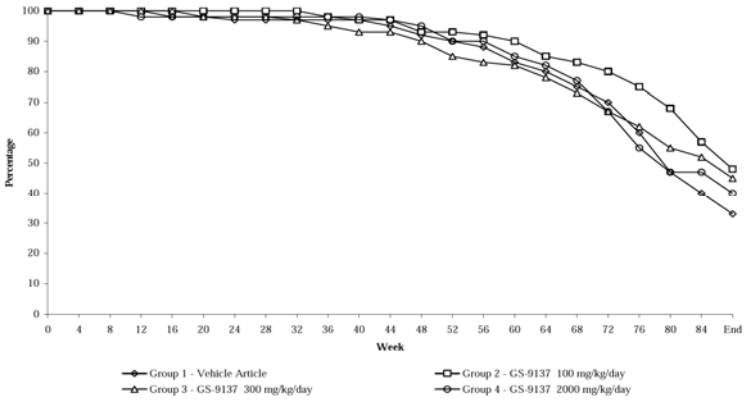
Mortality Rates (%) at the End of the Experiment

	Control	**100 mg**	**300 mg**	**2000 mg**
Male	61.7%	51.7%	58.3%	55.0%
Female	65.0%	60.0%	55.0%	51.7%

Percentage of survival in the high dose group at the end of Weeks 52 and 79

Percentage of survival	End of 52 week	End of 79 week
Male	85.0%	60.0%
Female	98.3%	66.7%

Figure 9. Survival Curves (%) – Males



For each decedent animal, the major factor considered to be contributory to its death was Determined. The cause of death or other contributory factors to death could not be determined for a small number of animals.

Figure 10. Survival Curves (%) – Females

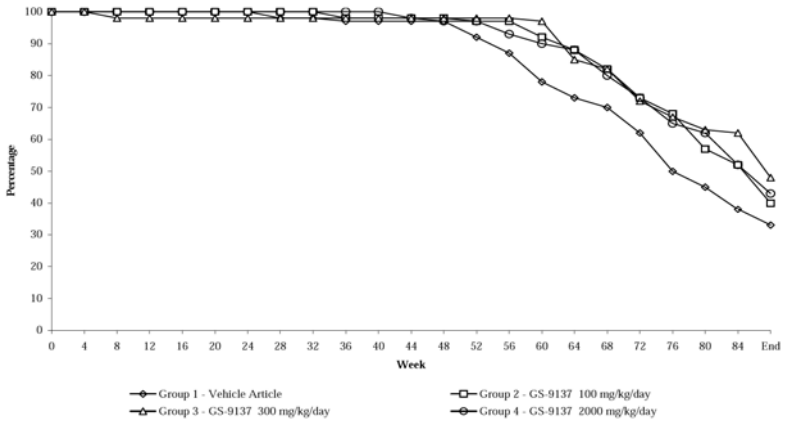


Table 37. Causes and Incidence of Preterminal Deaths

Sex	Male				Female			
Dose (mg/kg/day)	0	100	300	2000	0	100	300	2000
Tissue/Finding								
Neoplastic								
Pituitary	16	14	11	11	31	31	26	31
Brain/cranial cavity	—	1	—	1	—	1	—	—
Head	1	—	—	—	—	—	—	—
Mammary Gland	—	—	—	2	1	2	1	1
Ovary/Uterine	—	—	—	—	3	—	—	—
Prostate	1	—	—	—	—	—	—	—
Oral cavity	—	2	—	—	—	—	—	—
Liver	2	1	—	—	—	—	—	—
Heart	1	—	—	—	—	—	—	1
Skin/Subcutaneous tissue	—	4	—	3	—	—	—	—
Hemolymphatic tissue	1	—	1	1	1	—	1	—
Non-neoplastic								
Lung/Thorax	1	—	—	2	1	—	—	—
Kidney/Urinary tract	8	1	7	6	—	1	1	1
Heart	—	2	2	1	—	—	—	—
Rectum	1	—	—	—	—	—	—	—
Liver	—	—	1	1	—	—	—	—
Skin	—	—	1	—	—	—	—	—
Others	—	—	—	—	—	—	—	—
Undetermined	7	6	10	7	3	1	2	—
Total preterminal deaths	40	31	33	35	40	36	31	34

Clinical Signs: there were no GS-9137-related effects on the incidence of clinically observed masses as they were noted in varying proportions of animals from vehicle control and drug-treated groups, in both males and females. These data are summarized in the Table 37.

Table 38. Incidence of Clinically Observed Masses

Group Number Identification	Dose Level (mg/kg/day)	Masses			
		Males	%	Females	%
1/ Vehicle Control	0	21/60	35	38/60	63
2/ GS-9137	100	29/60	48	40/60	67
3/ GS-9137	300	20/60	33	31/60	52
4/ GS-9137	2000	26/60	43	43/60	72

The incidence of palpable masses is higher in females compared to males. This is mainly due to the presence of a high incidence of masses in the urogenital area in several females, including those assigned to the vehicle control group.

Clinical observations seen during this study were not considered GS-9137-related based upon their low and/or sporadic incidence and/or similarity to observations in vehicle controls.

Body Weights: there were no GS-9137-related effects on the body weights or body weight gains.

Figure 11. Group Mean Body Weights – Males

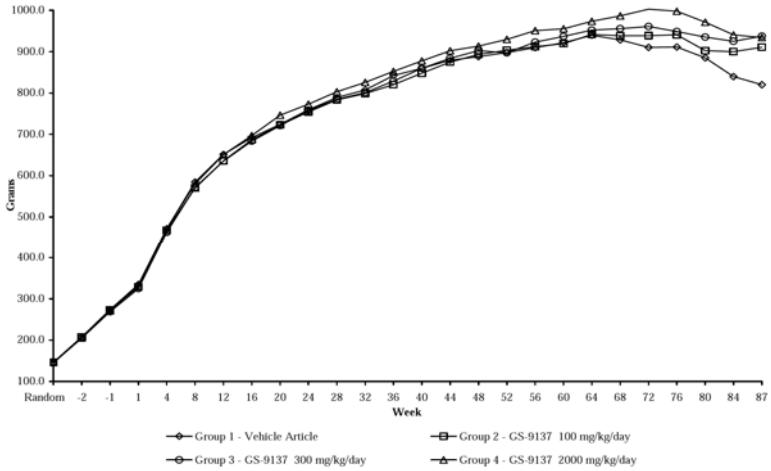
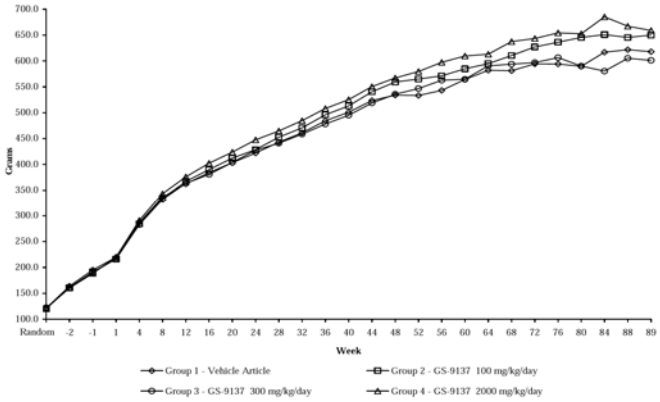


Figure 12. Group Mean Body Weights – Females



Feed Consumption: **t**here were no GS-9137-related effects on food consumption.

Gross Pathology: there were no GS-9137-related macroscopic findings. The various gross changes noted, including the masses reported at necropsy, were typical of those commonly encountered at necropsy in rats of this strain and age range. It was considered that the changes seen were spontaneous in origin and that their distribution among groups was not treatment related.

Histopathology: neoplastic Changes: tumor data analysis of the combinations of all organ/tumors as follow:

- Hemangiosarcoma from all sites;
- C-cell adenoma and carcinoma from Thyroid gland;
- Follicular adenoma and carcinoma from Thyroid gland

Please see Dr. Min's review for the analysis. In her review, the tumor rates and the p-values of the tested tumor types are listed in Tables 3A and 3B in the appendix for males and females, respectively. The criteria recommend the use of a significance level α=0.025 for rare tumors and α=0.005 for common tumors for a submission with two species.
The statistical report showed that in male rats the following tumor types showed p-values less than or equal to 0.05 either tests for dose response relationship and/or pair-wise comparisons between control and high dose group.

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pair-wise Comparisons

Organ	Tumor	Control N=60	Low N=60	Mid N=60	High N=60	P value Dose response	P value C vs H
Thyroid	Adenoma C-cell	1	4	1	6	0.033	0.058
	Carcinoma C-cell	-	-	1	-	0.489	-
	Adenoma C-cell + Carcinoma C-cell	1	4	2	6	0.040	0.058

Based on the criteria of adjustment for multiple testing of trend, the incidence of none of the above or any other tested tumor types in either sex was considered to have a statistically significant positive dose response relationship.

Peer Review: a peer review was performed.

Non Neoplastic: **t**here were no GS-9137-related non-neoplastic microscopic findings. The various findings noted were generally randomly distributed in control and treated groups, did not show a dose related pattern and were those commonly encountered in rats of this age and strain.

Toxicokinetics: Profile Characterization: GS-9137 exhibited rapid absorption following oral gavage administration with maximum plasma concentrations generally occurring at 0.5 hour post dose. The mean plasma concentrations declined slowly post C_{max} in an apparently mono-exponential manner. There was no influence of the dose level or repeated administration on the shape of mean plasma profiles.

Table 39. Toxicokinetic Parameters of GS-9137 in Albino Rat Plasma Following Oral Gavage Administration of GS-9137

Day 4 (Males)				
Group No.	Dose Level (mg/kg/day)	Tmax (h)	C_{max} (ng/mL)	$AUC_{(0\text{-tlast})}$ (ng•h/mL)
2	100	0.50	13667	64906
3	300	0.50	14867	45579
4	2000	2.00	26967	195051

Day 4 (Females)				
Group No.	Dose Level (mg/kg/day)	Tmax (h)	C_{max} (ng/mL)	$AUC_{(0\text{-tlast})}$ (ng•h/mL)
2	100	0.50	19133	42031
3	300	0.50	31600	89338
4	2000	0.50	44067	257506

Day 178 (Males)				
Group No.	Dose Level (mg/kg/day)	Tmax (h)	C_{max} (ng/mL)	$AUC_{(0\text{-tlast})}$ (ng•h/mL)
2	100	0.50	14167	37345
3	300	0.50	19067	62754
4	2000	2.00	36133	285152

Day 178 (Females)				
Group No.	Dose Level (mg/kg/day)	Tmax (h)	C_{max} (ng/mL)	$AUC_{(0\text{-tlast})}$ (ng•h/mL)
2	100	0.50	36667	121302
3	300	0.50	51767	262420
4	2000	0.50	71767	617381

The systemic exposure of GS-9137 was less than proportional to the dose in the dose range of 100 to 2000 mg/kg/day in both genders. The female rats generally exhibited higher systemic exposure compared to the male rats on all sampled days.

Dosing Solution Analysis: the CV for the calibration standards was ≤ 3% and the difference between the average response for the standards injected at the end compared with those injected at the beginning was within ±10%. Acceptance criteria with respect to system suitability were met.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Oral fertility and early embryonic development study of JTK-303 in female rats

Key study findings: JTK-303 was administered orally to groups of female rats (18 animals/group) for 14 days prior to mating and throughout the mating period to gestation day (GD) 7 at dose level of 0 (0.5% methylcellulose using purified water, vehicle control), 300 (low),

1000 (mid) or 2000 mg/kg/day (high). Following mating with untreated males, females were euthanized for necropsy along with uterine examination on GD 13 and the effects of the test article on the female fertility (in terms of copulation and fertility indices) and early embryonic development were evaluated. No deaths occurred in any dose group. No treatment related signs were seen in the in-life phase. No treatment related effects were seen in body weights or food consumption. No drug related changes were seen in the estrous cycle of the animals or in the copulation and fertility indices in any treated group. No treatment related effects were seen in the number of corpora lutea, the number of implantations, the implantation index, the number of live fetuses, the fetal viability, the number of dead embryos and fetuses or the post implantation deaths in any treated group.

Under the condition of this study, JTK-303 at doses up to 2000 mg/kg did not cause any toxicity in the female animals or on early embryonic development and thus concluded that the NOAEL was 2000 mg/kg/day for female rats. In the one month rat toxicology study, systemic exposure to JTK-303 at 2000 mg/kg/day dose level ($AUC_{0\text{-}24hr}$) was 695 µg*hr/ml in female rats on day 27.

Study no.: 04909-TX-019

Volume # and page #: 1 and 1-148

Conducting laboratory and location: (b) (4)

Date of study completion: January 7, 2005

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: Lot # R, 98.1%

Methods

Doses: groups of male and female Sprague-Dawley rats [Crj: CD (SD) IGS; 18 rats/sex/group] received JTK-303 via oral gavage at dose levels of 0 (0.5% methylcellulose using purified water, vehicle control), 300 (low), 1000 (mid) or 2000 mg/kg/day (high).

Species/strain: male and female rats/Crj: CD (SD) IGS

Number/sex/group: 18 rats/group

Route, formulation, volume, and infusion rate: oral gavage, 10 ml/kg

Satellite groups used for toxicokinetics: none

Study design: JTK-303 was administered orally to groups of female rats (18 animals/group) for 14 days prior to mating and throughout the mating period to gestation day (GD) 7 at dose level of 0 (0.5% methylcellulose using purified water, vehicle control), 300 (low), 1000 (mid) or 2000 mg/kg/day (high). Following mating with untreated males, females were euthanized for necropsy along with uterine examination on GD 13 and the effects of the test article on the female fertility (in terms of copulation and fertility indices) and early embryonic development were evaluated.

Observations and Results

Mortality: there were no deaths.

Clinical signs: whitish stools (mid or high), probably unabsorbed test article were noted. No other clinical signs were seen in the in-life phase.

Body weight: no treatment related changes

Food consumption: no change

Toxicokinetics: not done

Necropsy and Organ weights: no drug related changes.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): no treatment effects were seen.

Oral (Gavage) Fertility and General Reproduction Toxicity Study of GS-9137 in Male Rats

Key study findings: JTK-303 was administered orally via gavage to groups of male rats (25 animals/group) beginning 28 days prior to cohabitation with females (maximum 21 days; 24-26 female/group) and continuing through the day before sacrifice (total of 49 to 52 daily dosages) at dose level of 0 (0.5% methylcellulose using purified water, vehicle control), 300 (low), 1000 (mid) or 2000 mg/kg/day (high). All surviving rats were sacrificed on study days 50 to 53 and a gross necropsy was performed. Reproductive organs were weighed and retained for possible histopathological evaluation and sperm evaluations were conducted. Female rats with evidence of normal estrous cycling were mated to the treated male rats. The incidence of slight excess salivation was increased at the high dose only. No other clinical signs were seen in life phase. No treatment related effects were seen in body weights or food consumption. The weights of the epididymides, caudal epididymis, testes, seminal vesicles (with and without fluid) and prostate and the ratios of these organs weight to terminal body weight were unaffected by the treatment. All sperm parameters were normal.

All female rats survived to Caesarean sectioning on DG 13. Clinical and necropsy observations and body weights and feed consumption values were unremarkable. All Caesarean sectioning and litter parameters were unaffected by the treatment.

Under the condition of this study, JTK-303 at doses up to 2000 mg/kg did not cause any toxicity in the male animals and thus concluded that the NOAEL was 2000 mg/kg/day for male rats. In the one month rat toxicology study, systemic exposure to JTK-303 at 2000 mg/kg/day dose level ($AUC_{0\text{-}24hr}$) was 379 µg*hr/ml in male rats on day 27.
Study no.: TCA00075/TX-183-2003

Volume # and page #: 1 and 1-214

Conducting laboratory and location: (b) (4)

Date of study completion: September 27, 2006

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: Lot # S, 98.1%

Methods

Doses: JTK-303 was administered orally via gavage to groups of male rats (25 animals/group) beginning 28 days prior to cohabitation with females (maximum 21 days; 24-26 female/group) and continuing through the day before sacrifice (total of 49 to 52 daily dosages) at dose level of 0 (0.5% methylcellulose using purified water, vehicle control), 300 (low), 1000 (mid) or 2000 mg/kg/day (high).

Species/strain: male and female rats/Crj: CD (SD) IGS

Number/sex/group: 25 males; 24-26 female rats/group

Route, formulation, volume, and infusion rate: oral gavage, 10 ml/kg

Satellite groups used for toxicokinetics: none

Study design: All surviving rats were sacrificed on study days 50 to 53 and a gross necropsy was performed. Reproductive organs were weighed and retained for possible histopathological evaluation and sperm evaluations were conducted. Female rats with evidence of normal estrous cycling were mated to the treated male rats. All female rats were sacrificed on gestation day 13, Caesarean sectioned and a gross necropsy was performed. The number and distribution of corpora lutea were recorded. The uterus of each rat was excised and examined for pregnancy, number and distribution of implantation sites and viable and nonviable embryos. Placentae were examined for size, color and shape.

Observations and Results

Mortality: there were no deaths.

Clinical signs: the incidence of slight excess salivation was increased at the high dose male and female.

Body weight: no treatment related changes

Food consumption: no change

Mating and Fertility: all mating and fertility parameters [numbers of days in cohabitation, rats that mated, the fertility index (number of pregnancies per number of rats that mated), rats with confirmed mating dates during the first week of cohabitation and number of pregnancies per number of rats in cohabitation] were unaffected by the treatment.

Toxicokinetics: not done

Necropsy Observation: all necropsy observations were considered unrelated to GS-9137

Organ weights: Terminal body weights were comparable among the four dosage groups and did not significantly differ.

Sperm Evaluation: all sperm parameters evaluated were unaffected.

F0 Generation Female Rats: All female rats survived to Caesarean-sectioning on day 13 of gestation. Body weights and feed consumption values were unremarkable. The number of estrous stages per 14 days before cohabitation were comparable among the groups did not differ significantly. All Caesarean-sectioning and litter parameters were unaffected by the treatment. The litter averages for corpora lutea, implantations and viable and nonviable embryos were comparable among the four groups and did not significantly differ. No dam had a litter consisting of only nonviable embryos and all placentae appeared normal.

9.2 Embryonic Fetal Development

Study for Effects of JTK-303 on Embryo-Fetal Development in Rats

Key study findings: groups of pregnant Sprague-Dawley rats 19-20 rats/group received JTK-303 during organogenesis (days 7-17 post insemination) via oral gavage at dose levels of 0 (0.5% methylcellulose, vehicle control), 300 (low), 1000 (mid) or 2000 mg/kg/day (high). Maternal gross necropsy, Cesarean-sectioning and fetal evaluations were made. No deaths occurred in any dose group. No treatment related signs were seen in life phase. No treatment related effects were seen in body weights or food consumption. No drug related effects were seen in the number of corpora lutea, the number of implantations, the implantation index, the number of live fetuses, the fetal viability, the number of dead embryos and fetuses, the post implantation deaths, the fetal sex distribution and the fetal body weights. No treatment related findings were seen in the external, skeletal or visceral examination of the fetuses.

JTK-303 at doses up to 2000 mg/kg did not cause any toxicity in the dams or on embryonic fetal development and thus concluded that the NOAEL was 2000 mg/kg/day for the dams and embryonic/fetal development. Based on the body surface area factor, an equivalent dose in humans would be 324.67 mg/kg/day (19 g/day for a 60 kg person). At the NOAEL (2000 mg/kg/day), exposures (steady state AUC and Cmax) were 534.99 µg*hr/ml and 42.73 µg/ml, respectively.

Study no.: 04910-TX-020
Volume # and page #: 1 and 205-357
Conducting laboratory and location: (b) (4)
Date of study completion: January, 19, 2005
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity: **R, 98.1%**

Methods

Doses: 300 (low), 1000 (mid) or 2000 mg/kg/day (high)

Species/strain: Sprague-Dawley rats/Crj: CD (SD) IGS

Number/sex/group: 19-20 rats/group

Route, formulation, volume, and infusion rate: oral gavage

Satellite groups used for toxicokinetics: in a parallel pharmacokinetics study 5-6 inseminated rats received JTK-303 at the same dose levels from day 6 to 21 post insemination. On day 17, blood samples were collected and were analyzed by a validated analytical method.

Study design: groups of pregnant Sprague-Dawley rats 19-20 rats/group received JTK-303 during organogenesis (days 7-17 post insemination) via oral gavage at dose levels of 0 (0.5% methylcellulose, vehicle control), 300 (low), 1000 (mid) or 2000 mg/kg/day (high).

Parameters and endpoints evaluated: maternal gross necropsy, Cesarean-sectioning and fetal evaluation.

Observations and Results

Mortality (dams): there were no deaths.

Clinical signs (dams): whitish stools (mid or high), probably unabsorbed test article were noted. No other clinical signs were seen in life phase.

Body weight (dams): no effects.

Food consumption (dams): no effects.

Necropsy at Caesarean Section: there were no treatment-related findings in any treated group.

Organ Weights: No treatment-related changes were seen in any treated group.
Toxicokinetics: highest plasma concentrations were generally occurred between 2 and 4 hrs postdosing with mean values shown in Table 40. The mean values for the plasma concentration of the parent drug (Cmax and $AUC_{0\text{-}24hr}$) showed a dose-dependent increase at doses up to 2000 mg/kg. The mean $AUC_{0\text{-}24}$ hr values were similar on GDs 7 and 17, though the mean Cmax values were higher on GD 7 than on GD 17 for all treated groups.

Table 40. Toxicokinetics of JTK-303 on day 17 post insemination after repeated daily oral administration to pregnant rats

Dose (mg/kg/day)	$AUC_{0\text{-}24hr}$ (μg*hr/ml)	Cmax (μg/ml)	Tmax (hr)
300	184.39	20.60	2
1000	393.21	36.11	2.2
2000	534.99	42.73	3.9

Fetuses: Examinations at Caesarean Section: no treatment-related changes were seen in the number of corpora lutea, the number of implantations, the implantation index, the number of live fetuses, the fetal viability, the number of dead embryos and fetuses, post implantation deaths, the fetal sex distribution, the fetal body weights, the placental weights or the placental findings in any treated group.

External Examinations: anomalies were noted in two (0.7%), one (0.3%), one (0.7%) and two fetuses (0.7%) in the control, 300, 1000 and 2000 mg/kg groups, respectively, and the incidences were not significantly different between the control group and each of the treated groups. The types of anomalies seen were anasarca in one fetus each in the control and 1000 mg/kg groups, dwarfism in one fetus each in the control and 2000 mg/kg groups, absent eye bulge in one fetus in the 300 mg/kg group, and omphalocele in one fetus in the 2000 mg/kg group.

Skeletal Anomalies*:* no skeletal abnormalities were noted in the control and 2000 mg/kg groups.

Skeletal Variations: variations including incomplete ossification of the interparietal bones, supraoccipital bones, hyoid, cervical arches, sternebrae, bipartite ossification of the sternebrae, misshapen sternebrae, incomplete ossification of the thoracic centrum, bipartite ossification of the thoracic centrum, dumbbell ossification of the thoracic centrum, incomplete ossification of the pubis, unossified pubis, incomplete ossification of the ischium or supernumerary ribs were noted in 27 of 136 (19.4%) and 23 of 139 fetuses (17.5%) in the control and 2000 mg/kg groups, respectively. The incidences of the skeletal variations were not significantly different between the control and 2000 mg/kg groups.

Ossification: the number of sacral and coccygeal vertebrae and the number of metacarpals were not significantly different between the control and 2000 mg/kg groups.

Visceral Anomalies: membranous ventricular septum defects were noted in one of 143 (0.6%) and two of 150 fetuses (1.5%) in the control and 2000 mg/kg groups, respectively. The incidences of visceral anomalies were not significantly different between the control and 2000 mg/kg groups.

Visceral Variations: variations including thymic remnant in the neck, malpositioned subclavian branch, supernumerary right coronary orifice, abnormal lung lobation, small spleen, dilated renal pelvis, dilated ureter, malpositioned testis or malpositioned umbilical artery were noted in 24 of 143 (16.1%) and 20 of 150 fetuses (13.3%) in the control and 2000 mg/kg groups, respectively. The incidences of visceral variations were not significantly different between the control and 2000 mg/kg groups.

Oral Gavage Study for Effects on Embryo-fetal Development and Toxicokinetics with GS-9137 and Ritonavir in Rats

Key study findings: time-mated female Crl:CD(SD) rats were assigned to 7 main study groups (25/group). There were two unscheduled deaths during the study; the cause of death was known. Maternal necropsy findings were limited to three females with dilated renal pelvises: one female each in the Vehicle I-alone, Vehicle II-alone, and 10 mg/kg/day ritonavir & 100 mg/kg/day GS-9137 groups. There were an adequate number of litters per group for comprehensive fetal evaluations. Mean postimplantation loss was similar across groups, therefore there was no effect of the different vehicles, ritonavir, GS-9137, or the combination of GS-9137 and ritonavir on embryo/fetal viability. Other cesarean section data were unremarkable and mean covariate adjusted fetal weights were similar across all groups. There were no external, soft tissue or skeletal anomalies related to treatment with GS-9137, ritonavir or the combination of GS-9137 and ritonavir.

In this study, two vehicles [0.5% (w/v) Methylcellulose and Ethanol: water:propylene glycol] were tested separately and in combination. In addition, GS-9137 and ritonavir were administered daily via oral gavage either alone or in combination to pregnant rats during the period of organogenesis. Neither of the vehicles (alone or in combination) nor GS-9137 and ritonavir (alone or in combination) produced changes in maternal body weight, food consumption, cesarean section, fetal weight or fetal development parameters. Therefore NOAELs for maternal toxicity and embryo/fetal viability, growth and development for ritonavir and GS-9137 are 10 mg/kg/day ritonavir and 1000 mg/kg/day GS-9137 when used separately or in combination. At the NOEL, the mean AUC_{0-t} on GD 17 were 181 µg·h/mL for EVG, and 2.81 µg·h/mL for RTV when used in combination.

Study no.: TX-183-2008

Volume # and page #: electronic
Conducting laboratory and location: (b) (4)

Date of study completion: 29 June 2006
GLP compliance: yes
QA reports: yes

Drug, lot #, and % purity: 061240; 99.1%

Methods

Doses: study design. The vehicle/control articles were 0.5% (w/v) methylcellulose (MC) for GS-9137 and ethanol:reverse osmosis water: propylene glycol (43:15:42) for ritonavir.

Species/strain: time-mated female Crl:CD(SD) rats

Number/sex/group: study design

Route, formulation, volume, and infusion rate: oral gavage

Satellite groups used for toxicokinetics: study design

Study design: the dose was administered once daily beginning on Gestation Day (GD) 6 and continuing through GD 17. Additional groups of toxicokinetic animals were used to determine the maternal plasma concentrations of GS-9137 and/or ritonavir at selected timepoints following dose administration. At initiation of treatment, the animals were approximately 11 weeks old, and their body weights ranged from 197 to 286 g.

Animals were assigned to the following groups

Group	No of Animals Mated Females	GS-9137 Dose Level (mg/kg/day)	Ritonavir Dose Level (mg/kg/day)	Dose volume (ml/kg)
Toxicity Animals				
1 (Vehicle I)	25	0	0	5
2 (Vehicle II)	25	0	0	1
3 (Vehicle Controls II & I)	25	0	0	1,5
4 (GS-9137 alone)	25	1000	0	5
5 (Ritonavir alone)	25	0	10	1
6 (Ritonavir & GS-9137 Low Dose)	25	100	10	1,5
7 (Ritonavir & GS-9137 High Dose)	25	1000	10	1,5
Toxicokinetic Animals				
8 (Vehicle I)	3	0	0	5
9 (Vehicle II)	3	0	0	1
10 (Vehicle Controls II & I)	3	0	0	1,5
11 (GS-9137 alone)	6	1000	0	5
12 (Ritonavir alone)	6	0	10	1
13 (Ritonavir & GS-9137 Low Dose)	6	100	10	1,5
14 (Ritonavir & GS-9137 High Dose)	6	1000	10	1,5

Parameters and endpoints evaluated: assessment of maternal and embryo/fetal toxicity and teratogenic potential of GS-9137, and GS-9137 in combination with ritonavir, was based on mortality, clinical observations, body weights, food consumption, cesarean section data, and fetal external, soft tissue and skeletal evaluations.

Observations and Results

Mortality (dams): there were two unscheduled deaths during the study. One female (B06915) dosed with ritonavir alone was found dead on GD 11, and one female (B07055) dosed with 10 mg/kg/day ritonavir & 1000 mg/kg/day GS-9137 was found dead on GD 18. The last recorded clinical observations for both females were normal. Both females were normal and pregnant at necropsy. The cause of death could not be determined.

Clinical signs (dams): were generally unremarkable and sporadic. Alopecia was observed in females in most groups (including the Vehicle II and Vehicle II&I groups). All cases of alopecia occurred on the paw(s)/limb, and one animal had an additional area of alopecia in the sacral area. Other clinical signs included dark eye(s), soft feces, sore(s), and audible respiration prior to dosing.

Body weight (dams): treatment with GS-9137 and/or ritonavir produced no alteration in mean maternal body weight. Mean maternal body weight change from GD 0 to 20 was significantly increased in females treated with a combination of both Vehicles compared to each vehicle individually (10 and 14%, respectively). Females treated with GS-9137 only had significantly increased body weights (12%) upon comparison with Vehicle I (GS-9137 vehicle)-only group. All body weight changes were within the historical control data range None of the other treated groups had statistically significant alterations in mean body weight change.

Food consumption (dams): no effects.

Toxicokinetics: analysis are presented in Tables 41, 42, and 43. The toxicokinetic portion of this study demonstrated overall dose-related exposure on GD 6 and 17 for GS-9137, its metabolite GS-9200, and ritonavir for pregnant female rats administered GS-9137 alone at 1000 mg/kg/day, ritonavir alone at 10 mg/kg/day, and GS-9137 at 100 and 1000 mg/kg/day in combination with ritonavir at 10 mg/kg/day once daily by oral gavage during GD 6 to 17. In addition, ritonavir co-administration had no substantial effect on the exposure to GS-9137 on GD 6 and 17. Co-administration of GS-9137 at 100 and 1000 mg/kg/day with 10 mg/kg/day ritonavir resulted in decreased Cmax and AUC_{0-t} values for ritonavir relative to administration of ritonavir alone GD 6 and 17.

Table 41. Gestational Day 6 and 17 Toxicokinetic Parameters for GS-9137 Following Once Daily Oral Gavage Administration of GS-9137 With and Without Ritonavir to Female Rats

Group	Dose GS/R (mg/kg/day)	Day	Tmax (hr)	Cmax (ng/mL)	AUC_{0-t} (ng·hr/mL)	$t_{½}$ (hr)
11	1000/0	6	0.500	28500	140000	3.83
13	100/10	6	0.500	10900	58600	3.23
14	1000/10	6	2.00	30600	158000	6.43
11	1000/0	17	2.00	27300	226000	10.5
13	100/10	17	2.00	11800	59800	1.52
14	1000/10	17	2.00	27800	181000	2.29

Table 42. Gestational Day 6 and 17 Toxicokinetic Parameters for GS-9200 Following Once Daily Oral Gavage Administration of GS-9137 With and Without Ritonavir to Female Rat

11	1000/0	6	0.500	1940	10900	4.58
13	100/10	6	2.00	944	4520	2.47
14	1000/10	6	2.00	2880	12200	3.55
11	1000/0	17	2.00	2600	23000	17.3
13	100/10	17	2.00	1260	3810	NC
14	1000/10	17	2.00	2140	11400	2.28

Table 43. Gestational Day 6 and 17 Toxicokinetic Parameters for Ritonavir Following Once Daily Oral Gavage Administration of Ritonavir With and Without GS-9137 to Female Rats

Group	Dose GS/R (mg/kg/day)	Day	Tmax (hr)	Cmax (ng/mL)	AUC_{0-t} (ng·hr/mL)	$t_{½}$ (hr)
12	0/10	6	4.00	1200	6020	NC
13	100/10	6	2.00	512	2750	5.14
14	1000/10	6	2.00	941	3770	3.82
12	0/10	17	4.00	1620	7240	NC
13	100/10	17	4.00	968	5570	NC
14	1000/10	17	8.00	474	2810	NC

Postmortem observations: maternal necropsy findings were limited to three females with dilated renal pelvises: one female each in the Vehicle I-alone (B06926), Vehicle II-alone (B06929), and 10 mg/kg/day ritonavir&100 mg/kg/day GS-9137 (B06942) groups.

Gravid Uterine Weights, Corrected Terminal Weights, and Net Body Weight Changes: no treatment-related alterations in mean gravid uterine weights, corrected terminal weights, or net body weight changes were observed in any treatment group.

Reproductive parameters, necropsy findings, placental and fetal body weights, external and buccal examination: the pregnancy rates were 88% in the Vehicle I, Vehicle II&I, GS-9137-alone, and ritonavir-alone groups; 84% in the Vehicle II and 10 mg/kg/day ritonavir&100 mg/kg/day GS-9137 groups; and 72% in the 10 mg/kg/day ritonavir&1000 mg/kg/day GS-9137

group. The mean postimplantation was similar across groups, indicating that the different vehicles, GS-9137 and/or ritonavir had no effect on embryo/fetal viability.

There were no significant effects on mean covariate-adjusted fetal weights.

Visceral and skeletal examination: no fetal other external variations were noted. Fetal external malformations were limited to one 10 mg/kg/day ritonavir&1000 mg/kg/day GS-9137 fetus with a filamentous tail (B07007, Fetus 4). This malformation was within the (b) (4) historical control incidence.

Fetal soft tissue malformations were limited to a single incidence of situs inversus in the GS-9137-alone group (B06886, Fetus 4). The incidence of this finding is within the (b) (4) historical control range and considered within the normal variability of this strain of rat.

Skeletal Evaluations: although the fetal skeletal variations were those commonly seen in this strain of rat, a statistically significant change in fetal and/or litter incidence was noted in the following: fetal and litter incidence of incomplete ossification of the skull after treatment with GS-9137 alone or ritonavir alone as compared to those treated with the combination of Vehicle 1 and Vehicle 2 was significantly increased. Fetal incidence of incomplete ossification of the skull after treatment with both Vehicle 1 and Vehicle 2 was significantly decreased as compared with Vehicle 2 alone.

Fetal and/or litter incidence of incomplete ossification of the 5th/6th sternebra after combined treatment with Vehicle 1 and Vehicle 2, or ritonavir alone as compared to those treated with Vehicle 2 was significantly increased. Fetal incidence of incomplete ossification of the 5th/6th sternebra after treatment with 10 mg/kg/day ritonavir and 1000 mg/kg/day GS-9137 as compared to combined treatment with both Vehicle 1 and Vehicle 2 was significantly decreased.

Fetal incidence of 14th rudimentary rib after treatment with 10 mg/kg/day ritonavir and 1000 mg/kg/day GS-9137 as compared to those treated with both Vehicle 1 and Vehicle 2.

Fetal and/or litter incidence of total fetal skeletal variations after treatment with both Vehicle 1 and Vehicle 2, or ritonavir alone as compared to those treated with Vehicle 2 only.

In general, statistically significant increases of skeletal variations were seen in controls, increases in treated groups were seen when compared to one vehicle but not the other and although there were some significant increases in the incidences in a limited number of specific parameters, the fetal and litter incidences were within the Historical Control Database range and were therefore considered to be within the normal variability of this strain of rat.

Fetal skeletal malformations were limited to one fetus with major fusion of the sternebrae after treatment with Vehicle 1 only (B07008; fetus 6). Since, this malformation is within the (b) (4) Historical Control range and only occurred in a vehicle control group it is not attributed to test article.

Oral (stomach tube) development study of JTK-303 in rabbits

Key study findings: groups of pregnant New Zealand White rabbits (22 rabbits/group) received JTK-303 during organogenesis (days 7-19 post insemination) orally via stomach tube at dose levels of 0 (corn oil, vehicle control), 50 (low), 150 (mid) or 450 mg/kg/day (high). On day 19, blood samples were collected and were analyzed by a validated analytical method. There were no deaths. Clinical signs in dams included soft or liquid feces, scant feces and ungroomed coat; the incidence was not dosage dependent. Body weight (dams) and Food consumption (dams) values were statistically significantly ($p<0.05$ or $p<0.01$) reduced in the mid and high dosage dams on days 23 to 29. As a result of these reductions, reductions, body weight gains and absolute and relative feed consumption values for the entire postdosage period (DGs 20-29) were reduced in these groups. Body weight gains and food consumption were unaffected at the low dose. No drug related effects were seen in the number of corpora lutea, the number of implantations, the implantation index, the number of live fetuses, the fetal viability, the number of dead embryos and fetuses, the post implantation deaths, the fetal sex distribution and the fetal body weights. No treatment related findings were seen in the external, skeletal or visceral examination of the fetuses.

The maternal NOAEL was 50 mg/kg/day and the developmental NOAEL was 450 mg/kg/day in the rabbit. Based on the body surface area factor, equivalent doses in humans for dam and fetus would be 16 and 144 mg/kg/day (958 mg and 8.6 g/day for a 60 kg person, respectively). At the maternal NOAEL (50 mg/kg/day), exposures (steady state AUC and Cmax) were 1.45 µg*hr/ml and 0.09 µg/ml, respectively. At the developmental NOAEL (50 mg/kg/day), exposures (steady state AUC and Cmax) were 4.27 µg*hr/ml and 0.15 µg/ml, respectively.

Study no.: TOX-183-2002

Volume # and page #: 3 and 1-291

Conducting laboratory and location: (b) (4)

Date of study completion: August 31, 2006

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: S**,** 98.9%

Methods

Doses: 50 (low), 150 (mid) or 450 mg/kg/day (high)

Species/strain: New Zealand White rabbits/Hra:(NZW)SPF

Number/sex/group: 22 rabbits/group

Route, formulation, volume, and infusion rate: stomach tube (2 ml/kg)

Satellite groups used for toxicokinetics: 5 animals/sex/group
Study design: groups of pregnant New Zealand White rabbits (22 rabbits/group] received JTK-303 during organogenesis (days 7-19 post insemination) orally via stomach tube at dose levels of 0 (corn oil, vehicle control), 50 (low), 150 (mid) or 450 mg/kg/day (high). On day 19, blood samples were collected and were analyzed by a validated analytical method.

Parameters and endpoints evaluated: maternal gross necropsy, Cesarean-sectioning and fetal evaluation.

Observations and Results

Mortality (dams): there were no deaths. One doe (mid) was aborted and was sacrificed; it was a single abortion and was non-dosage dependent event.

Clinical signs (dams): included soft or liquid feces, scant feces and ungroomed coat; the incidence was not dosage dependent.

Body weight and body weight gain (dams): during the postdosage period (DGs 20 through 29), statistically significant reductions ($p<0.05$ or $p<0.01$) in body weight gain occurred in the 150 mg/kg/day dosage group on DGs 23 to 26 and in the 450 mg/kg/day dosage group on DGs 26 to 29. As a result, body weight gain for the entire postdosage period was reduced in the 150 mg/kg/day dosage group and significantly reduced ($p<0.05$) in the 450 mg/kg/day dosage group. Average maternal absolute body weights were comparable among the four dosage groups and did not significantly differ. Body weight gains were unaffected by the 50 mg/kg/day dosage of GS-9137.

Absolute and relative food consumption (dams): during the postdosage period, absolute and relative feed consumption values were reduced in the 150 and 450 mg/kg/day dosage groups on DGs 23 to 26 and 26 to 29; the reductions were statistically significant ($p<0.05$) for relative feed consumption on DGs 23 to 26 in the 150 and 450 mg/kg/day dosage groups and for absolute and relative feed consumption on DGs 26 to 29 in the 450 mg/kg/day dosage group. As a result of these reductions, absolute and relative feed consumption values for the entire postdosage period (DGs 20 to 29) were reduced and significantly reduced ($p<0.05$) in the 150 and 450 mg/kg/day dosage groups, respectively. Absolute and relative feed consumption values were unaffected by the 50 mg/kg/day dosage of GS-9137.

Caesarean-Sectioning and Litter Observations: pregnancy occurred in 18 (85.7%), 21 (95.4%), 20 (90.9%) and 21 (95.4%) does in the four respective dosage groups. As a result of the abortion in the 150 mg/kg/day dosage group described previously, Caesarean-sectioning observations were based on 18, 21, 19 and 21 pregnant does with one or more live fetuses in Groups I through IV, respectively.

No Caesarean-sectioning or litter parameters were affected by dosages of GS-9137 as high as 450 mg/kg/day. The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, percent resorbed conceptuses, and percent live male fetuses were comparable among the four dosage groups. A significant reduction ($p<0.05$) in the number of implantations in the 450 mg/kg/day dosage group was not considered related to the test article because the value was within the historical control ranges for this Testing Facility. No doe had a litter consisting of only resorbed conceptuses, and there were no dead fetuses. All placentae appeared normal.

Fetal Alterations: fetal evaluations were based on 160, 163, 146 and 163 live, DG 29 Caesarean-delivered fetuses in 18, 21, 19 and 21 litters in the 0 (Vehicle), 50, 150 and 450 mg/kg/day dosage groups, respectively. Each of these fetuses was examined for gross external, soft tissue and skeletal alterations and fetal ossification site averages.

No gross external, soft tissue or skeletal fetal alterations (malformations or variations) were caused by dosages of GS-9137 as high as 450 mg/kg/day. There were no dosage dependent differences in the litter or fetal incidences of any gross external, soft tissue or skeletal alterations. There were no biologically important differences among the four dosage groups in the average numbers of ossification sites per fetus.

Visceral and skeletal examination: no abnormal findings.

Fetal observations: no abnormal findings

Toxicokinetics: mean values are shown in Table 44. The systemic exposure as measured by Cmax and AUC increased with escalating doses of JTK-303 in rabbits, but increased less than proportionally to dose.

Table 44. Toxicokinetics of JTK-303 on day 17 post insemination after repeated daily oral administration to pregnant rabbits

Dose (mg/kg/day)	AUC $_{0\text{-}24hr}$ (μg*hr/ml)	Cmax (μg/ml)	Tmax (hr)
50	1.45	0.09	13.2
150	3.05	0.08	19.8
450	4.27	0.15	2.8

9.3 Prenatal and Postnatal Development

Oral (Gavage) Developmental and Perinatal/Postnatal Reproduction Toxicity Study of GS-9137 in Rats, Including a Postnatal Behavioral/Functional Evaluation and a 28-Day Juvenile Toxicity Evaluation

Key study findings: groups of presumed pregnant Crl:CD(SD) rats (25/group) received GS-9137 via oral gavage (10 ml/kg) at dose levels of 0 (0.5% aqueous methylcellulose as vehicle control), 300 (low), 1000 (mid) or 2000 mg/kg/day (high) daily from day 7 of gestation (DG 7)

through day 20 of lactation (DL 20) or DG 24 (rats that did not deliver a litter). In the developmental and perinatal/postnatal reproduction toxicity portion of the study, including the postnatal behavioral/functional evaluation, there were no adverse effects at the high dose. On the basis of these data, the maternal NOAEL for general toxicity of GS-9137 was 2000 mg/kg/day. The NOAELs for reproduction in the dams and viability and growth in the offspring were also 2000 mg/kg/day. In the juvenile toxicity evaluation portion of the study, the only test article-related observation was increased cecum weights at 2000 mg/kg/day for male rats and at 1000 and 2000 mg/kg/day for female rats (these increases were not statistically significant); however, these were no histopathological correlate. On the basis of these data, the NOAEL for toxicity of GS-9137 was 2000 mg/kg/day for juvenile male and female rats. The toxicokinetic portion of this study demonstrated dose-related exposure on Day 14 of lactation for the F0 pregnant female rats to GS-9137 and its metabolites GS-9200 and GS-9202. In addition, this study demonstrated overall dose-related exposure on Days 1 and 28 for the F1 generation male and female juvenile rats to GS-9137 and its metabolites GS-9200 and GS-9202 after single daily oral gavage doses of GS-9137 of 300, 1000, and 2000 mg/kg/day administered for 28 days.

At the maternal NOAEL for general toxicity (2000 mg/kg/day) and the NOAEL for reproduction in the dams and viability and growth in the offspring (2000 mg/kg/day), exposure (LD 14 AUC_{0-t}) was 408 µg*hr/ml. At the NOAEL for juvenile male and female toxicity, exposure ((Day 28 AUC_{0-t}) was 169 µg*hr/ml.

Study no.: TX-183-2006

Volume # and page #: electronic

Conducting laboratory and location: (b) (4)

Date of study completion: 22 October 2007

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: 061240; 96 %

Methods

Doses: groups of presumed pregnant Crl:CD(SD) rats (25/group) received GS-9137 via oral gavage (10 ml/kg) at dose levels of 0 (0.5% aqueous methylcellulose as vehicle control), 300 (low), 1000 (mid) or 2000 mg/kg/day (high) daily from day 7 of gestation (DG 7) through day 20 of lactation (DL 20) or DG 24 (rats that did not deliver a litter).

Species/strain: presumed pregnant rats (Crl: CD (SD)

Age & weights: 65 days of age on receipt; weights: 219-250 g at initiation of the study

Number/sex/group: 25 rats/group

Route, formulation, volume: oral gavage, 10 ml/kg

Satellite groups used for toxicokinetics: 3 animals/dose

Study design: F0 Generation Rats: observations for clinical signs, abortions, premature deliveries and deaths were made daily before and after dosage administration, and on the day sacrifice occurred. Rats were evaluated for adverse clinical signs observed during parturition, duration of gestation, litter sizes and pup viability at birth. Each litter was evaluated for viability at least twice daily. The pups in each litter were counted daily. Clinical observations were recorded once daily during the preweaning period. After completion of the 21-day postpartum period, surviving female rats were sacrificed and a gross necropsy was performed. The number and distribution of implantation sites were recorded. On DL 21, all pups not selected for continued evaluation were sacrificed and examined for gross lesions.
F1 Generation Rats - Postnatal Behavioral/Functional Evaluation: all F1 generation rats assigned to the postnatal behavioral/functional evaluation were examined for clinical observations at least once weekly during the postweaning period. Body weights for male rats were recorded weekly and when sacrifice occurred. Body weights for female rats were recorded weekly during the postweaning period and on DGs 0, 7, 10, 14, 17 and 21. Feed consumption values for the male and female rats were recorded at least weekly during the postweaning period except during cohabitation. Feed consumption values for female rats were also recorded on DGs 0, 7, 10, 14, 17 and 21. Male and female rats were evaluated for sexual maturation and for learning and memory in passive avoidance and watermaze tests. At approximately 90 days of age, the F1 generation rats were assigned to a 21-day cohabitation period. All male rats were sacrificed after completion of the cohabitation period and a gross necropsy was performed. Testes and epididymides weights were recorded. All female rats were sacrificed on DG 21, Caesarean-sectioned and a gross necropsy was performed. Each fetus (F2) was weighed and examined for sex and gross external alterations.

F1 Generation Rats - Juvenile Toxicity Evaluation: all F1 generation rats assigned to the juvenile toxicity evaluation were randomly assigned to four dosage groups (Groups I through IV), 10 rats/sex/group. Additionally, 9 male and 9 female pups per Group I, and 24 male and 24 female pups per Groups II through IV were selected for continued dosage administration in the toxicokinetic portion of the juvenile toxicity study. GS-9137 was administered orally via gavage once daily to these male and female rats from day 1 of study, (DS 1, day 22 postpartum) through DS 28 (day 49 postpartum) at dosages of 0 (Vehicle; 0.5% methylcellulose), 300, 1000 and 2000 mg/kg/day. All F1 generation rats were examined for clinical observations daily before and approximately one hour after dosing during the dosage period and once daily during the postdosage period. Body weights for male and female rats were recorded weekly and when sacrifice occurred. Feed consumption values for the male and female rats were recorded at least weekly during the dosage period. On the first and last days of administration, blood samples were collected from rats assigned to the toxicokinetic sample collection portion of the study. Male and female rats assigned to the toxicokinetic portion of the juvenile toxicity evaluation were sacrificed and discarded without further evaluation. Male and female rats assigned to the

main portion of the juvenile toxicity evaluation were sacrificed on DS 29 (day 50 postpartum) and a gross necropsy was performed. Selected organs were weighed and retained. Histological examination was performed on tissues from all animals.

Observations and Results

F0 Generation Rats Mortality: there were no test article-related deaths in the F0 generation female rats.

Clinical observations: there were no test article-related observations.

Necropsy: there were no test article-related necropsy observations.

Body weights and feed consumption: were unaffected.

Natural delivery observations: pregnancy occurred in 24 (96.0%), 23 (92.0%), 23 (92.0%) and 24 (96.0%) of the 25 mated female rats in the 0 (Vehicle), 300, 1000 and 2000 mg/kg/day dosage groups, respectively. Natural delivery and litter observations were unaffected by the treatment.

Clinical and necropsy- F1 Generation Pups**:** no clinical or necropsy observations were attributed in the F1 generation pups at any dose.

Toxicokinetics: F0 Generation Females on Day 14 of Lactation: dose-related exposure on DL 14 to GS-9137 and its metabolites GS-9200 and GS-9202. Exposure to GS-9137 and GS-9200 increased with dose, in a less than dose-proportional manner. Only limited plasma concentration data were available for GS-9202. Concentrations of GS-9137 in rat milk at 30 minutes postdosage on DL 14 increased with dose, in a less than dose-proportional manner. Neither of the two metabolites of GS-9137 (GS-9200 or GS-9202) were detected in the milk of any rats before or after dosage administration on DL 14 and GS-9137 was not detected in any milk samples before test article administration on DL 14.

Table 45. Day 14 of Lactation Toxicokinetic Parameters for GS-9137, GS-9200, and GS-9202 in Female F0 Rats

Dose (mg/kg/day)	Tmax (hr)	Cmax (ng/mL)	AUC_{0-t} (ng·hr/mL)	$t_{½}$ (hr)
GS-9137				
300	0.500	17500	67200	1.86
1000	0.500	34700	200000	3.05
2000	0.500	36000	408000	3.09
GS-9200				
300	0.500	3100	15800	1.51
1000	0.500	5260	22800	4.58
2000	2.00	7100	69400	2.84
GS-9202				
2000	0.500	30.0	57.8	NC

F1 Generation Rats - Postnatal Behavioral/Functional EvaluationMortality: there were no drug related deaths. All F1 generation male and female rats survived to scheduled necropsy.

Clinical signs: no drug related clinical observations occurred in either males or females at any dose level.

Body weight and feed consumption: there were no changes

Sexual maturation: was unaffected

Passive avoidance: there were no statistically significant or biologically important differences in the values for learning, short-term retention, long-term retention, or response inhibition in the F1 generation male and female rats, as evaluated by performance in a passive avoidance paradigm.

Watermaze: no biologically important differences occurred in watermaze performance of the F1 generation male and female rats regarding learning, short-term retention, long-term retention, or response inhibition.

Mating and fertility: there were no effects.

Necropsy observations: no changes were seen.

Terminal Body Weights, Testes and Epididymides Weights: terminal body weights were comparable among the four groups. Testes weights were significantly increased ($p \leq 0.05$) in the 300, 1000 and 2000 mg/kg/day dosage groups but it was comparable in all dose groups. These increases were 5% to 6% over the vehicle control group value.

Caesarean-Sectioning Observations: Caesarean-sectioning observations were based on 23 (92.0%), 17 (68.0%), 22 (88.0%) and 21 (84.0%) pregnant rats with one or more live fetuses in Groups I through IV, respectively. No Caesarean-sectioning or litter parameters were affected by the maternal dosages. No drug-related fetal gross alterations were seen.

F1 Generation Rats-juvenile toxicity Mortality: there were no test article-related deaths.

Clinical Observations: observations of slight excess salivation occurred in 2 of 10 male and female rats in the 2000 mg/kg/day dosage group and one female rat in the 1000 mg/kg/day. Two male rats in the 2000 mg/kg/day dosage group had an ungroomed coat on single days.

Body weight and feed consumption: there were no changes

Toxicokinetics: data are shown in Tables 46, 47 and 48.

GS-9137: On Day 28, the female Cmax and AUC values were 22600, 28300, and 31400 ng/ml and 51700, 122000, and 194000 ng·hr/mL, respectively. The male Cmax and AUC values were

10900, 19300, and 31500 ng/mL and 37400, 79100, and 144000 ng·hr/mL, respectively. Exposure increased with dose in the majority of cases, in a less than dose proportional manner.

GS-9200: On Day 28, the female Cmax and AUC values were 2520, 3410, and 3250 ng/mL and 6050, 15700, and 19400 ng·hr/mL, respectively. The male Cmax and AUC values were 2080, 2770, and 4610 ng/mL and 7600, 13500, and 20800 ng·hr/mL, respectively. Exposure increased with dose in the majority of cases, in a less than dose-proportional manner.

GS-9202: On Day 28, the female Cmax and AUC values were 37.7 ng/mL and 73.7 ng·hr/mL, respectively, at a dose of 2000 mg/kg/day. The male Cmax and AUC values were 89.0 and 74.3 ng/mL and 22.3 and 174 ng·hr/mL, at doses of 1000 and 2000 mg/kg/day, respectively. Exposure increased with dose in the majority of cases, in an approximately dose-proportional or less than dose-proportional manner.

Table 46. Day 1 and Day 28 Toxicokinetic Parameters for GS-9137 in juvenile rats

EVG (mg/kg/day)	Day	AUC_{0-t} (µg·h/mL)		C_{max} (µg/mL)	
		Male	Female	Male	Female
300	1	52.6	57.5	12.8	11.0
	28	37.4	51.7	10.9	22.6
1000	1	99.4	130	19.0	21.7
	28	79.1	122	19.3	28.3
2000	1	128	134	19.1	19.1
	28	144	194	31.5	31.4

Table 47. Day 1 and Day 28 Toxicokinetic Parameters for GS-9200 in juvenile rats

EVG (mg/kg/day)	Day	AUC_{0-t} (µg·h/mL)		C_{max} (µg/mL)	
		Male	Female	Male	Female
300	1	212	191	43.8	39.5
	28	7.60	6.05	2.08	2.52
1000	1	224	317	45.7	66.3
	28	13.5	15.7	2.77	3.41
2000	1	845	699	157	1551
	28	20.8	19.4	4.61	3.25

Table 48. Day 1 and Day 28 Toxicokinetic Parameters for GS-9202 in juvenile rats

EVG (mg/kg/day)	Day	AUC_{0-t} (µg·h/mL)		C_{max} (µg/mL)	
		Male	Female	Male	Female
300	1	0.879	0.838	0.268	0.241
	28	BLQ	BLQ	BLQ	BLQ
1000	1	1.37	2.43	0.637	0.797
	28	0.0223	BLQ	0.089	BLQ
2000	1	2.09	1.37	0.396	0.211
	28	0.174	0.0737	0.0743	0.0377

Necropsy observations: in rats that survived to scheduled sacrifice included slight dilation of the renal pelvis in single male rats in the 1000 and 2000 mg/kg/day dosage groups, red areas on the thymus of a single male rat in the 300 mg/kg/day dosage group, and a tan area on the papillary process of the liver in one female rat in the 2000 mg/kg/day dosage group.

Terminal Body Weights, Organ Weights and Ratios (%) of Organ Weights to Terminal Body Weight: weights of the cecum (with contents) and ratios of this weight to the terminal body weight and brain weight were increased for male rats in the 2000 mg/kg/day dosage group (14% to 18% over the control group values) and for female rats in the 1000 and 2000 mg/kg/day dosage groups (10% to 12% and 13% to 14% over control group values, respectively). However, these increases were not statistically significant. Terminal body weights were comparable among the four groups

Clinical Pathology: no changes were seen.

Histopathology: no changes were seen.

10 Special Toxicology Studies

Two week oral dose toxicity study in rats with 1-, 2- and 8-week recovery period (TX-018). JTK-303 was administered orally once daily at a dose level of 2000 mg/kg/day for 2 weeks to male Sprague-Dawley rats. At completion of the dosing period, and after 1- 2- or 8-week recovery period, the upper small intestine was resected (8 animals at each time point) and histopathologically examined in order to assess the reversibility of a histopathological change (lipid-like vacuoles) in the lamina propria. A control group of 8 animals received the vehicle only (0.5% methylcellulose solution) and was similarly evaluated after the dosing period. No deaths occurred in any group during the dosing or recovery period. Whitish stools, which may be derived from the color of the unabsorbed test article, were noted at 2000 mg/kg. Transient salivation probably attributed to the bitter taste of the test article solution was also observed in a few animals immediately after dosing. At 2000 mg/kg, there were no treatment-related differences in body weights or food consumption as compared to controls. At completion of 2-week dosing period, lipid-like vacuoles were observed in the lamina propria, mainly, in the upper small intestine (duodenum and jejunum). The severity of the finding was minimal to moderately severe. Oil red O, Sudan Black B and Nile Blue staining confirmed that the vacuoles were lipid

vacuoles containing mainly triglycerides. At completion of 1-, 2- and 8- week recovery period, lipid vacuoles were still present in all animals treated with 2000 mg/kg. However, none of the animals in the recovery groups had findings of "moderately severe" degree. This study demonstrated that the lipid vacuoles (consisting mainly of triglycerides) in the lamina propria of upper small intestine at 2000 mg/kg decreased in its severity after drug withdrawal.

Two-Week Repeated Oral Dose Study with Low Dose Levels of JTK-303 in Rats with Two-, Eight- and Sixteen-Week Recovery Period (TX-024). JTK-303 was administered orally once daily at a dose level of 100 mg/kg/day for two weeks to male Sprague-Dawley rats. On completion of the dosing period, and after a two-, eight- or sixteen-week recovery period, the upper small intestine was resected (10 animals at each time point) and examined histopathologically in order to assess the reversibility of the expected histopathological findings (lipid vacuoles) in the lamina propria. A control group of ten animals received the vehicle only (0.5% methylcellulose aqueous solution) and was similarly evaluated after the dosing period. No deaths occurred in any group during the dosing or recovery period. There were no findings in any group during the dosing or recovery period. There were no drug related effects on body weights and body weight gain in this study. There were no changes in food consumption. On completion of the two-week dosing period, there were the lipid vacuoles in the upper small intestinal lamina propria (duodenum and jejunum) in six of ten animals at 100 mg/kg, although the degree of the lipid vacuoles was minimal in each animal. After a two-week recovery period, the incidence and degree of the lipid vacuoles was comparable to those of the group sacrificed after the two-week repeated dosing. After an eight-week recovery period, lipid vacuoles were still observed, however the incidence of the lipid vacuoles was lower than that of the groups sacrificed after the two-week repeated dosing or after the two-week recovery period. After a sixteen-week recovery period, lipid vacuoles were not observed in any treated-animal. The lipid vacuoles in the lamina propria of the upper small intestine were slowly reversible, and completely disappeared after a sixteen-week recovery period following two-week repeated dosing with 100 mg/kg of JTK-303. The lipid vacuoles in the lamina propria in the upper small intestine were slowly reversible, and completely disappeared after the sixteen-week recovery period following a two-week repeated dosing with 100 mg/kg of JTK-303.

Four-Week Repeated Oral Dose Study of JTK-303 in Rats-Sequential Evaluation of JTK-303-Induced Histopathological Findings in the Small Intestine (TX-025). JTK-303 was administered orally once daily at a dose level of 2000 mg/kg/day for two or four weeks to male Sprague-Dawley rats. On completion of each dosing period, the upper small intestine was resected (eight animals at each time point) and examined histopathologically in order to assess the progression of the expected histopathological findings (lipid vacuoles) in the lamina propria. A control group of eight animals received the vehicle only (0.5% methylcellulose aqueous solution) and was similarly evaluated after the four-week dosing period. Lipid vacuoles in the lamina propria of the upper small intestine (duodenum and jejunum) were observed in all the animals after the two- and four-week repeated dosing. The degree of the lipid vacuoles was comparable between the two- and four-week repeated dosing groups. From the results of this study, the lipid vacuoles in the lamina propria of the upper small intestine were observed in all the animals after two- and four-week repeated dosing with JTK-303 at 2000 mg/kg. The degree of the lipid vacuoles was not increased in the animals after the four-week repeated dosing when

compared with that after the two-week repeated dosing. The lipid vacuoles were not progressive with prolongation of the dosing period for up to four weeks.

Two-Week Repeated Oral Dose Study of JTK-303 in Rats - Sequential Evaluation of JTK-303-Induced Histopathological Findings in the Small Intestine (TX-026). JTK-303 was administered orally once daily at a dose level of 2000 mg/kg/day for one, three, seven or 14 days to male Sprague-Dawley rats. At the end of each dosing period, the upper small intestine was resected (8 animals at each time point) and examined histopathologically in order to assess the progression of the expected histopathological findings (lipid vacuoles) in the lamina propria. A control group of eight animals received the vehicle only (0.5% methylcellulose aqueous solution) and was similarly evaluated after the 14-day dosing period. From the results of this study, lipid vacuoles in the upper small intestinal lamina propria were observed after single or repeated dosing with JTK-303 at 2000 mg/kg. The degree of the lipid vacuolation was increased after the 14-day repeated dosing when compared to that after 1-7 days repeated dosing.

Three-Day Oral Dose Study of JTK-303 in Spaced-Fed Rats (JTK303-TX-027). JTK-303 was administered orally once daily at 9:00 at a dose level of 2000 mg/kg/day for three days to eight male Sprague-Dawley rats fed a basal diet for 2 hours a day (spaced feeding from 19:00 to 21:00). On completion of the dosing period, the upper small intestine was resected and examined histopathologically in order to evaluate histopathological findings (JTK-303-induced lipid vacuoles) in the lamina propria. A control group of eight animals received the vehicle (0.5% methylcellulose aqueous solution) only under the same feeding conditions and was similarly evaluated after the dosing period. The animals used in this study were acclimated to the above feeding conditions for 20 days prior to initiation of dosing. Free-feeding control and JTK-303-treated groups were also set in order to compare the histopathological finding with that in the spaced feeding groups. In the free-feeding groups, the vehicle or JTK-303 was administered for three days in the same way as spaced-feeding groups to eight male Sprague-Dawley rats per group that had free access to the basal diet. On completion of the three-day dosing period, lipid vacuoles were observed mainly in the lamina propria in the upper small intestine in the JTK-303-treated free-feeding group. In the JTK-303-treated spaced-feeding group, the finding was very subtle when compared with the JTK-303-treated free-feeding group. There were no lipid vacuoles in the lamina propria of the upper small intestine in any control group. This study demonstrated that the JTK-303-induced lipid vacuoles in the lamina propria of upper small intestine were decreased in their severity by separating the times of administration of JTK-303 and feeding. From the results of this study, it is suggested that the mechanism of this pathological finding is related to the coexistence of food with high local concentrations of the compound in the small intestine and that fat in the diet is the source of the lipid in the vacuoles.

Two-Week Dietary Administration Study of JTK-303 in Rats (TX-028). JTK-303 was administered in the diet to groups of male Sprague-Dawley rats (8 animals/group) at levels of 1, 2 and 5% for two weeks. On completion of the dosing period, the upper small intestine was resected and examined histopathologically in order to investigate the relationship between the occurrence of JTK-303-induced lipid vacuoles in the lamina propria and local concentrations of JTK-303 in the gastrointestinal lumen. A control group of eight animals was fed the basal diet for two weeks. On completion of the two-week dosing period, lipid vacuoles were observed in the lamina propria, mainly, in the upper small intestine (duodenum and jejunum). The lipid

vacuoles were prominent in their incidence and severity at the 5% level. At the 1 and 2% levels, the lipid vacuoles were very subtle and there was a marked difference in the incidence and severity of the finding between the 1 or 2% and 5% levels. This study demonstrated that the lipid vacuoles in the lamina propria of upper small intestine were related to the local JTK-303 concentrations to which the gastrointestinal epithelium was exposed, rather than to systemic exposure to JTK-303

Ultrastructural Analyses of JTK-303-Induced Histopathological Findings in the Small Intestine in Rats after a Single Oral Dose of JTK-303 (JTK303-TX-029). The purpose of this study was to investigate the time sequence of the treatment-related histopathological and ultrastructural findings (lipid vacuoles in the upper small intestinal lamina propria) seen in free-fed rats receiving a single oral dose of JTK-303 at a dose level of 2000 mg/kg. A single oral dose of JTK-303 was administered at a dose level of 2000 mg/kg to male Sprague-Dawley rats. During the study, each animal had free access to diet. At 10, 20 and 30 minutes, and 1, 4, 8 and 24 hours post-dose, the upper small intestine was resected (three or six animals at each time point) and examined histopathologically and ultrastructurally in order to assess the time sequence of findings of the small intestine. A control group (one or two animals at each time point) received the vehicle (0.5% methylcellulose aqueous solution) and was similarly evaluated. The initial lipid-related findings occurred in the enterocytes covering the tip of intestinal villi 10 minutes after a single dose. Firstly it was difficult to identify dilated Golgi stacks with lipid particles, which was typical of the control ultrastructure. In contrast, lipid accumulation within the membrane-bound organelles was noticeable not only in the Golgi-associated vacuoles but also in the dilated rough endoplasmic reticulum. JTK-303 appears to initially affect the process of lipid re-synthesis in the enterocytes. Secondarily medium-density lipid droplets, which were identical to those produced in the lamina propria, were formed in the enterocytes. Altered lipid synthesis seems to result in the formation of unique lipid droplets in the cytoplasm. The lipid-related findings were accompanied by transient mitochondrial swelling in the enterocyte but there were no findings indicative of mitochondrial damage in the enterocytes, suggesting that the mitochondrial swelling was an adaptive change secondary to the lipid-related findings.

In the one-month and three-month repeated oral dose toxicity studies with JTK-303, the test article-induced lipid vacuoles were seen in the lamina propria of the small intestine, but no histopathological findings were observed in the intestinal epithelia. The present study, however, demonstrated that enterocytic findings preceded formation of the lipid vacuoles in the lamina propria. The initial lipid-related findings occurred in the enterocytes covering the tips of the intestinal villi 10 minutes after a single dose. Firstly it was difficult to identify dilated Golgi stacks with lipid particles, which are typical of the control ultrastructure. In contrast, lipid accumulation within the membrane-bound organelles was noticeable not only in the Golgi-associated vacuoles but also in the dilated RER. JTK-303 appears to initially affect the process of lipid re-synthesis in the enterocyte. Secondarily medium-density lipid droplets, which were identical to those produced in the lamina propria, were formed in the enterocytes. Altered lipid synthesis seems to result in the formation of the unique lipid droplets in the cytoplasm. The lipid droplets in the enterocytes increased in size and number up to 1 hour post-dose, but completely disappeared by 24 hours after a single dose.

During the course of repeated oral dosing with JTK-303, formation and disappearance of the lipid droplets are likely to be repeated daily in the rat enterocytes without causing any enterocytic dysfunction. A lack of epithelial findings in the routine repeated dose toxicity studies strongly supported this hypothesis. The present study suggests that effects on the lipid synthesis in the enterocytes were initiated shortly after a dose of JTK-303 but were reversible in a short time.

Immediately after dosing, small-sized lipid droplets appeared in the enterocyte, and then the larger lipid droplets tended to be located along the basement membrane as the lipid droplets increased in number and size. As time passed, the lipid droplets were also observed in the lamina propria. Some of the lipid droplets are likely to be transferred from the cytoplasm and others are likely to be newly formed in the lamina propria.

Mitochondrial swelling was observed in the enterocytes immediately after dosing. However, the finding was transient and completely disappeared by up to 4 hours after dosing indicating that the finding was reversible. In the time sequence, the onset and disappearance of mitochondrial swelling were identical to those of lipid-related findings in the enterocytes in general. Based on the observation of one animal at 10 minutes post-dose, mitochondrial swelling appeared to be preceded by lipid droplet formation. The mitochondrial swelling was not accompanied by any findings indicative of mitochondrial damage, such as the increased formation of autolysosomes containing embranous/myelin figures through breakdown, degradation or sequestration of the ingested mitochondria. In addition, no histopathological findings were observed in the enterocytes after three months repeated dosing with JTK-303. Thus, the finding was not suggestive of mitochondrial toxicity leading to cell death, but an adaptive or reactive change secondary to the lipid-related findings. Formation of JTK-303-induced lipid droplets in the lamina propria was preceded by enterocytes with noticeable lipid accumulation within the membrane-bound organelles and lipid droplet formation. The findings in the enterocytes were initiated shortly after a single dose but were clearly reversible as they completely disappeared by 24 hours post-dose. It was confirmed that the findings were related to alteration of the lipid absorption process.

Impurities Toxicology

28-day Oral Gavage Bridging Study with GS-9137 in Female Rats (TOX-183-2010). The objectives of the study were to evaluate the possible toxic effects and to qualify potential impurities between different lots of GS-9137 when administered orally by gavage to female rats for a minimum of 28 days. GS-9137 in the vehicle, 0.5% methylcellulose in deionized water, was administered orally by gavage once daily for 28 consecutive days to 3 toxicology groups (Groups 2-4) and 3 toxicokinetic groups (Groups 2A-4A) of female Crl:CD(SD) rats. Dosage levels were 2,000 mg/kg/day of GS-9137-A, and 300 and 2,000 mg/kg/day of GS-9137-B. Concurrent control groups (Groups 1 and 1A) received the vehicle on a comparable regimen. The dosage volume was 10 mL/kg for all groups. Groups 1-4 each consisted of 10 animals. Group 1A consisted of 3 animals, and Groups 2A-4A each consisted of 9 animals. Following 4 weeks of dose administration, all groups were euthanized. Doses: GS-9137-A: 2,000 mg/kg/day (Lot no. 061240; 99.1% pure); GS-9137-B: 300 mg/kg/day (Lot no. 062091; 96.2% pure); GS-9137-B: 2,000 mg/kg/day (Lot no. 062091). All toxicology animals survived to the scheduled

necropsy. Beginning on study day 14, some rats from the 2,000 mg/kg/day GS-9137-A and GS-9137-B groups had clinical findings of light brown feces which persisted through the end of the study. There were no drug related effects on body weights and body weight gain or food consumption in this study. There was an increased percent and absolute eosinophil counts (2000 mg/kg/day GS-9137-B) and large unstained cells counts (300 mg/kg/day GS-9137-B). These group mean differences were not considered to be test article-related because the values did not show a dose-related response, involved percent cell counts, and/or were within reference ranges for absolute cell counts. The pH of urine in the females administered 300 mg/kg/day GS-9137-B was statistically significantly decreased compared to the control group. There was an increase in cecum weights at the scheduled necropsy. The changes are shown in Table 49.

Table 49. Cecum weights in rats

Organ	Direction and (magnitude) of change	Dosage Level(s) (mg/kg/day)	Histologic Correlate
Cecum with contents (absolute)	41%* Higher	2000-A	None
Cecum with contents (rel. to body weight.)	33%* Higher	2000-A	None
Cecum with contents (rel. to brain weight)	43%* Higher	2000-A	None
Cecum with contents (absolute)	37%* Higher	2000-B	None
Cecum with contents (rel. to body weight.)	32%* Higher	2000-B	None
Cecum with contents (rel. to brain weight)	38%* Higher	2000-B	None

* = significantly ($p<0.01$) different from the control group using Dunnett's test.

There were no other test article-related effects on organ weights. No other statistically significant differences were observed when the control and test article-treated groups were compared. Dark red areas in the thymus noted grossly in all control and test article-treated groups correlated with hemorrhage observed during microscopic examination. There were no histologic changes associated with administration of either formulation of GS-9137. The higher weights of cecum with contents noted in animals administered either 2000 mg/kg/day GS-9137-A or 2000 mg/kg/day GS-9137-B were not accompanied by any microscopic alterations. Toxicokinetics: summary of mean toxicokinetic parameters is shown in Table 50, 51 and 52. Plasma concentrations of GS-9137 and its metabolites GS-9200 and GS-9202 were monitored in Sprague Dawley rats after daily oral gavage administration of two lots of GS-9137 [GS-9137-A (lot number 061240) and GS-9137-B (lot number 062091)] for 28 days ((b) (4) Study No. (b) (4) 604001, Gilead Sciences, Inc. Study No. TX-183-2010). The dose of GS-9137-A was 2000 mg/kg/day and the doses of GS-9137-B were 300 and 2000 mg/kg/day. Toxicokinetic analysis was performed on Day 0 and Day 23.
GS-9137: On Day 0 the Cmax, AUC_{0-t} and Tmax values for GS-9137 were virtually identical (ratios of GS-9137-A/GS-9137-B = 1.00 to 1.07). On Day 23, the AUC_{0-t} and Tmax values were

virtually identical (ratios of GS-9137-A/GS-9137-B = 1.00 to 1.02); the Cmax ratio was 0.762.

Table 50. Day 0 and Day 23 Toxicokinetic Parameters for GS-9137 Following Once Daily Oral Gavage Administration of GS-9137-A and GS-9137-B to Female Rats for 28 Days

Group	Dose (mg/kg/day)	Day	Tmax (hr)	Cmax (ng/mL)	AUC0-t (ng·hr/mL)	t½ (hr)
2A	2000	0	1.00	43800	174000	2.65
3A	300	0	0.500	19100	47500	2.68
4A	2000	0	1.00	43700	163000	2.59
2A	2000	23	1.00	36500	173000	3.05
3A	300	23	0.500	19200	60000	31.9
4A	2000	23	1.00	47900	169000	2.88

GS-9200: on Day 0 the Cmax, AUC0-t, and Tmax values for GS-9200 were virtually identical (ratios of GS-9137-A/GS-9137-B = 1.00 to 1.11). On Day 23, the AUC0-t values were virtually identical (ratio = 1.06). The Cmax ratio was 0.676. The Tmax occurred at 1.00 (GS-9137-B) to 2.00 (GS-9137-A) hr.

Table 51. Day 0 and Day 23 Toxicokinetic Parameters for GS-9200 Following Once Daily Oral Gavage Administration of GS-9137-A and GS-9137-B to Female Rats for 28 Days

Group	Dose (mg/kg/day)	Day	Tmax (hr)	Cmax (ng/mL)	AUC0-t (ng·hr/mL)	t½ (hr)
2A	2000	0	1.00	3120	11800	40.7
3A	300	0	1.00	1620	4250	1.43
4A	2000	0	1.00	2960	10600	3.70
2A	2000	23	2.00	2600	12900	1.85
3A	300	23	1.00	1110	3120	8.02
4A	2000	23	1.00	3840	12200	1.16

GS-9202: there were limited plasma concentration data available for this metabolite; consequently, the toxicokinetic parameters should be interpreted with caution. On Day 0 and Day 23 the ratios of GS-9137-A/GS-9137-B for Cmax were 0.688 to 0.810 and for AUC0-t they were 0.280 to 0.545. It was difficult to compare the AUC0-t values since they were calculated over different periods of time. The Tmax occurred at 1.00 hr.

Table 52. Day 0 and Day 23 Toxicokinetic Parameters for GS-9202 Following Once Daily Oral Gavage Administration of GS-9137-A and GS-9137-B to Female Rats for 28 Days

Group	Dose (mg/kg/day)	Day	Tmax (hr)	Cmax (ng/mL)	AUC0-t (ng·hr/mL)	t½ (hr)
2A	2000	0	1.00	162	58.8	NC
4A	2000	0	1.00	200	210	NC
2A	2000	23	1.00	137	144	NC
4A	2000	23	1.00	199	264	2.80

The toxicokinetic portion of this study demonstrated overall dose-related exposure for GS-9137 and its metabolites GS-9200 and GS-9202 for female rats administered 300 and 2000 mg/kg of GS-9137 daily for 28 days in an oral toxicity study. In addition, the comparability of GS-9137-A (lot number 061240) and GS-9137-B (lot number 062091)

was demonstrated. The administration of GS-9137 orally (gavage) to Crl:CD(SD) rats for 28 days at the dosage level of 2,000 mg/kg/day resulted in increased weight of the cecum with contents and a clinical finding of light brown feces. Based on these results, the NOEL for GS-9137 was 300 mg/kg/day and the NOAEL was 2,000 mg/kg/day. Both GS-9137-A (Lot no. 061240) and GS-9137-B (Lot no. 062091) with various impurity levels had similar findings.

28-Day Oral Gavage Qualification Study with GS-9137 in Rats (TX-183-2023). The purpose of this study was to evaluate the potential toxicity and qualify the potential impurities by comparing a pure lot (GS-9137-A) to a lot spiked with impurities (GS-9137-B) administered daily via oral gavage to female rats for at least 28 days. Female Crl:CD(SD) rats were assigned to four toxicity groups (10 animals/group) and were administered vehicle control article [0.5% (w/v) methylcellulose] and 2000 mg/kg/day GS-9137-A or 300 and 2000 mg/kg/day GS-9137-B in a dose volume of 10 mL/kg. Blood was collected for toxicokinetic analysis from satellite groups of toxicokinetic animals (3 animals/vehicle control group and 9 animals/treated group) on Days 1 and 28. Assessment of toxicity was based on mortality, clinical signs, body weights, food consumption, and clinical and anatomic pathology. All animals survived to their scheduled sacrifice. No GS-9137-related effects on clinical signs, mean body weight, mean food consumption, or clinical pathology were observed for either lot. No macroscopic or microscopic findings were attributable to either lot of GS-9137. Exposure to GS-9137 increased with the increase in dose level from 300 to 2000 mg/kg/day GS-9137-B. Increases in Cmax and AUC_{0-t} were generally less than dose proportional between 300 and 2000 mg/kg/day GS-9137-B. Values for Cmax at 2000 mg/kg/day GS-9137-A and GS-9137-B were generally similar on Days 1 and 28. Values for AUC_{0-t} at 2000 mg/kg/day GS-9137-B were generally greater than 2-fold higher than values at 2000 mg/kg/day GS-9137-A on Days 1 and 28. No accumulation of GS-9137 was apparent after multiple dosing of either lot. Administration of GS-9137-A and GS-9137-B once daily by oral gavage to female rats for 29 days was well tolerated up to 2000 mg/kg/day, the highest dose administered. Although systemic exposure to 2000 mg/kg/day GS-9137-B was generally greater than 2-fold exposure to 2000 mg/kg/day GS-9137-A at both intervals (Days 1 and 28), overall findings indicated no appreciable difference in toxicity between the two lots of GS-9137 at 2000 mg/kg/day. The NOAEL was considered to be 2000 mg/kg/day for both GS-9137-A and GS-9137-B (Day 28 Cmax values of 77,400 and 70,667 ng/mL, respectively, and AUC_{0-t} values of 294,720 and 746,457 ng·hr/mL, respectively).

In Silico Evaluation of Potential Genotoxicity and Carcinogenicity for Intermediates and Impurities of GS-9137 (TX-183-2024). See Appendix C.

28-Day Oral Gavage Qualification Toxicity and Toxicokinetic Study with GS-9350/GS-9137 Tablets in Rats (TX-236-2002). The purpose of this study was to evaluate the potential toxicity and qualify potential impurities between different lots of GS-9350/GS-9137 tablets (degraded and nondegraded) administered daily via oral gavage to female rats for 28 days. Female Crl:CD(SD) rats were assigned to 5 toxicity groups (10/group) and administered vehicle [0.5% (w/v) sodium carboxymethylcellulose 10% (w/v) propylene glycol, prepared in 40 mM sodium acetate buffer, pH adjusted to 4 (± 0.1)], or nondegraded and degraded GS-9350/GS-9137 crushed tablets at30/30 and 50/50 mg/kg/day in a dose volume of 10 mL/kg. All animals survived to their scheduled sacrifice. No GS-9350/GS-9137-related effects on clinical signs, mean body weight, or mean food consumption were observed for nondegraded or degraded GS-

9350/GS-9137. No macroscopic or microscopic findings were attributed to nondegraded or degraded GS-9350/GS-9137. Clinical pathology changes were minor, similar with both lots of the test article (nondegraded and degraded), and not considered adverse. Hematologic changes were limited to a mild but statistically significant increase in platelet count in animals administered 50/50 mg/kg/day degraded GS-9350/GS-9137. Clinical chemistry changes included mild, but significantly increased globulin levels resulting in higher total protein and lower albumin-to-globulin ratio, and minimally increased cholesterol levels with both test article lots. Significantly increased mean absolute and relative (to brain and terminal body weight) liver weights were noted in all GS-9350/GS-9137-treated animals compared to control. Changes in absolute liver weight were similar for both lots. These differences were considered test article-related, were not associated with any histopathological changes, and not considered adverse. Exposure profiles for nondegraded and degraded lots of GS-9350 and GS-9137 were comparable at the corresponding dose levels on Days 1 and 28. Exposure to GS-9350 and GS-9137 increased with the increase in dose level, and increases in Cmax and AUC0-t were generally dose-proportional. No accumulation was observed for either test article after multiple dosing. Decreases in GS-9137 Cmax and AUC0-t values were observed on Day 28 compared with Day 1. Two lots of GS-9350/GS-9137 ground tablets (nondegraded and degraded) given once daily by oral gavage to female rats for 28 days were well tolerated at 30/30 and 50/50 mg/kg/day GS-9350/GS-9137. Exposure profiles for nondegraded and degraded lots of GS-9350 and GS-9137 were comparable at corresponding dose levels on Days 1 and 28. The NOAEL was 50/50 mg/kg/day GS-9350/GS-9137 for both nondegraded and degraded tablets. At this dose, exposures on Day 28 were: GS-9350 Cmax: 4920 and 4837 ng/mL, AUC_{0-t}: 35884 and 25832 ng•hr/mL; GS-9137 Cmax: 15367 and 10343 ng/mL, AUC_{0-t}: 44406 and 40584 ng•hr/mL for nondegraded and degraded tablets, respectively.

Local Tolerance Studies

GS-9137: The bovine corneal opacity and permeability assay (TX-183-2021). The Bovine Corneal Opacity and Permeability Assay (BCOP) was performed to assess the in vitro ocular irritancy potential of GS-9137. Imidazole was tested in parallel as a positive control. The assay uses isolated bovine corneas as a means of assessing the ocular corrosivity or severe irritancy potential of test substances in vitro. The isolated corneas were obtained as a by-product of the meat production industry. Two endpoints, corneal opacity and permeability, were measured and combined to give an In Vitro Irritancy Score which can be used to classify and rank test substances as potential eye irritants according to a guideline. GS-9137 (20% w/w) elicited an In Vitro Irritancy Score of 6.1 ± 2.0 and is predicted to be a non-corrosive/non-severe eye irritant. The In Vitro Irritancy Score of imidazole (positive control) was 161.8 ± 25.5, thereby confirming the ability of the assay to detect corrosive/severe irritants.

GS-9137: Skin Irritation to the Rabbit (TX-183-2020). A study was performed to assess the skin irritation potential of GS-9137 to the rabbit. Three female rabbits received a single four hour, semi-occlusive, dermal administration of approximately 0.5 g of GS-9137 as supplied and were observed for four days. No dermal reaction was observed in any animal throughout the duration of the study. The means of scores for these reactions at approximately 24, 48 and 72 hours after administration, calculated separately for each animal, are summarized below:

Means of scores at approximately 24, 48 and 72 hours		
Animal number	**Erythema**	**Oedema**
11	0.0	0.0
130	0.0	0.0
132	0.0	0.0
EC trigger values*	≥2.3	≥2.3

*Classification according to regulation (EC) 1272/2008 is triggered if means of scores for either effect are ≥ 2.3 for two or three animals (or if effects persist to Day 14 in at least two animals).

The Primary Irritation Index was calculated to be 0.0; GS-9137 was classified as 'non-irritant.'

Immunotoxicity Studies

Four week oral gavage immunotoxicity study in rats (TX-011). To evaluate the immunotoxicity potential of EVG, Crj:CD(SD)IGS rats (10/sex/group) were orally administered EVG at 0 (vehicle: 0.5% MC), 300, 1000, and 2000 mg/kg/day for a period of 4 weeks in a GLP-compliant study. Assessment of toxicity was based on mortality, body weight, selected organ weights, effects on the production of sheep red blood cell (SRBC)-specific antibodies, and the evaluation of splenic lymphocyte subsets. For animals used in the evaluation of antibody production, SRBC were administered intravenously to immunize the animals 5 days prior to the last dose. Twenty-four hours after the last dose, serum was collected from these animals and the titers of SRBC-specific antibodies were measured by enzyme-linked immunosorbent assay (ELISA). In animals where splenic lymphocyte subsets were evaluated, splenic cellularity was determined using a hemocytometer, and the ratio and absolute numbers of the lymphocyte subsets were evaluated using flow cytometry. There were no preterminal mortalities, treatment-related clinical signs, and no treatment-related changes in body weights, body weight gains, absolute and relative weights of the thymus, spleen or adrenals, anti-SRBC antibody titers or splenic cellularity. The changes in the ratios of each lymphocyte subset were generally slight and there were no treatment-related changes in the absolute cell numbers for each subset except for a decrease in absolute CD45RA+ cells in the 1000 mg/kg/day males. As there were no similar decreases in absolute CD45RA+ cells in the high dose animals, and there were no treatment-related effects on humoral immune function, the effects on the splenic lymphocyte subsets were of no toxicological significance. In conclusion, EVG was not considered immunotoxic at doses up to 2000 mg/kg/day.

Other Toxicity Studies

Single Intravenous Dose Toxicity Study of JTK-303 in Dogs (TX-008). In a preliminary single oral dose toxicokinetic study of JTK-303 in dogs dose levels: 30, 100 and 300 mg/kg, suspension in corn oil), vomiting was notable for up to 8 hours after dosing in almost all the animals at 100 and 300 mg/kg. The Cmax values of the parent drug in plasma in these groups were 8.77 to 18.05 μg/mL.

In the present study, in order to determine whether the above-mentioned vomiting was caused by the direct effect of JTK-303 on the digestive tract, a single dose of JTK-303 was administered intravenously to 2 male beagle dogs at a dose level of 20 mg/kg. Observation of the clinical signs and determination of the plasma concentrations of the parent drug were performed. After dosing, vomiting did not occur and the Cmin values of the parent drug in plasma (individual values: 16.02 and 27.47 μg/mL) were comparable to or higher than the Cmax values after the oral dosing of JTK-303 at 100 and 300 mg/kg. In summary, vomiting was not observed following the intravenous dosing of JTK-303, although the plasma concentrations of the parent drug were higher than those obtained after the oral dosing in which vomiting was observed. Therefore, the vomiting observed after the oral dosing of JTK-303 was considered to be caused by the direct effect of JTK-303 on the digestive tract.

Study of Synergistic Effects of JTK-303 and Nonsteroidal Anti-Inflammatory Drugs (NSAID) in Mice (JTK303-TX-009). The induction of convulsions by synergistic drug interaction of JTK-303 and nonsteroidal anti-inflammatory drugs (NSAID) was examined by coadministration of JTK-303 and fenbufen to male ddY mice. Enoxacin was used for the positive control. Doses of 2000 mg/kg JTK-303, 200 mg/kg enoxacin and 400 mg/kg fenbufen were administered. The vehicle used was 0.5 % (w/v) methylcellulose solution. No convulsions or animal deaths occurred with the coadministration of JTK-303 and fenbufen. Moreover, no convulsions or animal deaths occurred with the coadministration of vehicle and fenbufen, vehicle and JTK-303, or vehicle and enoxacin. The concentration of the parent drug in plasma after a single oral dose of 2000 mg/kg JTK-303 reached its peak value at 1 hour after dosing, the Cmax value was 94.99 μg/mL and the AUC0-8hr value was 505.95 μg•hr/mL. Positive controls: clonic convulsions were observed in all 8 mice coadministered enoxacin and fenbufen up to 1 hour after dosing. Seven of these mice died after tonic convulsions were observed. In addition, the surviving mouse also died within 2 hours after dosing, therefore all 8 mice died.

Therefore, it is considered that under the conditions of this study the mice were exposed to an adequate amount of JTK-303 when a dose of 2000 mg/kg of JTK-303 was administered orally and that coadministration with nonsteroidal anti-inflammatory drugs such as fenbufen does not induce convulsions.

GS-9137: Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (Individual animal approach) (TX-183-2022). This study was performed to assess the skin sensitization potential of GS-9137 using the local lymph node assay (LLNA). Preliminary investigations were performed at 25 and 50% w/v with 2 mice per concentration to establish the highest concentration of test substance, which did not lead to systemic toxicity or excessive local irritation. The main study consisted of three treated groups, each comprising five female mice receiving GS-9137 at concentrations of 10, 25 or 50% w/v. Similarly constituted groups received the vehicle, dimethylformamide (DMF), or positive control substance, 25% v/v hexyl cinnamic aldehyde (HCA). The mice were treated by daily application of 25 μL of the appropriate concentration of GS-9137or control (vehicle or positive) to the dorsal surface of both ears for three consecutive days. The proliferative response of the lymph node cells (LNC) from the draining auricular lymph nodes was assessed five days following the initial application, by measurement of the incorporation of ^{3}H-methyl Thymidine (HMT) by β-scintillation counting of LNC suspensions. The response was expressed as radioactive disintegrations per minute per

lymph node (dpm/node) and as the ratio of HMT incorporation into LNC of test nodes relative to that recorded for control nodes (test/control ratio), termed as Stimulation Index (SI). The test substance is regarded as a sensitizer if at least one concentration of the chemical has a SI of three or more. All animals survived to the terminal sacrifice. No GS-9137-related adverse clinical observations, dermal reactions, ear thickness or body weight changes were noted during the preliminary or main phases of the study. The SI obtained for 10, 25 and 50% w/v were 1.0, 0.7 and 1.0, respectively, which indicates that GS-9137 did not show the potential to induce skin sensitization. The SI for the positive control substance HCA was 8.8, which demonstrates the validity of this study. GS-9137 is not regarded to have the potential to cause skin sensitization at the levels administered in this study.

Single Oral Dose Phototoxicity Study of JTK-303 in Mice (JTK-303-TX-010). An oral dose of 2000 mg/kg of JTK-303 was administered to male ICR mice, and the mice were then irradiated with long-wavelength UV light (dose: 21.6 J/cm2) for 4 hours to investigate the phototoxic potential of JTK-303. As a positive control, mice were similarly treated with a dose of 200 mg/kg enoxacin (ENX) in 0.5 % (w/v) methylcellulose solution. No erythema of the ear and no change in the thickness of the ear (which was measured as an indicator of edema) were found in the JTK-303 experimental group, the vehicle control group irradiated with UV light, or in the ENX group not irradiated with UV light. On the other hand, in all 6 positive control animals treated with ENX and irradiated with UV light, erythema was present and ear thickness was increased by about 50%. The parent drug concentrations in the plasma reached peak value at 0.5 hours after dosing, the C_{max} value was 54.22 μg/mL and the $AUC_{0\text{-}8hr}$ value was 260.20 μg•hr/mL. Moreover, the parent drug concentration at 4 hours after dosing, which was the end of the irradiation period, persisted at 72% of C_{max} (38.90 μg/mL). From the above results, we concluded that an oral dose of 2000 mg/kg of JTK-303 gave a sufficient exposure dose in mice and that JTK-303 was not phototoxic under the conditions of this test.

11 Integrated Summary and Safety Evaluation

Elvitegravir, a new chemical entity that belongs to the class of HIV-1 integrase strand-transfer inhibitors, is being evaluated as a component of the E/C/F/T (Elvitegravir 150 mg/Cobicistat 150 mg/Emtricitabine 200 mg/Tenofovir DF 300 mg Tablet) for the treatment of HIV-1 infection.

Elvitegravir demonstrated no significant effect on vital organ systems in rats and dogs in the safety pharmacology studies. In the central nervous, renal/urinary and GI systems, there were no adverse effects in rats at doses up to 2000 mg/kg. In cardiovascular and respiratory systems in conscious beagle dogs, EVG did not show any significant effects on blood pressure, heart rate, electrocardiogram, respiratory rate, or the degree of oxygen saturation at doses up to 100 mg/kg. In a hERG assay, EVG had no effect on the tail current at concentrations up to 1 μM.

Elvitegravir showed modest bioavailability in rats and dogs. In rats, EVG was rapidly absorbed and widely distributed, although it was excluded from the central nervous system and eye. Binding to human plasma and purified human albumin was high. EVG was extensively metabolized by oxidation, glucuronidation and combinations of the two. The most abundant metabolites were common between mouse, rat, rabbit, dog and human. The predominant metabolite was GS-9202 (M1), with lesser amounts of GS-9200 (M4) and M7 (JTP-74488, a

glucuronide of M1). Following administration of [^{14}C]-EVG in a human mass balance study, 94.8% of the dose was recovered in feces, consistent with the hepatobiliary excretion of EVG; 6.7% of the administered dose was recovered in urine, primarily as glucuronide metabolites, with no unchanged EVG observed. The potential for enterohepatic recirculation was low. In rats, low levels of EVG, but not its metabolites, were detectable in milk.

In single- or repeat-dose nonclinical studies with EVG, no clinically-relevant target-organ toxicity was observed. However, two non-adverse findings, not considered relevant to clinical use, were observed in rats and dogs.

In rats, cecal weights and/or its contents were increased at doses ≥ 300 mg/kg/day, with dilatation of the cecum observed at ≥ 1000 mg/kg/day (3-, 6-month studies). In dogs, dilatation of the cecum was observed in males at 100 mg/kg/day only in the 4-week repeat-dose study. These observations were not accompanied by any histological changes in the cecum or GI adverse events. Similar changes in the cecum have been reported with antibacterial quinolones which affect the GI microflora. Elvitegravir has a quinolone moiety and was confirmed to have antibacterial activity in the reverse mutation assay (23.4 μg/plate). Although the activity was much weaker than that of the antibacterial quinolones, the changes in the cecum were considered to be due to the antibacterial activity of high local concentrations of EVG in the GI tract.

Lipid-like vacuoles were observed in the lamina propria in the upper small intestine (duodenum and/or jejunum) in rats, with increased incidence and severity at doses ≥ 1000 mg/kg/day. The incidence and severity did not increase with long term dosing, and there was no evidence of toxicity or any adverse tissue reactions associated with these vacuoles. The cause of the vacuolization was considered related to the high local EVG concentrations to which the GI epithelium was exposed. In a series of mechanistic studies (2-week repeated oral dose toxicity study in rats with a 1-, 2-, and 8-week recovery period), the vacuoles were shown to contain mainly triglycerides, tended to disappear slowly after withdrawal of treatment with EVG, and may be related to the lipid absorption process although there were no changes in plasma lipid parameters or adverse clinical observations. In dogs, lipid vacuoles containing mainly triglycerides were observed in the upper small intestinal lamina propria in both sexes at doses of 30 (mid) and 100 mg/kg/day (high) in the 39-week dog study. Similar to rats, these observations were also not accompanied by any GI adverse events or histological changes in the cecum and the small intestines, and they were not considered adverse. Furthermore, in the 2-year rat carcinogenicity study, there were no notable findings in the upper small intestine, further suggesting that the presence of the vacuoles was not adverse.

The NOAELs for EVG were determined to be 2000 mg/kg/day in mice and rats, and 100 mg/kg/day for dogs. The exposures based on plasma AUC values at the NOAELs in the animals were approximately 2- to 3-fold (mice), 20- to 36-fold (rats), and 2- to 3-fold (dogs) higher than the AUC in patients treated once daily with EVG at 150 mg as part of the E/C/F/T.

Estimated safety margins (Table 53) were calculated based on exposure after repeat dosing (AUC_{0-t}) from the 13-week mouse, 6-month rat, and 9-month dog studies with EVG, as well as exposure to EVG when administered with COBI or with ritonavir in rats. Calculations of the safety margins are based on a human AUCtau value of 23 μg•h/mL following administration

of 150 mg EVG as part of the E/C/F/T for the treatment of HIV-1 infection. While the margin of safety was approximately 2-fold in the 13-week mouse study, in the 2-year mouse carcinogenicity study (EVG alone or in combination with ritonavir) no adverse effects were noted at an exposure margin of approximately 14-fold. Elvitegravir exposure in the chronic toxicity studies (26-week rat and 39-week dog) exceeded the exposure at the clinical dose.

Table 53. Estimated Safety Margins for EVG 150 mg as Part of the E/C/F/T Based on Exposure (AUC) at Animal NOAELs

Species Gender	Study Type	NOAEL Dose (mg/kg/day)	AUC_{0-t} (µg•h/mL)	Safety Margin[a]
Mouse				
Male - Female	13-week Toxicity	2000	44 - 59	1.9 – 2.6 X
Rat				
Male - Female	26-week Toxicity	2000	460 - 836	20 – 36 X
Rat + 10 mg/kg RTV				
Male - Female	13-week Combination Toxicity	1000	140 - 167	6.1 – 7.3 X
Rat + 30 mg/kg COBI				
Male - Female	13-week Combination Toxicity	1000	183 - 201	8.0 – 8.7 X
Dog				
Male - Female	39-week Toxicity	100	54 - 66	2.3 – 2.9 X

COBI, cobicistat; EVG, elvitegravir; NOAEL, no observed adverse effect level; RTV, ritonavir
a = Human AUCtau 23 µg•h/mL

Elvitegravir induced a slight increase in the number of cells with chromosomal structural aberrations at levels greater than or equal to 55 µg/mL in Chinese Hamster Lung cells when tested in the 6-hour treatment without metabolic (S9) activation. However, no evidence of chromosomal aberrations was observed after 24-hour treatment without S9 up to 45 µg/mL, or in the presence of S9 up to 175 µg/mL. EVG may have a weak potential to induce chromosomal aberrations. No mutagenic activity of EVG was detected in the bacterial reverse mutation test (Ames) using 4 different strains of *Salmonella typhimurium* and in 1 strain of *Escherichia coli*. The highest dose (750 µg/mL) of EVG that could be tested was limited by the growth inhibition (antibacterial) effects of EVG on the tester strains. Elvitegravir exhibited antibacterial activity at 23.4 µg/plate and above depending on the strain and test conditions. Elvitegravir did not show any significant increases in the incidence of micronucleated immature erythrocytes or any significant decreases in the proportion of immature erythrocytes. The systemic exposure to the parent drug (C_{max}) on Day 1 was 43.5 µg/mL in males and 68.3 µg /mL in females dosed at 2000 mg/kg.

In the 2-year carcinogenicity studies, there was no increase in EVG-related tumor incidence in mice at doses up to 2000 mg/kg/day (2.4- to 3.8-fold the human systemic exposure at the therapeutic dose of 150 mg/day). Similarly in rats at doses up to 2000 mg/day/day (12- to 27-fold the human systemic exposure at the therapeutic dose), no EVG-related increase in tumor

incidence was found. In the mouse study, high-dose EVG (2000 mg/kg/day) was also dosed in combination with ritonavir (25 mg/kg/day). No drug-related increases in tumor incidence were noted in these animals at exposures approximately 14-fold the human systemic exposure at the therapeutic EVG dose.

Table 54. Estimated Safety Margins for EVG 150 mg Based on Exposure (AUC) at the High Dose in the Carcinogenicity Studies.

Species Gender	Study Type	High Dose (mg/kg/day)	AUC_{0-t} (µg*hr/ml)	Safety Margin[a]
Mouse				
Male	2-Year Carcinogenicity	2000	54.2	2.4
Female		2000	86.6	3.8
Mouse	2-Year Carcinogenicity			
Male	EVG +	2000+25	318	13.8
Female	ritonavir	(ritonavir)	333	14.5
Rat				
Male	88-90 Week Carcinogenicity	2000	285	12.4
Female		2000	617	26.8

a = Human AUCtau 23 µg•h/mL

There were no EVG-related adverse effects observed in fertility studies in male and female rats. The NOAEL for reproductive parameters in the fertility studies was 2000 mg/kg/day in rats at exposures approximately 16- to 30-fold higher than human therapeutic exposure. In the embryo-fetal development studies in rats, there were no effects on Caesarean-sectioning, litter parameters, gross external, soft tissue or skeletal fetal alterations (malformations or variations) at dose levels up to 2000 mg/kg/day. The developmental NOAEL for EVG was 2000 mg/kg/day at exposures 23-fold higher than human therapeutic exposure. In a combination embryo-fetal development study with EVG and ritonavir, the NOAELs were 10 mg/kg/day for ritonavir and 1000 mg/kg/day EVG when administered separately or in combination at exposures approximately 8-fold higher EVG than therapeutic exposure. In rabbits, the maternal NOAEL of EVG was 50 mg/kg/day (low). The 150 and 450 mg/kg/day dosages were associated with reduced body weight gains and feed consumption during the post-dosage period. There were no adverse effects on embryo-fetal development and the developmental NOAEL was 450 mg/kg/day at exposures 0.2-fold higher than human therapeutic exposure. In the developmental and perinatal/postnatal reproductive toxicology studies, including postnatal behavioral/functional and juvenile toxicity evaluation in rats, there were no adverse effects at dosages up to 2000 mg/kg/day. The maternal NOAEL for general toxicity of EVG and the NOAEL for reproduction in the dams and viability and growth in the offspring were 2000 mg/kg/day at exposures 18-fold higher than human therapeutic exposures. In the juvenile toxicity evaluation portion of the study, the drug-related observation was increased cecum weights at 2000 mg/kg/day (high) for male rats and at 1000 (mid) and 2000 mg/kg/day (high) for female rats. There were no histopathological correlates for this finding in the rat. The NOAEL for toxicity of EVG was 2000 mg/kg/day for juvenile animals at exposures 7-fold higher than human therapeutic exposures.

Elvitegravir was secreted in the milk of nursing rats in the pre/postnatal study. At the 2000 mg/kg/day dose level, the EVG milk: plasma ratio was 0.1.

Table 55. Estimated Safety Margins for EVG 150 mg Based on Exposure (AUC) at NOAELs in the Reproductive Toxicology Studies

Reproductive Toxicology Study	NOAEL (mg/kg/day)	AUC_{0-t} (µg*hr/ml)	Safety Margin[a]
Fertility and General Reproduction in Rat			
Male	2000	379	16.5
Female	2000	695	30
Embryo-Fetal Development in Rat			
Maternal	2000	535	23.3
Fetal	2000		
Embryo-Fetal Development in Combination with Ritonavir in Rat			
Maternal	1000	181	8
Fetal	1000		
Embryo-Fetal Developmental in Rabbit			
Maternal	50	1.45	0.06
Fetal	450	4.27	0.2
Perinatal Postnatal Toxicity in Rat			
Maternal General Toxicity	2000	408	18
Reproduction in Dams	2000		
Juvenile Toxicity:			
Male	2000	134	5.8
Female	2000	194	8.4

a = Human AUCtau 23 µg•h/mL

Elvitegravir was neither irritating to skin or eyes, nor showed potential for phototoxicity. The immunotoxicity of EVG was evaluated in a 28-day study in rats at doses up to 1000 mg/kg/day and was not found to be immunotoxic in the rats. In a local lymph node assay in mice, EVG did not show potential to induce skin sensitization.

The most prominent metabolites of EVG were similar across rat, mice, rabbit and humans. The most prominent, GS-9200 and GS-9202 were studied in repeat dose mouse and rat studies, including the pre-/postnatal developmental toxicity and juvenile toxicity studies. Over 18 impurities and degradation products related to EVG have been identified in batches of the active pharmaceutical ingredient or drug product. Based on impurity profiles, the multiple GLP batches of EVG tested in the toxicology program were considered to be representative of the GMP material and support the specified limits of impurities proposed for commercial production.

The overall nonclinical development program with EVG has not identified any specific target organ toxicities or cause for concern.

12 Attachments: Exec CAC Minutes dated February 7, 2012

Executive CAC

Date of Meeting: February 07, 2012

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Anne Pilaro, Ph.D., DHOT, Alternate Member
Peyton (Laine) Myers, DAVP, Pharm/Tox Acting Team Leader
Pritam Verma, Ph.D., DAVP, Presenting Reviewer

Author of Minutes: Pritam Verma, Ph.D.

The following brief summary reflects the Committee discussion and its recommendations. Detailed study information can be found in Dr. Verma's review.

NDA #: 203-100

Drug Name: Elvitegravir (GS-9137)

Sponsor: Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404

Background: Elvitegravir (GS-9137) is a new chemical entity that belongs to a new class of HIV-1 integrase strand-transfer inhibitors that prevent integration of HIV-1 genetic material into the host-cell genome. The integration of the viral genome into the host cell genome is an essential step of the HIV replication cycle and is catalyzed by the viral integrase.

Rat Carcinogenicity Study: The oncogenicity potential of GS-9137 was investigated in rats/Crl:CD(SD) with oral gavage doses of 100, 300 and 2000 mg/kg/day in comparison with a vehicle control (0.5% methylcellulose) for a period of 88 and 90 weeks in males and females, respectively. The protocol was approved by the Exec CAC. However, the studies were terminated early because of survival issues in the vehicle controls.

No drug-related neoplasms were seen in the rat.

Mouse Carcinogenicity Study: The oncogenicity potential of GS-9137 was investigated in male and female mice/ Crl:CD1(ICR) at oral gavage dose levels of 0 (0.5% methylcellulose), 200, 600, and 2000 mg/kg/day GS-9137 and 0 [ethanol:water:propylene glycol (43:15:42)], 25 mg/kg/day ritonavir, and 2000 mg/kg/day GS-9137 in combination with 25 mg/kg/day ritonavir for a period of 104 weeks. The protocol was approved by the

Exec CAC.

No drug-related neoplasms were seen either with GS-9137 alone or in combination with ritonavir in the mouse.

Executive CAC Recommendations and Conclusions:

Rat:

The committee considered the carcinogenicity study in the rat to be acceptable despite the suboptimal duration. The Committee concurred that there were no drug-related neoplasms in the rat.

Mouse:

The Committee concurred that there were no drug-related neoplasms in the mouse.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\
/Division File, DAVP
/HGhantous, DAVP
/PVerma, DAVP
/SMin, DAVP
/ASeifried, OND IO

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	203-100 (Appendix B)
Supporting document/s:	1
Applicant's letter date:	10/26/2011
CDER stamp date:	10/27/2011
Product:	Elvitegravir 150 mg/Cobicistat 150 mg/Emtricitabine 200 mg/Tenofovir DF 300 mg Tablet (E/C/F/T) Appendix B: Cobicstat 150 mg
Indication:	Pharmcoenhancer
Applicant:	Gilead Sciences, Inc. 333 Lakeside Drive Foster City, CA 94404
Review Division:	Division of Antiviral Products
Reviewer:	L. Peyton Myers, PhD
Supervisor/Team Leader:	Hanan Ghantous, PhD, DABT
Division Director:	Debra Birnkrant, MD
Project Manager:	Stacey Min, PharmD

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 203-100 are owned by Gilead Sciences, Inc. or are data for which Gilead Sciences, Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 203-100 that Gilead Sciences, Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 203-100.

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1 Executive Summary

1.1 Introduction

Cobicistat (COBI, GS-9350) is a new chemical entity that belongs to the class of pharmcoenhancers. COBI inhibits human CYP3A4 with high affinity and is planned to be used with other anti-HIV drug products that are CYP3A4 substrates, in order to increase the exposure of those drug products. COBI itself has no antiviral activity.

This NDA is submitted for a single tablet regimen that contains a fixed-dose combination of elvitegravir 150 mg (EVG), cobicistat 150 mg (COBI), emtricitabine 200 mg (FTC) and tenofovir 300 mg (TDF) or E/C/F/T, for the treatment of HIV-1 infection. Thus, COBI 150 mg as one of the components of the E/C/F/T, is being evaluated in this review.

1.2 Brief Discussion of Nonclinical Findings

All nonclinical studies required to support chronic use have been performed and submitted as a part of the nonclinical assessment for COBI. The only exception is the carcinogenicity studies. The Division agreed with the Sponsor that these studies can be submitted post-marketing.

No significant effects were noted that would preclude approval of COBI as a pharmacoenhancer in an HIV combination drug product.

The most concerning safety pharmacological effects with COBI were limited to the cardiovascular system. In *in vitro* studies, COBI inhibited hERG potassium current (IC_{50} 1.8 μM) and the hCav1.2 L-type calcium channel (IC_{50} 6 μM). In further *in vitro* characterization in rabbit hearts, COBI caused negative inotropic effects and shortening of the APD at ≥ 1 μM in rabbit Purkinje fibers. COBI also produced negative inotropic effects (PR interval prolongation, and produced decreases in left ventricular [LV] function) at concentrations ≥ 1.5 μM in a follow up study in rabbit hearts. In *in vivo* studies in beagle dogs, COBI showed potential cardiotoxicity. Mild prolongation in PR intervals were noted primarily from 1 to 6 hours postdose, but it was within upper limits of normal. The Sponsor evaluated the potential effects of COBI on ECG parameters in humans due to the safety signal in the nonclinical studies. At 250 mg dose and higher, there were no effects on QT interval, but there was evidence of PR interval prolongation. Five subjects dosed 400 mg and 2 subjects dosed 250 mg of COBI had asymptomatic absolute PR>200 ms post-baseline. However, at the 150 mg dose (the proposed clinical dose), there were no cardiac effects noted. COBI had limited central nervous system effects in rats at 150 mg/kg and higher (increased salivation, decreases in arousal, decreases in locomotor and motor activities, and decreases in body temperature). None of these effects were significantly noted at the clinical dose in humans.

COBI was highly protein bound (98-99%) and was widely distributed with most of the drug in the GI and lesser amounts in the liver, adrenal, kidney, and pituitary. After oral dosing, bioavailability was low or low/moderate, likely due to high first-pass elimination. COBI had multiple metabolites (>50 to >80) in rats, mice, and dogs as well as in human hepatic microsomal fractions. All species tested had the major human metabolites (M31, M26, M21,

M39). The parent drug as well as the M31 and M21 metabolites were excreted in the feces (79-89%) in all species tested. In rats and dogs, roughly 63-69% of the drug was recovered in the bile. Less than 2% of the drug was recovered in the urine.

COBI has multiple predicted drug-drug interactions based on inhibition of CYP3A enzymes as well as inhibition of several renal transporters. COBI slightly induced CYP3A in the rat, whereas in the dog it caused inhibition. However, in humans, it appears that CYP3A is inhibited, which supports the primary mechanism of action of CYP3A. A clinical concern for COBI was the inhibition of renal transporters (mainly MATE1) which may increase serum creatinine (without affecting aGFR) as well as inhibition of p-gp may increase tenofovir renal exposure in humans. The Sponsor plans to evaluate the effects of COBI on tenofovir in humans to address these concerns. The Agency may request additional studies to further evaluate a potential COBI/TDF interaction.

Toxicology was assessed in rodents (mice and rats) as well as in dogs. In single dose studies in rats and mice, COBI was well tolerated in rats up to 500 mg/kg. Mice, however did not tolerate COBI and were euthanized moribund at 300 mg/kg after a single dose. The maximum tolerated single dose was 100 mg/kg in mice. In repeat dose toxicology studies, mice were dosed up to 13 weeks (NOAEL = 5 or 50 mg/kg in males and females, respectively). Dogs and rats were dosed up to 26 weeks (NOAEL = 30 mg/kg) and 39 weeks (NOAEL = 10 mg/kg), respectively. The target organ in all species was mainly the liver (rat, mouse, and dog) as well as the thyroid (rat). This was expected since CYP inhibition has been associated with compensatory mechanisms in the liver of exposed animals such as: microsomal enzyme induction, increases in liver weights, and minimal/mild hepatocellular hypertrophy. A notable finding which was found in all species and appeared to be dose dependent was a change in urinalysis parameters. Urinalysis changes were related to urine dilution from increased water consumption associated with salivation. Although the animals increased the water intake as well as increased urine output, it was not associated with adverse effects. These findings were also consistent with slight increases in polyuria noted in clinical trials.

COBI is not genotoxic as evaluated by the Ames assay, chromosomal aberration assay, as well as an *in vivo* rat micronucleus assay. Carcinogenicity studies were not submitted with this NDA. The Division agreed with the Sponsor that the Carcinogenicity studies would be submitted post-marketing. It should be noted that a single male rat at the high dose (100 mg/kg) had a thyroid follicular cell carcinoma after 26 weeks of dosing. Most of the animals at the 100 mg/kg dose had follicular cell hypertrophy in the thyroid and thyroid hormone changes. The thyroid changes were attributed to the adaptive changes in the liver caused by CYP inhibition. The thyroid and liver findings have been frequently reported in rodents exposed to microsomal enzyme inducers. No other carcinomas were noted in the other repeat dose toxicology studies.

A full reproductive toxicology panel was performed. No effects were noted on fertility or general reproduction with NOAELs corresponding to 3.9-fold (males) and 5.1-fold (females) exposures over the human therapeutic exposures. There were no effects no early embryonic development, embryo-fetal development (rats and rabbits), postnatal development or lactation. Maternal NOAELs corresponded to exposure margins of 1.7 (rats) and 4.3 (rabbits) over the human therapeutic exposures. COBI was excreted in the milk of lactating rats with milk to

plasma ratios ranging from 1.3 to 1.9. Juvenile F1 pups tolerated direct exposure to COBI up to 75 mg/kg with adaptive liver and thyroid changes noted, which were similar to adult findings. The NOAEL for maternal toxicity of COBI was 75 mg/kg/day (including reproduction, viability, growth and development). The NOAEL was also 75 mg/kg/day in juvenile animals at exposures ~2.5-fold higher than human therapeutic exposures.

The overall nonclinical program of COBI was considered adequate to support the safety of COBI as part of the E/C/F/T.

1.3 Recommendations

1.3.1 Approvability

There are no nonclinical pharmacology and toxicology issues which would preclude the approval of this NDA.

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

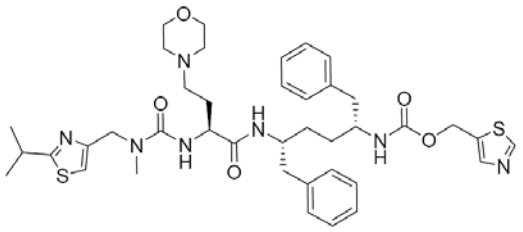
Labeling is included in the main E/C/F/T NDA.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional)

Generic Name
Cobicistat
Code Name
GS-9350
Chemical Name
5-Thiazolylmethyl [(1R,4R)-4-[[2-[[methyl[[(2S)-2-(1-methylethyl)-4-thiazolyl]methyl]carbamoyl]amino]-4-(4-morpholinyl)-1-oxobutyl]amino]-2,4-bis(phenylmethyl)butyl]carbamate
Molecular Formula/Molecular Weight
$C_{40}H_{53}N_7O_5S_2$ MW 776.0
Structure or Biochemical Description



Pharmacologic Class
Pharmacoenhancer

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND (b) (4)

2.3 Drug Formulation

The elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (EVG/COBI/FTC/TDF) tablet is an immediate-release tablet containing elvitegravir 150 mg (EVG), cobicistat 150 mg (COBI), emtricitabine 200 mg (FTC), and tenofovir disoproxil fumarate 300 mg (TDF, equivalent to 245 mg of tenofovir disoproxil).

Table 56- 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) (			USP, Ph. Eur.	
Film Coat				
(b) (			In-House	
			USP, Ph. Eur.	

2.4 Comments on Novel Excipients

No novel excipients of concern.

Silicone dioxide levels may be up to 180 mg/day. This exposure is slightly higher than the maximum of 170 mg listed in the IID. Although there is a report of clinical sarcoidosis associated with ingested colloidal silicon dioxide (Sola et al., 2009), this excipient is generally considered to be relatively non-toxic (WHO, 1974; FDA, 1979). Given the indication and lack of overt toxicity associated with silicon dioxide, the proposed levels are considered acceptable.

2.5 Comments on Impurities/Degradants of Concern

The proposed specifications for most impurities in the cobicistat drug substance were deemed acceptable based on repeat dose general toxicology studies, in silico evaluation of mutagenic potential, and the serious nature of the clinical indication. However, [redacted] were considered potentially mutagenic [redacted] In addition, [redacted] are known to be mutagenic in the Ames assay. Exposures to the expected or known mutagenic impurities will be controlled to appropriate levels as described in the FDA Draft Impurity Guidance.

For further information, please see Appendix C of the E/C/F/T review by Dr. Mark Powley.

2.6 Proposed Clinical Population and Dosing Regimen

Naïve HIV patients. Fixed dose combination once-a-day oral pill

2.7 Regulatory Background

EVG (Elvitegravir) and COBI components are new chemical entities. Emtricitabine and Tenofovir DF are approved products. Only COBI is discussed in Appendix B.

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacology

- AD-216-2028 – Inhibition of Human CYP3A Activity by GS-9350 *In Vitro*

Secondary Pharmacology

- PC-216-2003 – Cytotoxicity Profile of GS-9350
- PC-216-2004 – In vitro Effect of GS-9350 on Human Adipocytes

Safety Pharmacology

- TX-216-2006 – A Pharmacological Assessment of the Effect of GS-9350 on the Central Nervous System of the Albino Rat
- TX-216-2009 – Effects of GS-9350 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells
- TX-216-2015 – Effects of GS-9350 and GS-017415 on Cardiac Ion Channels Expressed in Human Embryonic Kidney Cells
- PC-216-2007 – Effect of GS-9350 on Isolated Rabbit Hearts
- PC-216-2009 – An Examination of the Cardiovascular Effects of GS-9350, Atazanavir and GS-9350 + Atazanavir on the Isolated Heart of the Female Rabbit (Langendorff Method)
- TX-216-2008 – A Pharmacological Assessment of the Effect of GS-9350 on the Cardiovascular System of the Beagle Dog Using Telemetry
- TX-216-2007 – A Pharmacological Assessment of the Effect of GS-9350 on the Respiratory System of the Albino Rat

PK/ADME Methods

- BA-216-2005 – Abbreviated Validation of a Method for the Determination of GS-9350 in Mouse Plasma by HPLC with MS/MS Detection

- BA-216-2010 – Abbreviated Validation of a Method for the Determination of GS-9612 in Mouse Plasma by HPLC with MS/MS Detection
- BA-216-2202 – Validation of a Method for the Determination of GS-9350 in Rat Plasma by HPLC with MS/MS Detection
- BA-216-2008 – Validation of a Method for the Determination of GS-9612 in Rat Plasma by HPLC with MS/MS Detection
- BA-216-2013 – Validation of a Method for the Determination of GS-9350 in Rat Milk by HPLC with MS/MS Detection
- BA-216-2004 – Abbreviated Validation of a Method for the Determination of GS-9350 in Rabbit Plasma by HPLC with MS/MS Detection
- BA-216-2003 – Abbreviated Validation of a Method for the Determination of GS-9350 in Dog Plasma by HPLC with MS/MS Detection
- BA-216-2009 – Abbreviated Validation of a Method for the Determination of GS-9612 in Dog Plasma by HPLC with MS/MS Detection
- BA-216-2006 – Validation of a Method for the Determination of Atazanavir in Rat Plasma by HPLC with MS/MS Detection
- BA-216-2007 – Quantitative Determination of GS-9137 and GS-9350 in Rat Plasma by LC/MS/MS

Absorption

- AD-216-2023 – Bi-directional Permeability of GS-9350 and Ritonavir in Caco-2 Cell Monolayers
- AD-216-2020 – Pharmacokinetics of GS-9350 in SD Rats
- AD-216-2021 – Pharmacokinetics of GS-9350 in Beagle Dogs
- AD-216-2022 – Pharmacokinetics of GS-9350 in Cynomolgus Monkeys
- PC-216-2013-PK – Determination of the Pharmacokinetics of GS-9350 Following a Single Oral gavage Dose to Male and Female 0001178-W (Wild-type) Mice
- AD-216-2042 – Pharmacokinetics of GS-9350 After Oral Doses in Various Formulations in Beagle Dogs
- AD-216-2061 – Pharmacokinetics of EVT, FTC, TDF, and GS-9350 after Oral dosing in Various Formulations in Beagle Dogs

Distribution

- AD-216-2076 – Plasma Protein Binding of GS-9350 in CD-1 Mice
- AD-216-2026 – Plasma Protein Binding of GS-9350
- AD-216-2034 – Pharmacokinetics, Distribution, Metabolism, and Excretion of ^{14}C-GS-9350 following Oral Administration to Rats
- AD-216-2060 – Whole Body Autoradiography (WBA) of Rats Following Oral Administration of ^{14}C-GS-9350

Metabolism

- AD-216-2074 – Identification of Major Metabolits of GS-9350 in CD-1 Mouse Microsomes *In Vitro*
- AD-216-2038 – Identification of Major Metabolits of GS-9350 *In Vitro*
- AD-216-2073 – Pharmacokinetics, Metabolism, and Excretion of ^{14}C-GS-9350 following Oral Administration to Mice

- AD-216-2082 – Metabolite Profiling and Identification of Rat Plasma, Bile, Urine, and Feces Following Oral Administratino to Mice
- AD-216-2101 – Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Intact and Bile Duct-Cannulated Dogs after Oral Administration of ^{14}C-GS-9350
- AD-216-2024 – In Vitro Metabolism of GS-9350 in Hepatocytes and Hepatic Subcellular Fractions from Rat, Dog, Monkey, and Human
- AD-216-2025 – Cytochrome P450 Phenotyping for GS-9350

Excretion

- AD-216-2073 – Pharmacokinetics, Metabolism, and Excretion of 14C-GS-9350 Following Oral Administration to Mice
- AD-216-2034 – Pharmacokinetics, Distribution, Metabolism, and Excretion of 14C-GS-9350 Following Oral Administration to Rats
- AD-216-2067 - AD-216-2067 Mass Balance of Radioactivity after Oral Administration of [14C] GS-9350 to Naïve Male Beagle Dogs
- AD-216-2068 – AD-216-2068 Mass Balance of Radioactivity after Oral Administration of [14C] GS-9350 to Naïve Male Bile Duct-Cannulated Beagle Dogs

PK and Drug Interactions

- AD-216-2072 – Inhibition of P-glycoprotein-dependent Bidirectional Transport of Digoxin Through Caco-2 Cell Monolayers by COBI
- AD-216-2104 – Inhibition of Breast Cancer Resistance Protein-Dependent Bidirectional Transport of Prazosin through Monolayers of Caco-2 Cells by COBI
- AD-216-2103 – Bidirectional Permeability of COBI Through Monolayers of P-glycoprotein and BCRP Overexpressing Cells
- AD-216-2028 – Human CYP3A Mechanism-Based Inhibition Potential of COBI In Vitro
- AD-216-2075 – In Vitro Assessment of Human UGT1A1 Inhibition Potential of COBI
- AD-216-2027 – Induction of Metabolizing Enzymes by COBI In Vitro
- AD-216-2071 – In Vitro Assessment of the Induction Potential of COBI in Primary Cultures of Human Hepatocytes
- AD-216-2039 – Induction of Rat Metabolizing Enzymes By COBI In Vitro
- AD-216-2030 – Interaction of COBI with Human MRP1, MRP2, and MDR1
- AD-216-2105 – In Vitro Interaction Studies of COBI and RTV With Human OAT1 and OAT3 Transporters
- AD-216-2099 – In Vitro Assessment of COBI and RTV Inhibition of Human Breast Cancer Resistance Protein
- AD-216-2093 – In Vitro Interaction Studies of COBI with Human OCT2 Uptake Transporter
- AD-216-2098 – In Vitro Interaction Studies of COBI and RTV With Human OCTN1 Transporter
- AD-216-2094 – In Vitro Interaction Studies of COBI with Human MATE1 and MATE2-K Efflux Transporters
- AD-216-2100 – In Vitro Assessment of COBI and RTV Inhibition of Human OATP1B1 and OATP1B3

Single Dose Toxicology

- TX-216-2003 – Single-Dose Oral Gavage Toxicity Study with GS-9350 in Rats
- PC-216-2013 – Collection of Samples for Determination of the Pharmacokinetics of GS-9350 Following a Single Oral Gavage Dose to Male and Female 001178-W (wild type) Mice

Repeat Dose Toxicology

- TX-216-2026 – 3-Month Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in CD-1 Mice
- TX-216-2025 – 2-Week Non-GLP Oral Gavage dose Range-Finding Toxicity and TK study of GS-9350 in CD-1 mice
- TX-216-2032 – 14-Day Oral Gavage Toxicity and Toxicokinetic Study of GS-9350 in Mice
- TX-216-2041 – 4-Week Dose Range-finding Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in CByB6F1-Tg(HRAS)2Jic (wild type) Mice
- TX-216-2027 / TX-216-2027-TK – Five Day Oral Toxicity and TK Study of GS-9350 and Atazanavir in Female SD Rats
- TX-216-2001 / TX-216-2001-TK – 14-Day Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 and GS-017415 in Rats
- TX-216-2004 – 4-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Rats with a 4-Week Recovery
- TX-216-2017 – 26-Week Oral Gavage Toxicity and TK Study with GS-9350 in Rats with a 13-Week Recovery Period.
- TX-216-2024 – 90-Day Oral Gavage Bridging Study with GS-9350 and Atazanavir in Rats with a 1-Month Recovery Period
- TX-216-2002 / TX-216-2002-TK – 7-Day Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 and GS-017415 in Dogs
- TX-216-2005 – 4-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Dogs with a 4-Week Recovery Phase
- TX-216-2016 – 39-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Dogs with a 13-Week Interim Necropsy and a 13-Week Recovery Period

Genotoxicity

- TX-216-2010 – GS-9350 *Salmonella-Escherichia Coli*/Mammalian-Microsome Reverse Mutation Assay
- TX-216-2011 – GS-9350 L5178Y+/- Mouse Lymphoma Forward Mutation Assay
- TX-216-2012 – GS-9350 Rat Micronucleus Test

Carcinogenicity

- Carcinogenicity studies were not submitted to the NDA.

Reproducive and Developmental Toxicity

- TX-216-2023 – Oral Gavage Study of Fertility and Early Embryonic Development to Implantation with GS-9350 in Rats
- TX-216-2018 – Oral Gavage Dose Range-Finding Developmental Toxicity and Toxicokinetic Study with GS-9350 in Rats
- TX-216-2020 – Oral Gavage Study for Effects on Embryo-fetal Development with GS-9350 in Rats
- TX-216-2019 – Oral Gavage Dose Range-finding Developmental Toxicity and Toxicokinetic Study with GS-9350 in Rabbits

- TX-216-2021 – Oral Gavage Study for Effects on Embryo-fetal Development and Toxicokinetics with GS-9350 in Rabbits
- TX-216-2033 – An Oral Pre and Postnatal Development Study, Including a 28-day Juvenile Toxicity Evaluation, of GS-9350 in the Rat

Local Tolerance

- TX-216-2044 – GS-9350: Skin Irritation to the Rabbit
- TX-216-2043 – GS-9350: The Bovine Corneal Opacity and Permeability Assay

Antigenicity and Immunotoxicity

- TX-216-2042 – GS-9350: Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (Individual animal approach)
- TX-216-2022 – 4-Week Oral Gavage T-Cell Dependent Antibody Assay with GS-9350 in Rats

3.2 Studies Not Reviewed

None. All submitted studies were reviewed.

3.3 Previous Reviews Referenced

None. Prior reviews were incorporated into the final review for this NDA.

4 Pharmacology

4.1 Primary Pharmacology

Study title:	Inhibition of Human CYP3A Activity by GS-9350 *In Vitro*
Study no.	AD-216-2028

Summary findings: The purpose of this study was to assess the potential for GS-9350 to inhibit the catalytic activity of human cytochromes P450 of the CYP3A family. GS-9350 was found to be a potent inhibitor of CYP3A (see table below). Inhibition was increased by preincubation with NADPH, suggesting that GS-9350 is a mechanism-based inhibitor of human CYP3A. GS-9350 showed similar potency to ritonavir, an approved clinical inhibitor of CYP3A activity.

Table 57 - Effects of GS-9350 and Ritonavir on Various Activities Catalyzed by Human Hepatic Microsomal CYP3A Enzymes

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

Table 58 - Human Hepatic Microsomal CYP3A Inactivation Kinetic Parameters for GS-9350 and Ritonavir

Parameter	Inhibitor	
	GS-9350	Ritonavir
K_I (μM)	1.07	0.26
k_{inact} (min^{-1})	0.47	0.23

Parameters were determined using midazolam 1'-hydroxylase as the CYP3A activity.

4.2 Secondary Pharmacology

Study title:	Cytotoxicity Profile of GS-9350
Study no.	PC-216-2003

Summary findings:
The cytotoxicity of GS-9350 was determined in standard *in vitro* assays in human lymphoid MT-2 and hepatoblastoma HepG2 cell lines, and compared with that of RTV. GS-9350 showed similar or lower cytotoxicity than RTV. GS-9350 had CC_{50} values of 88.6 and 44 μM, for MT-2 and HepG2, respectively. RTV had CC_{50} values of 37.6 and 64 μM, for MT-2 and HepG2, respectively. This indicated both a similarity between GS-9350 and RTV as well as a lack of significant cytotoxicity for GS-9350.

Study title:	In Vitro Effect of GS-9350 on Human Adipocytes
Study no.	PC-216-2004

Summary findings: Chronic treatment of HIV-infected patients with RTV is known to induce metabolic syndrome, which is believed to be at least in part linked to the demonstrated effects of RTV on adipocyte functions such as differentiation-associated lipid accumulation and insulin-stimulated glucose uptake. Therefore, the effects of GS-9350 on adipocyte functions were

evaluated using *in vitro* assays, and compared to RTV and atazanavir (ATV), a PI with minimal clinical metabolic adverse effects. Whereas RTV showed a strong effect both on lipid accumulation and insulin-stimulated glucose uptake in adipocytes, both GS-9350 and ATV exhibited no effect on lipid accumulation and a substantially less pronounced effect on glucose uptake at the tested concentrations. Taken together, the results of present studies suggest a lower potential of GS-9350 for the clinically relevant metabolism disorders when compared to RTV.

Table 59 - Effect of GS0-935- on Insulin-Stimulated Glucose Uptake

Compounds	Lipid Accumulation[a] EC_{50} ± SD (μM)	Glucose Uptake[a] % inhibition at 10 μM ± SD
GS-9350	> 30	9.5 ± 6.4
Atazanavir	> 30	0.4 ± 0.9
Ritonavir	16 ± 8	55 ± 10

a Data shown represent the mean and standard deviation from 3 independent experiments.

4.3 Safety Pharmacology

Study title:	A Pharmacological Assessment of the Effect of GS-9350 on the Central Nervous System of the Albino Rat
Study no.	TX-216-2006

Summary findings:
Female albino rats (b) (4) were evaluated for CNS effects of GS-9350 (50, 150 or 500 mg/kg) utilizing a FOB as well as motor activity evaluation at pre-dose, 0, 5, 2, and 6 hour post-treatment. No effects were noted at 50 mg/kg. At all time points in the higher doses (150 and 500 mg/kg), there were decreases in arousal incidence, locomotor activity, as well as motor activity. Motor activity was significantly decreased at 2 hours post-dose and did not resolve in the highest dose (500 mg/kg).

Furthermore, at 150 and 500 mg/kg, body temperature dropped 4 and 8%, respectively. In the mid-dose (150 mg/kg), the body temperature decrease was maximal at 2 hours post-dose and returned to baseline by 6 hours. The decrease in the highest dose (500 mg/kg) was maximal at the last time point (6 hours), and did not resolve. Moderate salivation was noted at 30 minutes in the 150 and 500 mg/kg groups.

There were signs of neurotoxicity (decreased body temperature, locomotor activity, motor activity, and arousal incidence) at 150 and 500 mg/kg in rats. The effects were most notable at 2 and 6 hours post-dose. The NOAEL for this study was 50 mg/kg for a single oral gavage of GS-9350.

Study title:	Effects of GS-9350 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells
Study no.	TX-216-2009

Summary findings: The objective of this study was to examine the in vitro effects of GS-9350 on the hERG channel current. GS-9350 inhibited hERG current by (mean ± SEM; n = 3) 3.2 ± 0.5% at 0.3 μM, 37.1 ± 2.2% at 1 μM, 64.2 ± 0.9% at 3 μM and 89.5 ± 0.6% at 10 μM versus 0.8 ± 0.2% in the vehicle control. HERG inhibition at all four concentrations was statistically significant ($P<0.05$) when compared to vehicle control values.

GS-9350 bound the hERG potassium channel at a low concentration (IC_{50} = 1.8 μM) indicating a possible signal for cardiotoxicity.

Study title:	Effects of GS-9350 and GS-017415 on Cardiac Ion Channels Expressed in Human Embryonic Kidney Cells
Study no.	TX-216-2015

Summary findings:

The objective of this study was to examine the in vitro effects of GS-9350 (cobicistat) and GS-017415 (ritonavir) on three human ion channels (potassium, sodium, calcium) that make major contributions to the generation, shape and duration of the human cardiac action potential.

GS-9350 strongly bound to the hERG potassium channel (IC_{50} = 1.85 μM) as well as exhibiting moderate binding to the hCaV1.2 L-type calcium channel (IC_{50} = 5.99 μM), and weak binding to the hNaV1.5 sodium channel channel (IC_{50} = 86.5 μM).

The strong binding of GS-9350 to the potassium channel and moderate binding to the calcium channel in vitro indicates that GS-9350 may interfere with cardiac function and cause cardiotoxicity in vivo.

Study title:	Effect of GS-9350 on Isolated Rabbit Hearts
Study no.	PC-216-2007

Summary findings:
The objective of this study was to evaluate the effects of GS-9350 on isolated rabbit hearts at concentrations of 0.3, 1, 3 and 10 μM. GS-9350 was associated with negative inotropic effects and shortening of the action potential duration on the isolated rabbit heart at concentrations ≥1 μM. At ≥3 μM, decreases in the QT interval, increases in the PR interval and the RR interval were noted. There were no notable effects on the QRS interval, triangulation and stability. There were no remarkable effects noted on hemodynamic, electrophysiologic and electrocardiographic parameters at a concentration of 0.3 μM GS-9350.

Study title:	An Examination of the Cardiovascular Effects of GS-9350, Atazanavir and GS-9350 + Atazanavir on the Isolated Heart of the Female Rabbit (Langendorff Method)
Study no.	PC-216-2009

Summary findings:
The isolated rabbit heart was utilized to determine the effects of GS-9350 alone (0.15, 0.45, 1.5, and 4.5 μM), atazanavir (ATV) alone (1.5, 4.5, 15, and 45 μM), as well as a combination of ATV (fixed, 1.5 μM) and escalating concentrations of GS-9350 (0.045, 0.15, 0.45, and 1.5 μM) on cardiac hemodynamic and electrophysiologic parameters.

All three regimens (GS-9350, ATV, and GS-9350 and ATV in combination) were associated with negative inotropic effects on the isolated rabbit heart. When 1.5 μM GS-9350 was co-administered with 1.5 μM ATV, effects on LV function were similar to the decreases noted at 1.5 μM GS-9350, alone. Decreases in heart rate and increases in the PR interval were noted with both compounds alone, and with the highest concentration of the combination (1.5 μM GS-9350/1.5 μM ATV). There were no notable effects of the combination on QRS, QT interval, monophasic action potential duration and triangulation, and there was no association with the early after depolarizations. There were no remarkable effects noted on hemodynamic, electrophysiologic and electrocardiographic parameters at concentrations of 0.45 μM GS-9350, 4.5 μM ATV, and with the combination at 0.45 μM GS-9350 plus 1.5 μM ATV.

Study title:	A Pharmacological Assessment of the Effect of GS-9350 on the Cardiovascular System of the Beagle Dog Using Telemetry
Study no.	TX-216-2008

Summary findings:
The purpose of this study was to evaluate the pharmacological effects of GS-9350 on hemodynamic and electrocardiographic parameters in the beagle dog *via* telemetry following an oral gavage administration.

In a dose escalation design, each of five male dogs received vehicle (95% PG, 5% EtOH (with 0.005M HCl)), 5, 15 and 45 mg/kg GS-9350 as a single oral gavage with a minimum of 2 days between each dose. The dosing was a crossover design (see below).

Table 60 - Cardiovascular Study in Dogs - Crossover Design

Animal Number	Dosing Schedule (0 (Vehicle), 5, 15 and 45 mg/kg of Test Article)			
	Dose 1	Dose 2	Dose 3	Dose 4
101	0	5	15	45
105	0	5	15	45
103	0	5	15	45
104 (Dose 1) 106 (Dose 2-3-4)	0	5	15	45

There were no deaths in this study. Emesis was observed in 3 out of 4 animals following doses of 15 and 45 mg/kg. For two animals (Animal Nos. 103 and 106), emesis was noted up to 5 and 8 times, respectively, and for up to 2 hours following dosing with 45 mg/kg.

There were no compound-related effects on any hemodynamic parameters, no qualitative waveform abnormalities and no effects on heart rate or QRS interval parameters.

Oral administration of GS-9350 caused an increase in the mean PR interval, predominantly following the high dose (45 mg/kg) and sporadically at the mid dose (15 mg/kg). The magnitude of the mean PR interval prolongation was mild. The PR interval increases were up to 12.2 msec compared to predose values, however, the range of absolute PR interval values was 91.8-99.6 msec which did not exceed the upper limits of normal for a dog (130 msec) at any timepoint. Oral administration of GS-9350 caused a mild increase in the mean QTc interval only following the high dose. The magnitude of the QTc interval prolongation was mild (<4%) and is unlikely to be biologically important. There was no effect of the low dose (5 mg/kg) on any quantitative ECG parameter.

Although there were mild PR increased and mild QTc prolongation at 45 mg/kg, the findings are likely not adverse. Therefore the NOAEL was the high dose of 45 mg/kg in dogs.

Study title:	A Pharmacological Assessment of the Effect of GS-9350 on the Respiratory System of the Albino Rat
Study no.	TX-216-2007

Summary findings:
The purpose of this study was to evaluate the pharmacological effects of GS-9350 on the respiratory system of the female albino rat after oral gavage of GS-9350.

Group/Identification	Dose Level (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Females
1/ Vehicle Control	0	0	10	6
2/ GS-9350	50	5	10	6
3/ GS-9350	150	15	10	6
4/ GS-9350	500	50	10	6

GS-9350 was administered at 50, 150, and 500 mg/kg (single oral gavage) in female albino rats. No effects on respiratory rate, tidal volume, or derive minute volume was noted. Rats did have erected fur, decreased activity and salivation at all doses. The NOAEL for respiratory effects after a single oral dose of GS-9350 was 500 mg/kg.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1.1 PK/ADME - Methods

Methods were validated to determine the levels of GS-9350 (COBI) in mouse plasma (BA-216-2005 and BA-216-2010), rat plasma (BA-216-2202 and BA-216-2008), rat milk (BA-216-2013), rabbit plasma (BA-216-2004), and dog plasma (BA-216-2003 and BA-216-2009). Validation methods were also peformed for atazanavir in rat plasma (BA-216-2006) as well as a combination of elvitegravir (GS-9137) and COBI (GS-9350) in rat plasma (BA-216-2007). The methods were valid. No issues were identified.

5.1.2 PK/ADME - Absorption and PK

Study title:	Bi-directional Permeability of GS-9350 and Ritonavir in Caco-2 Cell Monolayers
Study no.	AD-216-2023

Summary findings:
The purpose of the present study was to predict the oral absorption of GS-9350 and ritonavir by performing bi-directional permeability studies in vitro using a human colon carcinoma cell line (Caco-2).

Similar to ritonavir, GS-9350 was found to have high forward permeability through Caco-2 cells and showed no evidence for marked efflux. The permeability of GS-9350 through Caco-2 monolayers suggests that complete absorption of the soluble dose through the intestinal wall should occur in vivo.

Table 61 - Bidirectional Permeability of COBI Through Caco-2 Cell Monolayers

Direction	Target Conc. (μM)	Initial Conc. (μM)	Recovery (%)	P_{app} (10^{-6} cm/s)	Efflux Ratio
Cell-Free	1	1.2	ND	9.45	
Forward		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

Source: Report AD-216-2023

Single Dose PK Following IV Infusion
Mean PK parameters for COBI following IV administration to Sprague-Dawley rats (AD-216-2020), beagle dogs (AD-216-2021), and cynomolgus monkeys (AD-216-2022) are summarized in the tables and graphs below.

At 30 minutes post-dose, systemic clearance (CL) of COBI was high in males of all species and was close to hepatic blood flow in each case. The volume of distribution (V_{ss}) in rats was equal to the total body water but was higher in dogs and monkeys.

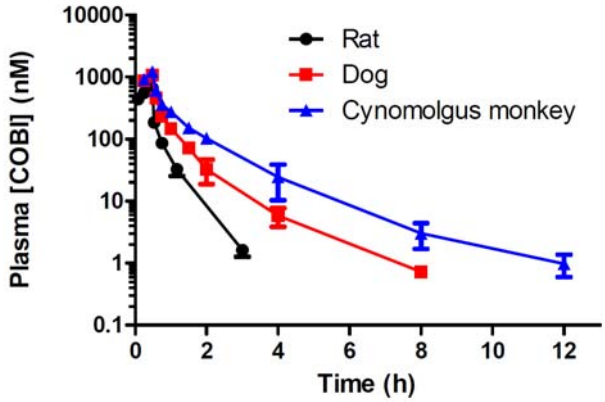
Table 62 - Mean Plasma PK Parameters for COBI Following 30-Minute IV Infusion at 1 mg/kg to Sprague-Dawley Rats, Beagle Dogs, and Cynomolgus Monkeys

Species	Sex	C_{max} (nM)	$AUC_{0-\infty}$ (nM•h)	CL (L/h/kg)	V_{ss} (L/kg)	$t_{½}$ (h)
Rat	Male	664 ± 31.4	351 ± 12.6	3.59 ± 0.14	0.76 ± 0.14	0.40 ± 0.02
	Female	890 ± 74.3	566 ± 50.1	2.37 ± 0.18	0.70 ± 0.09	0.35 ± 0.01
Dog	Male	924 ± 267	565 ± 155	2.18 ± 0.69	1.33 ± 0.69	1.02 ± 0.04
Monkey	Male	1222 ± 41.1	977 ± 83.7	1.36 ± 0.14	1.31 ± 0.12	1.42 ± 0.07

COBI = cobicistat; SD = standard deviation

Source: Reports AD-216-2020 (rat), AD-216-2021 (dog), and AD-216-2022 (monkey)

Figure 13- Mean Plasma PK Parameters for COBI Following 30-Minute IV Infusion at 1 mg/kg to Sprague-Dawley Rats, Beagle Dogs, and Cynomolgus Monkeys



COBI = cobicistat; SD = standard deviation

Source: Reports AD-216-2020 (rat), AD-216-2021 (dog), and AD-216-2022 (monkey)

Single Dose PK Following Oral Gavage

The mean plasma PK in rats (AD-216-2020), dogs (AD-216-2021), and monkeys (AD-216-2022) is summarized below. CL (clearance) was high in males of all species. CL in females was slightly lower than males. The V_{ss} (volume of distribution) was equal to (rats) or larger than (dog and monkey) the total body volume of water.

At doses of 5 or 6 mg/kg, oral bioavailability of COBI was moderate in the rat (33%) and low in the dog and monkey (11% and 7%, respectively). The high clearance values in these species indicate the potential for high hepatic metabolic first-pass extraction following oral absorption in

these species. Comparing bioavailability and predicted hepatic extraction values, it is likely that a substantial proportion (> 50%) of the dose was absorbed from the gastrointestinal (GI) tract.

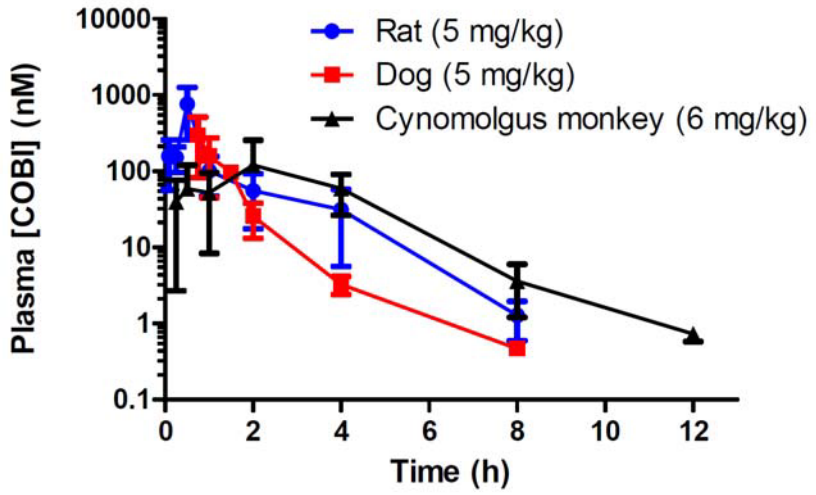
Table 63 - Mean Plasma PK after Oral Administration of COBI in Rats, Dogs, and Monkeys

Species	Dose (mg/kg)	t_{max} (h)	C_{max} (nM)	$t_{½}$ (h)	$AUC_{0-\infty}$ (nM•h)	%F
Rat	5	0.50 ± 0.00	764 ± 506	0.92 ± 0.22	594 ± 42.6	33 ± 3
Dog	5	1.00 ± 0.43	313 ± 186	1.12 ± 0.14	331 ± 130	11 ± 4
Monkey	6	2.17 ± 1.76	161 ± 102	1.36 ± 0.21	445 ± 280	7.3 ± 4.6

COBI = cobicistat; SD = standard deviation

Source: Reports AD-216-2020 (rat), AD-216-2021 (dog) and AD-216-2022 (monkey)

Figure 14 - Mean Plasma PK after Oral Administration of COBI in Rats, Dogs, and Monkeys



COBI = cobicistat; SD = standard deviation

Source: Reports AD-216-2020 (rat), AD-216-2021 (dog), and AD-216-2022 (monkey)

Single Dose Escalation PK Following Oral Gavage

Exploratory dose escalation was performed in mice (PC-216-2013-PK), rats (AD-216-2012), and dogs (AD-216-2020).

In the mice, the animals dosed at 300 mg/kg were moribund and euthanized 4 hours after dosing. There were no remarkable sex differences in exposure, and exposure increased in a roughly dose-proportional manner from 30 to 100 mg/kg.

Table 64 - Mean Plasma Pharmacokinetic Parameters Following Oral Administration of Increasing Doses of COBI in Solution to CByB6F1-Tg(HRAS)2Jic Mice

Dose (mg/kg)	Sex	t_{max} (h)	C_{max} (ng/mL)	t_{last} (h)	C_{24h} (ng/mL)	AUC_{0-t} (ng•h/mL)
30	M	2.0	5940	24	1.67	35535
	F	1.0	10158	24	1.28	46306
100	M	2.0	11130	24	1418	108796
	F	1.0	16205	24	1532	128930
300	M	4.0	29392	4[a]	NC[a]	NC[a]
	F	2.0	23464	4[a]	NC[a]	NC[a]

COBI = cobicistat; NC = not calculated
a Animals moribund and euthanized after 4 hours, so parameters were not calculated
Source: Report PC-216-2013-PK

In the male rat and the male dog, there was a greater than proportional increase in AUC_{0-t} as the dose was increased from 5 to 25 mg/kg and 10 to 30 mg/kg, respectively, likely reflecting saturation of first-pass metabolism. Following saturation, the change in AUC_{0-t} was near proportional as the dose was increased further, from 25 to 100 mg/kg in the male rat and 30 to 100 mg/kg in the male dog. In the female rat, as seen after intravenous administration, exposures were higher than in the males at both doses consistent with the known gender difference in CYP3A expression in this species. The increase in AUC_{0-t} in female rats, when the dose was increased from 25 to 110 mg/kg, was greater than dose proportional.

Tablet formulation studies

Two studies were performed in beagle dogs with COBI alone (AD-216-2042) or with the combination of EVG/FTC/TDF/COBI (AD-216-2061) with various oral formlulations in pentagrastin pretreated dogs. Pentagrastin (6 µg/kg pentagastrin, intramuscular injection 20 minutes prior to the oral dosing of the test articles) was performed to decrease vomiting.

After an oral dose of GS-9350 aqueous solution at 1 mg/kg in pentagastrin pretreated dogs, the plasma concentration of GS-9350 reached an average C_{max} of 89.2 nM at approximately 0.7 hr.

Several tablet formulations of GS-9350 were also evaluated in pentagastrin pretreated dogs at 1 mg/kg. Compared with the solution formulation, the plasma exposure of GS-9350 was 2 to 3-fold lower from tablets. However similar exposure was achieved at 1 mg/kg dose level with different API percentage (b) (4) in the tablets.

The comparative exposures of elvitegravir (EVG), emtricitabine (FTC), tenofovir disoprixil fumarate (TDF) and GS-9350 were evaluated by dosing the agents orally in pentagastrin pretreated male beagle dogs. Doses were fixed at 37.5 mg EVG, 50 mg FTC, 75 mg TDF, and 25 mg GS-9350. Baseline exposure was determined after simultaneous administration of three separate tablets (EVG, GS-9350, and FTC+TDF) [redacted] This report describes the results for two representative single tablet combinations; a bilayer [redacted] Each of these gave exposure values similar to those obtained in the multi-tablet control arm.

5.1.3 PK/ADME - Distribution

Plasma binding of cobicistat in the CD1 mouse, Sprague-Dawley rat, beagle dog, cynomolgus monkey, and human (studies AD-216-2076 and AD-216-2026) was assessed by equilibrium dialysis against isotonic phosphate buffer at 37°C for 3 hours (the time determined to achieve equilibration).

The plasma protein binding of COBI was determined to be moderately high in all species, ranging from 90.9% to 97.7% over the concentration range 1 to 30 µM. Binding to mouse, rat, and monkey plasma showed modest concentration dependence.

Table 65 - Protein Binding for COBI in Mouse, Rat, Dog, Monkey and Human Plasma

Plasma source	Fraction Unbound (%)			
	1 µM COBI	10 µM COBI	30 µM COBI	Mean
Mouse	3.31 ± 0.14	4.78 ± 0.27	6.15 ± 0.48	4.75
Rat	2.33 ± 0.06	5.34 ± 0.24	8.51 ± 0.48	5.40
Dog	5.68 ± 0.60	6.46 ± 0.60	6.33 ± 0.40	6.16
Monkey	4.31 ± 0.50	6.17 ± 0.50	9.13 ± 0.30	6.54
Human	6.33 ± 0.80	8.92 ± 0.90	7.54 ± 0.60	7.60

COBI = cobicistat; SD = standard deviation
Source: Reports AD-216-2076 (mouse) and AD-216-2026 (other species)

^{14}C-COBI was administered orally as a solution to male albino Sprague-Dawley rats (AD-216-2034) and pigmented Long Evans rats (AD-216-2060) at a target dose of 10 mg/kg and 200-250 µCi/kg.

After oral administration of ^{14}C-COBI to rats, radioactivity was widely distributed to most tissues by 0.25 hour postdose. Almost all of the tissues reached maximum radioactive concentration by 1 hour postdose. Generally, the radioactivity was preferentially distributed into glandular tissues and organs of elimination. The tissues showing the highest concentrations of radioactivity, excluding the GI tract, included liver, adrenal, kidney, and pituitary. The tissues with the lowest C_{max} values were eye, spinal cord, and brain, bone, seminal vesicles, epididymis, and testes (with concentrations all < 400 ng COBI equivalent/g tissue).

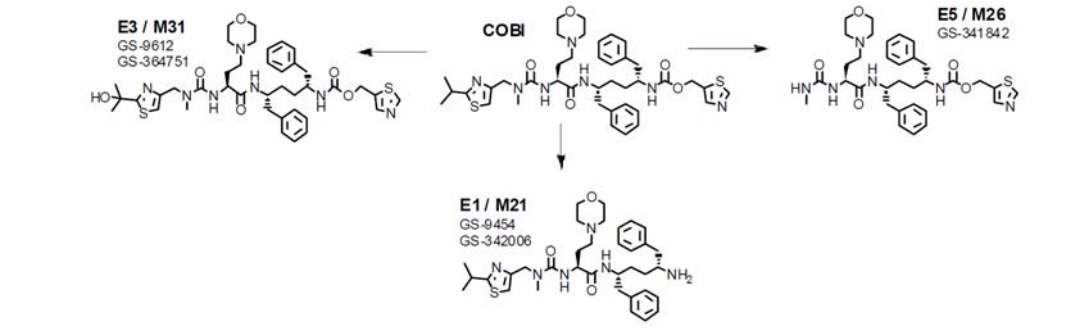
Low levels of radioactivity in the brain, spinal cord, and testes suggest minimal transport across the blood:brain and blood:testes barriers. Compared to albino rats, the pigmented rats showed very similar patterns of distribution of radioactivity, but with higher concentrations in the uveal tract of the eye. There were also higher concentrations of radioactivity in pigmented skin compared to nonpigmented skin, suggesting that COBI was associated with melanin.

In the Sprague-Dawley rats, clearance from most tissues was not complete by 24 hours postdose; however, radioactivity showed a time-dependent decrease in all tissues examined over the sampling period. Also, in an excretion study an average total of 6.9% of dosed radiolabel was recovered in excreta between 24 and 168 hours postdose. In the Long Evans rats, there was detectable radioactivity in pigmented tissues and some other tissues at 72 hours postdose, but dosimetry analysis showed that concentrations were declining, indicating association with the tissues was reversible.

5.1.4 PK/ADME - Metabolism

In preliminary studies, COBI metabolites were identified using human hepatocytes as well as rat, mouse, dog, and human hepatic microsomal fractions. In all species the major *in vitro* metabolites (M31, M26, and M21) were found. These findings were summarized in studies *AD-216-2074* and *AD-216-2038*.

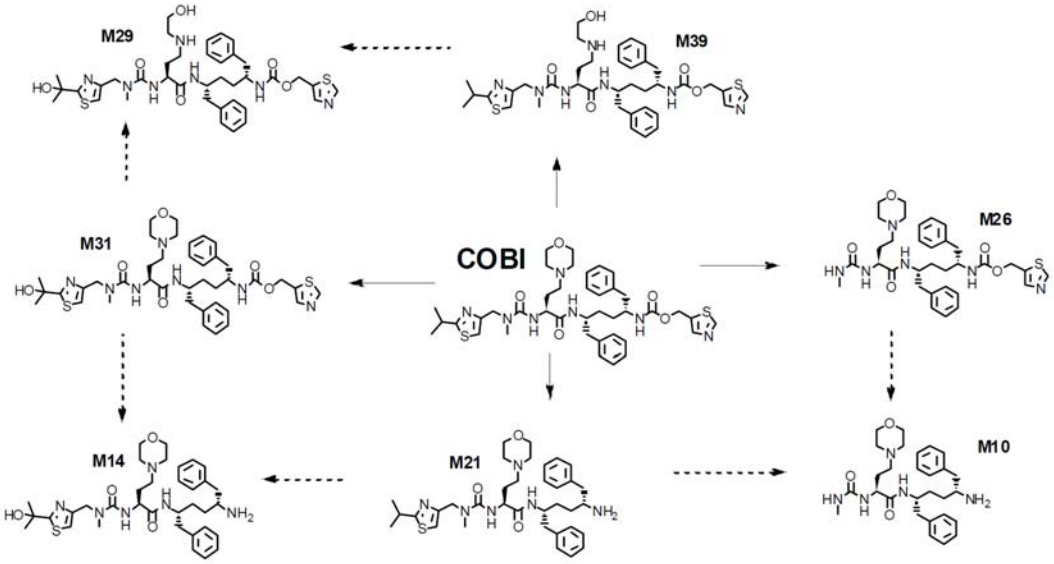
Figure 15 - Common Primary Pathways for Metabolism of COBI by Mouse, Rat, Dog, and Human (*In Vitro*)



COBI = cobicistat
Source: Reports AD-216-2074 and AD-216-2038

Identification of the COBI metabolites from *in vivo* studies was performed in mice (AD-216-2073), rats (AD-216-2082), dogs (AD-216-2101), and humans (GS-US-216-0111). The three primary oxidative metabolites (M21, M26, and M31) as well as M39 (a primary deethylation metabolite) were common to all species *in vivo* which corresponded to the *in vitro* studies. The most common secondary metabolites were formed by combinations of these primary reactions and all possible pairwise combinations of the common primary reactions, M21, M26, M31, and M39 were detected.

Figure 16 - Common Primary and Secondary Pathways for Metabolism of COBI by Mouse, Rat, Dog, and Human (*In vivo*)



COBI and all metabolites were detected in samples from mouse, rat, dog and human, except M29 (not in human).
Dashed arrows indicate combinations of primary metabolic pathways, not proven routes of metabolism.
Source: Reports AD-216-2073 (mouse), AD-216-2082 (rat), AD-216-2101 (dog), and GS-US-216-0111 (human)

Study title:	In Vitro Metabolism of GS-9350 in Hepatocytes and Hepatic Subcellular Fractions from Rat, Dog, Monkey, and Human
Study no.	AD-216-2024

Summary findings:
Both GS-9350 and ritonavir (RTV) exhibited low to medium stability in hepatic microsomes of monkey, dog and rat (listed in order of increasing stability), and higher stability in human. In human cryopreserved hepatocytes, the rates of metabolism of both GS-9350 and RTV were low.

Table 66 - In Vitro Rate of Metabolism of COBI and RTV at 3µM in Hepatic Microsomes

Species	$t_{½}$ (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

Study title:	Cytochrome P450 Phenotyping for GS-9350
Study no.	AD-216-2025

Summary findings:
GS-9350 was either stable or metabolized slowly by all CYP450 enzymes tested. The highest turnover rate was observed with CYP2D6. The apparent stability in the presence of CYP3A4 is likely a consequence of efficient inactivation of the enzyme by GS-9350. The low metabolism by the major CYP450 enzymes tested indicates a low potential for drug-drug interactions affecting the pharmacokinetics of GS-9350 when co-dosed with CYP450 inhibitors.

Table 67 - Rates of Metabolism of COBI and RTV Catalyzed by Major Human CYP450 Enzymes

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.

5.1.4 PK/ADME - Excretion

After oral administration of ^{14}C-COBI to mice (AD-216-2073), rats (AD-216-2034), and dogs (AD-216-2067), recovery of radioactivity was high (≥ 86.1% in all groups) with the majority being found in feces (≤ 2.06% in urine). Recovery was largely complete by 48 hours postdose. After oral administration of ^{14}C-COBI to bile duct-cannulated animals, an average of 69.3% and 63.9% of dosed radioactivity was recovered in bile in rats (AD-216-2034) and dogs (AD-216-2068), respectively.

Excretion of COBI in rat milk was examined as part of the postnatal development study (see Reproductive toxicology section). In summary, two hours after treatment of lactating females (postnatal Day 10) with COBI, mean milk/plasma concentration ratios were 1.3, 1.9, and 1.7 after doses of 10, 30, and 75 mg/kg/day, respectively (study TX-216-2033), indicating that COBI is excreted into milk in rats.

5.1.4 PK/ADME - Drug Interaction

CYP Activity

The intended pharmacological action of COBI is inhibition of human CYP3A enzymes. This ability was confirmed in vitro using multiple activities catalyzed by human hepatic microsomal fractions and also by clinical studies. Cobicistat is a potent mechanism-based inhibitor of human CYP3A with inactivation kinetics (kinact 0.47 min-1, KI 1.1 μM), similar to those of RTV (AD-216-2028). Inhibition of CYP3A is relatively specific as COBI does not inhibit human CYP1A2, CYP2C9, or CYP2C19, is a very weak inhibitor of CYP2C8 (IC50 30.1 μM), a weak inhibitor of CYP2D6 (IC50 9.2 μM), and a modest inhibitor of CYP2B6 (IC50 2.8 μM) (AD 216-2029 and AD-216-2070). This is in contrast to RTV, which is a more potent inhibitor of CYP2D6 (IC50 3.4 μM), CYP2C9 (IC50 3.9 μM), and CYP2C8 (IC50 5.5 μM). This higher specificity for COBI has been confirmed in clinical drug interaction studies in which COBI had no effect on the pharmacokinetics of efavirenz (CYP2B6) and little effect on the pharmacokinetics of desipramine (CYP2D6) ([GS-US-216-0112]). Cobicistat is a weak inhibitor of human hepatic microsomal UGT1A1 activity (IC50 16.3 μM), being less potent than RTV (IC50 4.7 μM) and ATV (IC50 0.8 μM) (AD-216-2075).

Induction Liability

In xenobiotic receptor transactivation studies, COBI showed no ability to activate human AhR and was a very weak activator of human PXR (2.2-fold activation at 10 μM, compared to 10.1-fold activation by 10 μM RTV; AD-216-2027). This was confirmed in human hepatocyte studies where COBI, at concentrations up to 30 μM, increased CYP1A2 activity and mRNA and protein by < 2% of the positive control and increased CYP3A4 mRNA expression by an average of 27.4% (AD-216-2071). CYP3A activity was below that of the vehicle control, due to mechanism-based inhibition by COBI, but a slight increase in immunodetectable CYP3A was detected. Other targets for induction (uridine diphosphate glucuronosyltransferase 1A1 [UGT1A1] mRNA, MDR1 mRNA, and CYP2B6 mRNA and protein) were all unaffected or weakly affected by COBI treatment. Ritonavir is a potent inducer in human hepatocytes and cell lines and is known to cause clinical drug interactions through induction of multiple phase I and phase II enzymes. In contrast to its lack of effect on human PXR, COBI activates rodent PXR and increases the expression of proteins regulated by this receptor, such as rat CYP3A, UGT1A1, and presumably OATP2 (AD-216-2039).

Transporter Activity

Cobicistat showed negligible or weak inhibition of the efflux transporters MDR1, MRP1, MRP2, MRP4, BCRP, and MATE2-K, and the renal uptake transporters OAT1 and OAT3. Cobicistat is a weak inhibitor of the renal uptake transporter, OCT2 (unbound Cmax/IC50 = 0.01), and a more potent inhibitor of the hepatic uptake transporters OATP1B1 and OATP1B3 ([I]1/IC50 0.4 and 0.7, respectively), and the renal efflux transporters OCTN1 and MATE1 (unbound Cmax/IC50 0.04 and 0.05, respectively).

The effects of COBI and RTV on the activities of human transporters are summarized in the table below.

Table 68 - Effects of COBI and RTV 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]	2.6.5.15.16 (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	S_2	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

5.2 Toxicokinetics

Toxicokinetic data are reviewed as part of the toxicity studies. See Section 6 for the TK data included in the toxicology reviews.

6 General Toxicology

NOTE: In multiple toxicology studies, the Sponsor referred to the purity of the test article as ~50% pure. This is a typographical error. The purity of the stock of GS-9350 was 97.5% (see below). This stock was then diluted in ethanol to a solution of roughly 50% for dosing. The 50% solution was recorded as the "purity" of GS-9350 instead of clearly delineating that this was the concentration of the working stock solution.

> GS-9350, Lot No. B ([(b) (4)] Material ID No. 30145), at the time of preparation stated an overall correction factor of 0.974 and was later recertified to be 0.975, supplied [(b) (4)] by Gilead Sciences, Inc., recertification date stated as 31 July 2008 and was later recertified to be 31 January 2009

6.1 Single-Dose Toxicity

Study title:	Single-Dose Oral Gavage Toxicity Study with GS-9350 in Rats
Study no.	TX-216-2003

Summary findings:
The purpose of this study was to evaluate the potential toxicity of GS-9350, following a single oral gavage administration to rats followed by a 14-day observation period to assess the reversibility, persistence, or delayed occurrence of effects.

Four groups of male and female Crl:CD(SD) rats (5 per sex/group) received control article or 125, 250, or 500 mg of test article/kg of body weight (mg/kg) at a dose volume of 10 mL/kg. Additional groups of animals (3/sex/group) were assigned for toxicokinetic analysis.

Single oral doses of GS-9350 caused no effects at 125 mg/kg. Effects (mainly hepatic findings), were first noted at 250 mg/kg and increased at 500 mg/kg. Clinical chemistry findings were significant at day 3. Animals dosed 500 mg/kg had a slight (not significant) decrease in body weight which correlated to a decrease in food consumption in this group.

The sponsor proposes a NOAEL of 500 mg/kg for a single dose. The decreased food consumption is not considered adverse (with no significant body weight loss). Hepatic effects were noted at 500 and 250 mg/kg, but they were minimal and not adverse. Therefore, the sponsor's assessment of 500 mg/kg for the NOAEL is acceptable.

Study title:	Collection of Samples for Determination of the Pharmacokinetics of GS-9350 Following a Single Oral Gavage Dose to Male and Female 001178-W (wild type) Mice
Study no.	PC-216-2013

Summary findings:
This study was conducted to collect samples for investigating the pharmacokinetics of GS-9350 when administered once via oral gavage to male and female 001178-W mice (wild type for RasH2 transgenic mice).

Table 69 - Study Design for Study PC-216-2013

Group	No. of Animals		Dose Level	Dose Concentration
	Male	Female	(mg/kg)	(mg/mL)
1 (Low)	24	24	30	3
2 (Mid)	24	24	100	10
3 (High)	24	24	300	30

GS-9350-related severe clinical signs were observed after a single oral dose of 300 mg/kg to male and female 001178-W mice (wild type for RasH2 transgenic mice). Blood collection for this group occurred only up through the 4-hour postdose collection interval. No abnormal macroscopic observations were noted at unscheduled sacrifices. No GS-9350-related clinical signs were observed at 30 or 100 mg/kg. NOAEL in the wild type controls for the RasH2 mice was 100 mg/kg by a single oral gavage.

Following the single oral administration of GS-9350, the systemic exposure of GS-9350 was increased with the increase in dose level from 30 mg/kg to 100 mg/kg/day in both males and females. The increase in AUC_{0-24} was roughly dose proportional at 30 and 100 mg/kg. There were no remarkable sex differences (< 2-fold) in exposure. At the 300 mg/kg dose, male and female mice were euthanized moribund after the 4-hour bleed, and AUC_{0-24} was not calculated. At dose levels of 30, 100 and 300 mg/kg, GS-9350 reached C_{max} of 10158 and 5940, 16205 and 11130, and 23464 and 29392 ng/mL, in females and males, respectively. At dose levels of 30 and 100 mg/kg, AUC_{0-24} was 46306 and 35535, and 128930 and 108796 ng•h /mL in females and males, respectively.

Table 70 - Mean PK Parameters of COBI Following Single Oral Doses of COBI in Female and Male 001178-W Mice

Dosage (mg/kg)	Gender	AUC_{0-24} (ng•h /mL)	C_{max} (ng/mL)	T_{max} (hr)	C_{24hr} (ng/mL)
30	Female	46306	10158	1.0	1.28
	Male	35535	5940	2.0	1.67
100	Female	128930	16205	1.0	1532
	Male	108796	11130	2.0	1418
300	Female	NC	23464	2.0	NC
	Male	NC	29392	4.0	NC

NC: not calculated due to insufficient data.

6.2 Repeat-Dose Toxicity

Study title: 2-Week Non-GLP Oral Gavage dose Range-Finding Toxicity and TK study of GS-9350 in CD-1 mice

Study no.:	TX-216-2025
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	30 June 2008
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	See below.

Test Article	Lot No.	Storage	Purity	Retest Date
GS-9350	3168-157-8	REF, PFL	50.5%	31 Aug 2008

REF Refrigerated (2 to 8°C).
PFL Protected from light.

Key Study Findings

This study evaluated the toxicity and determined the toxicokinetics of GS-9350 when administered daily by oral gavage to mice for at least 2 weeks.

Initial doses were 0, 10, 30, 100, 300 mg/kg. Due to deaths at 300 mg/kg/day on Day 1 or Day 2 (1 male and 5 females), the high dose was reduced to 200 mg/kg/day beginning on Day 3, and animals were replaced with an animal of similar body weight and of the same sex.

Increases in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were noted at dose levels of 30 mg/kg/day, and above. Increases in ALT (7.1- to 7.3-fold versus controls) and AST activity (5.0- to 4.1-fold versus controls) were considered adverse in animals given 300/200 mg/kg/day. Mild increases in globulin and associated decreases in albumin-to-globulin (A:G) ratios, noted at 100 mg/kg/day, and above, were not considered adverse. The remaining clinical chemistry and urinalysis test results were generally unremarkable.

Based on mortality and marked increases in ALT and AST levels in both sexes at 300/200 mg/kg/day, the NOAEL of GS-9350 when administered daily via oral gavage to CD-1 mice for at least 2 weeks is 100 mg/kg/day in males and females (Day 14 C_{max}: 11997 and 13933 ng/mL, AUC_{0-t}: 134451 and 75695 ng·hr/mL, in males and females, respectively).

Methods

Doses:	See table below (up to 300 mg/kg)
Frequency of dosing:	Daily
Route of administration:	Oral
Dose volume:	20 mg/ml
Formulation/Vehicle:	95% propylene glycol 5% ethanol, pH 2.3 wit HCl
Species/Strain:	CD-1 mice (Harlan Sprague Dawley (b) (4))
Number/Sex/Group:	See table below.
Age:	~ 7 wks old
Weight:	25.4 to 36 g (male) and 21.7 to 33.3 g (female)
Satellite groups:	See table below
Unique study design:	None – range finding study
Deviation from study protocol:	None noted.

Table 71 - Mouse Range-Finding Study: Study Design

Group	No. of Animals Male	No. of Animals Female	Dose Level (mg/kg/day) [b]	Dose Concentration (mg/mL) [b]
Toxicity Animals				
1 (Control) [a]	5	5	0	0
2 (Low)	5	5	10	1
3 (Mid)	5	5	30	3
4 (Mid-High)	5	5	100	10
5 (High) [b]	5	5	300/200	30/20
Toxicokinetic Animals				
6 (Control) [a]	6	6	0	0
7 (Low)	36	36	10	1
8 (Mid)	36	36	30	3
9 (Mid-High)	36	36	100	10
10 (High) [b]	36	36	300/200	30/20

a Groups 1 and 6 received control article only [95% propylene glycol (PG), 5% ethanol (EtOH) with 1.5 eq. HCl, pH adjusted to 2.3 with HCl].

b Due to animal deaths in the high dose group, Group 5 and 10 animals were not dosed on Day 2. Beginning on Day 3, the Group 5 and 10 dose level and dose concentration were reduced to 200 mg/kg/day and 20 mg/mL, respectively.

Observations and Results

Toxicokinetics

Table 72 - Mouse 2-Week Range Finding Study: Toxicokinetics

Dose Group	Dose Level (mg/kg/day)	Gender	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)
		Day 1			
7	10	M	519	2.00	2152
		F	720	4.00	4721
8	30	M	4103	2.00	27830
		F	6690	2.00	24534
9	100	M	10070	1.00	106633
		F	12767	2.00	84801
10	300	M	31100	4.00	382318
		F	22267	4.00	376113
		Day 14			
7	10	M	2290	1.00	7145
		F	971	1.00	3748
8	30	M	6600	2.00	27681
		F	7173	1.00	32404
9	100	M	11997	4.00	134451
		F	13933	2.00	75695
10	200	M	15733	1.00	100723
		F	16767	4.00	289685

Study title: 14-Day Oral Gavage Toxicity and Toxicokinetic Study of GS-9350 in Mice

Study no.:	TX-216-2032
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	8 Sept 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	See below

Test Article[a]	Lot No.	Storage	Purity	Retest Date
GS-9350 (provided as a 52.9% solution in EtOH)	9350-AC-2E op 189 Crop 2	In a refrigerator, set to maintain 2 to 8°C, and protected from light	98.6%	30 Nov 2009

a See Test Article Analytical Report. Information on synthesis methods, composition, or other characteristics that defines the test article is on file with the sponsor.

Key Study Findings

This study compared the toxicity and determined the toxicokinetics of GS-9350 when administered in pH-adjusted and pH-unadjusted vehicles daily via oral gavage to mice for 14 days.

17 animals were found dead on study (15 were related to gavage error). Two mice died unrelated to gavage error: female (30 mg/kg, day 9, undetermined causes), male (50 mg/kg, day 13, undetermined causes). Since other animals survived to sacrifice at much higher doses, and there were no drug-related clinical correlates in these two animals, the deaths were unlikely to be drug related. The gavage error was attributed by the Sponsor to a pH-adjusted vehicle compared to non-pH-adjusted vehicle since most of the deaths occurred in the pH-adjusted vehicle groups.

Due to the lack of any significant drug-related findings (mild liver enzyme changes and increased liver weights), the high dose in both males and females was the NOAEL.

NOAEL (males): 50 mg/kg/day in males (Day 14 AUC_{0-t} of 50,999 and 54,813 ng·hr/mL in pH-adjusted and pH-unadjusted vehicles, respectively)
NOAEL (females): 100 mg/kg/day in females (Day 14 AUC_{0-t} 150,989 and 169,700 ng·hr/mL in pH-adjusted and pH-unadjusted vehicles, respectively).

Methods

Doses:	See table below (0, 5, 15, 30, 50, 100 mg/kg)
Frequency of dosing:	Daily
Route of administration:	Gavage
Dose volume:	5 ml/kg
Formulation/Vehicle:	Vehicle Control Article 1 was 95% propylene glycol (v/v) and 5% EtOH (v/v), pH 2.3 (+0.1) with hydrochloric acid. Vehicle Control Article 2 was 95% propylene glycol (v/v) and 5% EtOH (v/v).
Species/Strain:	CD-1 mice (Charles River)
Number/Sex/Group:	See table below
Age:	6.9 to 7.7 wks old
Weight:	27.8 to 38.8 g for males 19.3 to 30.4 g for females.
Satellite groups:	TK animals – see below
Unique study design:	Different vehicle formulations among groups – see below
Deviation from study protocol:	None noted that would significantly alter study outcome or interpretation.

Table 73 - Mouse 2-Week Toxicology Study: Study Design

	No. of Animals		Dose Level (mg/kg/day)		Dose Concentration (mg/mL)	
Group	Male	Female	Male	Female	Male	Female
Toxicity Animals[d]						
1 (Control)[a]	24	24	0	0	0	0
2 (Vehicle Control 1)[b]	24	24	0	0	0	0
3 (Low)[b]	24	24	5	10	1	2
4 (Mid)[b]	24	24	15	30	3	6
5 (High)[b]	24	24	50	100	10	20
6 (Vehicle Control 2)[c]	24	24	0	0	0	0
7 (Low)[c]	24	24	5	10	1	2
8 (Mid)[c]	24	24	15	30	3	6
9 (High)[c]	24	24	50	100	10	20

a Animals in Group 1 received the control article [reverse osmosis (RO) water] only.
b Animals in Groups 2 through 5 received Vehicle Control Article 1 [95% propylene glycol (v/v) and 5% ethanol (EtOH; v/v), pH adjusted to 2.3 (± 0.1)] or GS-9350 in Vehicle Control Article 1.
c Animals in Groups 6 through 9 received Vehicle Control Article 2 [95% propylene glycol (v/v) and 5% EtOH (v/v)] or GS-9350 in Vehicle Control Article 2.
d Animals designated for the predose toxicokinetic blood collection on Day 14 (three animals/sex/group) were dosed for 13 days.

Observations and Results

Toxicokinetics

Exposure to GS-9350 generally increased with the increase in dose level from 5 to 50 mg/kg/day in males, and from 10 to 100 mg/kg/day in females. The increases in C_{max} and AUC_{0-t} for males and females were generally greater than dose proportional following administration of GS-9350 in both Vehicle 1 and Vehicle 2. Exposures (based on C_{max} and AUC_{0-t} values) were generally similar after administration of GS-9350 in Vehicle 1 or Vehicle 2.

Table 74 - Mouse 2-Week Toxicology Study: Toxicokinetic Analysis

Dose Group	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng·hr/mL)	C_{last} (ng/mL)	T_{last} (hr)
		GS-9350 in Vehicle 1 (95% propylene glycol, 5% ethanol, pH 2.3)					
3	5	M	242	2.00	594	77.2	4.00
	10	F	906	2.00	2638	147	8.00
4	15	M	1630	2.00	7576	10.7	8.00
	30	F	6553	4.00	43932	5.06	12.0
5	50	M	8837	2.00	50999	12.0	12.0
	100	F	14633	2.00	150989	16.0	24.0
		GS-9350 in Vehicle 2 (95% propylene glycol, 5% ethanol)					
7	5	M	241	1.00	885	16.4	8.00
	10	F	1870	1.00	4143	6.37	8.00
8	15	M	3767	1.00	10962	23.5	8.00
	30	F	8367	1.00	36043	7.07	12.0
9	50	M	9443	1.00	54813	34.2	12.0
	100	F	19767	4.00	169700	90.5	24.0

Study title: 4-Week Dose Range-finding Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in CByB6F1-Tg(HRAS)2Jic (wild type) Mice

Study no.:	TX-216-2041
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	13 Sept 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	See below

Test Article[a]	Lot No.	Storage	Purity	Retest Date[a]	Reserve (Archive) Sample
GS-9350 Foam	3793-143-21	Refrigerated (2 to 8°C), protected from light, with desiccant	99.1%	31 Dec 2010	Collected

a See Test Article Analytical Report. Information on synthesis methods, composition, or other characteristics defining the test article is on file with the Sponsor.

Key Study Findings

This study evaluated the potential toxicity and toxicokinetics profile of GS-9350 when administered daily via oral gavage to CByB6F1-Tg(HRAS)2Jic (wild type) mice for 29 days.

Mild, dose-dependent increases in alanine aminotransferase (ALT, < 3-fold) and aspartate aminotransferase (AST, < 2-fold) activities were noted in males given GS-9350. In females, similar increases in ALT (< 3-fold) and AST (< 2-fold) were noted in the 100 mg/kg/day group, only. These changes correlated with increased liver weights at 100 mg/kg/day and with hepatocellular hypertrophy in males at 100 mg/kg/day. Minimal increases in mean cholesterol concentration were noted in males at 30 and 100 mg/kg/day and in females at 100 mg/kg/day, and minimal increases in triglyceride concentrations were noted in males and females at 100 mg/kg/day. These changes were due to adaptive changes in the liver due to CYP inhibition by the drug and not considered adverse at these doses.

Due to the lack of any significant findings or adverse events at any dose, the NOAEL for CByB6F1-Tg(HRAS)2Jic (wild type) mice by oral gavage for 29 days was the high dose of 100 mg/kg/day. This dose level corresponded to a Week 4 AUC_{0-t} of 65297 and 90127 ng·hr/mL for males and females, respectively.

Methods

Doses:	0, 10, 30, 100 mg/kg (see below)
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	5 ml/kg/day
Formulation/Vehicle:	The vehicle control article was 10% (v/v) propylene glycol (PG) in 40 mM acetate buffer, pH 4.0 (± 0.1)
Species/Strain:	CByB6F1-Tg(HRAS)2Jic (wild type) mice
Number/Sex/Group:	See below
Age:	6-8 wks old
Weight:	21.9 to 27.8 g for males 16.9 to 23.8 g for females
Satellite groups:	TK groups, see below
Unique study design:	None.
Deviation from study protocol:	Minor deviation noted regarding documentation of the storage conditions for the second set of bioanalytical plasma samples. It should not affect study outcome or study interpretation.

Table 75 - Mouse Tg(HRAS) Wild Type Range-Finding Study: Study Design

Group[a]	No. of Animals Male	No. of Animals Female	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
Toxicity Animals				
1 (Control)	10	10	0	0
2 (Low)	10	10	10	2.0
3 (Mid)	10	10	30	6.0
4 (High)	10	10	100	20.0
Toxicokinetic Animals[b]				
5 (Control)	4	4	0	0
6 (Low)	21	21	10	2.0
7 (Mid)	21	21	30	6.0
8 (High)	21	21	100	20.0

a Groups 1 and 5 received 10% (v/v) propylene glycol in 40 mM acetate buffer, pH 4.0 (± 0.1), which also served as the vehicle for GS-9350 formulations. The dose volume was 5 mL/kg.
b Toxicokinetic animals were included for the purpose of blood sample collections.

Observations and Results

Toxicokinetics

All concentration values of GS-9350 in the control group were below the lower limit of quantitation. Exposure to GS-9350 increased with the increase in dose level from 10 to 100 mg/kg/day. Increases in AUC_{0-t} were more than dose proportional between 10 and 30 mg/kg/day, dose proportional in females between 30 and 100 mg/kg/day, and slightly less than dose proportional in males between 30 and 100 mg/kg/day. There were no notable sex differences (< 2-fold) in C_{max} and AUC_{0-t} values.

Table 76 - Mouse Tg(HRAS) Wild Type Range-Finding Study: Toxicokinetic Analysis

Dose Group	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng·hr/mL)	T_{last} (hr)	C_{last} (ng/mL)
6	10	M	2623	2.00	6576	8.00	6.35
		F	1677	2.00	3845	8.00	7.06
7	30	M	6473	4.00	33210	12.0	6.02
		F	7700	2.00	30801	8.00	49.0
8	100	M	10953	2.00	65297	12.0	130
		F	13400	2.00	90127	12.0	174

Study title: 3-Month Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in CD-1 Mice

Study no.:	TX-216-2026
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11 Aug 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-9350, Lot 3168-157-8, 50.5% pure (as a 50.5 wt% solution in ethanol).

Key Study Findings

There were an abnormally high rate of gavage-related or non-drug related deaths. 15 animals (10 males and 5 females) died on study which were not related to drug exposure. There were no major toxicological findings. Liver weight increases (high dose males and females) associated with histological findings (males only) with AST and ALT increases (males only). TK analysis indicates that there was no accumulation after repeat exposure to GS-9350. There was a slightly higher Cmax and AUC in females, compared to males in all dosed groups.

The NOAEL for this study is 50 mg/kg/day in females (based on the absence of any toxicologically significant findings) and 5 mg/kg/day in males (based on 3-fold increases in mean ALT and AST levels at 15 mg/kg/day and 9- to 11- fold increases in ALT and AST at 50 mg/kg/day).

Methods

Doses:	0, 5, 15, 50 mg/kg/day (see below)
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	95% propylene glycol (PG), 5% ethanol (EtOH) with 1.5 eq. HCl, pH adjusted to 2.3 with HCl.
Species/Strain:	Male and female Hsd:ICR(CD-1®)
Number/Sex/Group:	See table below (15/sex/group)
Age:	~7 weeks old at study start
Weight:	30.0 to 39.3 g and 23.8 to 31.2 g for toxicity males and females, respectively 30.7 to 38.6 g and 20.6 to 31.7 g for toxicokinetic males and females, respectively
Satellite groups:	TK animals (8/sex/vehicle group and 40/sex/treatment group)
Unique study design:	None
Deviation from study protocol:	No significant deviations noted.

Table 77 - 3 Month Oral Tox Study in Mice: Study Design

Group [a]	No. of Animals Male	No. of Animals Female	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
Toxicity Animals				
1 (Control)	15	15	0	0
2 (Low)	15	15	5	1
3 (Mid)	15	15	15	3
4 (High)	15	15	50	10
Toxicokinetic Animals [b]				
5 (Control)	8	8	0	0
6 (Low)	40	40	5	1
7 (Mid)	40	40	15	3
8 (High)	40	40	50	10

a Groups 1 and 5 received the formulation vehicle (95% propylene glycol, 5% ethanol with 1.5 eq. HCl, pH-adjusted to 2.3 with HCl) only.

b Two extra animals/sex and four extra animals/sex were included in Group 5 and Groups 6-8, respectively. Due to unscheduled deaths on study, several extra toxicokinetic animals were treated as toxicity animals for clinical pathology assessment and other terminal endpoints (terminal sacrifice, necropsy, organ weights, bone marrow smears, electron microscopy collection, tissue preservation, and histopathology) as per protocol and amendment specifications.

Observations and Results

Mortality

Twice daily (AM and PM). 15 unscheduled deaths were noted.

Gavage error: 9 animals (5 males and 4 females)

Found dead or sacrificed moribund: (6 animals)

- 0 mg/kg (2 males on days 59 and 91)
- 15 mg/kg (2 males on days 66 and 70)
- 50 mg/kg (1 male on day 50 and 1 female on day 65)

None of the deaths were considered treatment-related on the basis of clinical signs, histologic findings, and the lack of a dose-response relationship.

Clinical Signs

Twice daily. A dose-related increase in struggling during dosing of GS-9350

Body Weights

Predose then weekly. No change.

Feed Consumption

Weekly for toxicity animals. No change.

Ophthalmoscopy

Predose, then week 13. No change.

Hematology

At sacrifice. No change.

Clinical Chemistry

At sacrifice. ALT was increased 5- and 17-fold at 15 and 50 mg/kg (respectively) in males and 5-fold at 50 mg/kg in females. AST was increased (4.9-fold) in males at 50 mg/kg.

Urinalysis

Overnight prior to sacrifice. No change.

Gross Pathology

At sacrifice. No major changes noted.

Organ Weights

At sacrifice. Significant increase in absolute and relative liver weights (20-30%) in males and females at 50 mg/kg

Histopathology

Adequate Battery - yes

Peer Review - no

Histological Findings (At sacrifice.) Minimal hepatocellular centrilobular hypertrophy was evident in 7/14 males given 50 mg/kg/day

Toxicokinetics

Collected on day 1 and day 13.

Table 78 - GS-9350 in Mouse Plasma

Dose Group	GS-9350 Dose Level (mg/kg/day	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	C_{last} (ng/mL)	T_{last} (hr)
			Day 1				
6	5	M	378	1.00	470	183	2.00
		F	436	1.00	1065	46.0	4.00
7	15	M	3607	1.00	7573	387	4.00
		F	4457	1.00	9152	180	4.00
8	50	M	8943	1.00	32478	182	8.00
		F	11400	1.00	46449	484	8.00
			Week 13				
6	5	M	236	1.00	931	6.53	8.00
		F	256	2.00	1194	5.91	8.00
7	15	M	3527	1.00	16300	21.8	8.00
		F	2725	2.00	11316	7.02	8.00
8	50	M	8477	1.00	46201	3135	8.00
		F	11373	1.00	60128	2314	8.00

Table 79 - GS-9612 (metabolite of GS-9350) in Mouse Plasma

Dose Group	GS-9350 Dose Level (mg/kg/day	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	C_{last} (ng/mL)	T_{last} (hr)	M/P Ratio[a] (%) C_{max}	M/P Ratio[a] (%) AUC_{0-t}
					Day 1				
6	5	M	6.63	1.00	9.22	5.17	2.00	1.8	2.0
		F	9.26	1.00	20.7	2.14	4.00	2.1	1.9
7	15	M	118	1.00	301	29.3	4.00	3.3	4.0
		F	120	1.00	294	15.8	4.00	2.7	3.2
8	50	M	239	1.00	1037	9.63	8.00	2.7	3.2
		F	355	2.00	1436	15.2	8.00	3.1	3.1
					Week 13				
6	5	M	10.1	4.00	24.3	10.1	4.00	4.3	2.6
		F	7.46	1.00	21.5	5.92	4.00	2.9	1.8
7	15	M	99.0	4.00	469	1.85	8.00	2.8	2.9
		F	76.8	4.00	222	76.8	4.00	2.8	2.0
8	50	M	209	4.00	1110	55.6	8.00	2.5	2.4
		F	239	4.00	1382	50.3	8.00	2.1	2.3

a Metabolite (GS-9612) to parent (GS-9350) ratio

Exposure to GS-9350 increased more than dose proportionally between 5 and 15 mg/kg/day, and dose proportionally between 15 and 50 mg/kg/day. In general, no marked (>2-fold) sex differences were observed in C_{max} and AUC_{0-t} values after GS-9350 exposure in mice. No accumulation of GS-9340 was observed after multiple dosing in mice.

Metabolite GS-9612 exposure, increased more than dose proportionally between 5 and 15 mg/kg/day, and dose proportionally between 15 and 50 mg/kg/day. In general, no marked (> 2-fold) sex differences were observed in GS-9612 exposure. No accumulation of GS-9612 was observed after multiple dosing of GS-9350 in mice. The percent C_{max} and AUC_{0-t} metabolite to parent ratios ranged from 1.8 to 4.3%, indicating that GS-9350 is not extensively converted to GS-9612 in mice following oral gavage of GS-9350.

Dosing Solution Analysis

The stability of samples prepared at 0.9259 to 277.8 mg/mL was confirmed for 15 days at room temperature under (b) (4) Study 6511-334 (Gilead BA-216-2001).

Samples prepared at 0.9259 and 277.8 mg/mL were confirmed to be solutions under (b) (4) Study 6511-334 (Gilead BA-216-2001); therefore, homogeneity analysis was not necessary.

Study title: 4-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Rats with a 4-Week Recovery

Study no.:	TX-216-2004
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	21 Aug 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-9350, Lot# 3168-121-23, 92.9% pure

Key Study Findings

There was an oral discharge and skin/coat findings (cold to the touch, rough/yellow haircoat) at 50 mg/kg and increasing at 100 mg/kg. There was a lower red cell mass (RBC count, hemoglobin, hematrcrit) at 100 mg/kg accompanied by a mild increase in platelets at 50 and 100 mg/kg. Clinical chemistry findings at 50 and 100 mg/kg were limited to mildly increased albumin, decreased ASP, increased Ca, decreased Cl, and decreased A:G ratio. There was also an increase in urine volume and pH with a decrease in specific gravity at 50 and 100 mg/kg. Liver findings were limited to an increase in weight at 50 and 100 mg/kg with a slight hepatcellular hypertrophy at 100 mg/kg. Thyroid findings were limited to a slight follicular cell hyperplasia/hypertrophy at 50 and 100 mg/kg.

The sponsor proposed a NOAEL of 100 mg/kg. Based on clinical observations, clinical chemistry, and urinalysis findings at 100 mg/kg as well as the liver and thyroid findings at 100 mg/kg, the NOAEL was 50 mg/kg.

Methods

Doses:	0, 10, 20, 50, 100 mg/kg (see table below)
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	10 ml/kg
Formulation/Vehicle:	15% Self-Emulsifying Drug Delivery System (SEDDS) (6% Labrasol, 6% Solutol HS-15, 1.5% ethanol, 1.5% propylene glycol) in reverse osmosis water
Species/Strain:	SD Rats (Charles River)
Number/Sex/Group:	See table below
Age:	56 to 62 days old
Weight:	255-347 g for males; 170-232 g for females
Satellite groups:	See table below
Unique study design:	
Deviation from study protocol:	

Group[a]	No. of Animals[b] Male	Female	Dose Level (mg/kg/day)	Dose Concentration[c] (mg/mL)
Toxicity Animals				
1 (Control)	15	15	0	0
2 (Low)	10	10	10	1
3 (Mid-Low)	10	10	20	2
4 (Mid-High)	15	15	50	5
5 (High)	15	15	100	10
Toxicokinetic Animals				
6 (Control)	3	3	0	0
7 (Low)	9	9	10	1
8 (Mid-Low)	9	9	20	2
9 (Mid-High)	9	9	50	5
10 (High)	9	9	100	10

a Groups 1 and 6 received control article only.
b Toxicity animals designated for recovery phase sacrifice (five animals/sex in Groups 1, 4, and 5) underwent at least 4 weeks of recovery following dose administration.
c All dose concentrations were adjusted for purity (correction factor = 1.08).

Table excerpted from Sponsor

Observations and Results

Mortality

Checked 2x daily. None related to drug administration. One TK female (10 mg/kg group) died on day 4 due to technical problems with blood collection.

Clinical Signs

Checked 2x daily. Oral discharge and skin/pelage findings (alopecia, cold to touch, red or yellow haircoat and rough haircoat) were noted more frequently (and dose dependently) in treated animals and are considered test article-related. The clinical signs were not present during the recovery phase and therefore are considered reversible.

Body Weights

Predose, then weekly. No changes noted.

Feed Consumption

Weekly for toxicity animals. No changes noted.

Ophthalmoscopy

Predose, then week 4. No changes noted.

Hematology

Day 28 of dosing and day 24 of recovery. Findings were few and limited to the 50 and 100 mg/kg doses.

There were several findings associated with lower red cell mass (i.e., red blood cell count, hemoglobin, and hematocrit) and mildly higher absolute reticulocyte count for all animals at 100 mg/kg/day. Reticulocyte count tended to be slightly higher for females at 20 or 50 mg/kg/day.

At the 50 and 100 mg/kg doses, there was a minimal to mild increase in platelet count. This was associated with a minimally lower PT time for females dosed at 50 or 100 mg/kg/day.

There was also a mild, but higher absolute neutrophil count for males given 100 mg/kg/day and monocyte count for males at 50 or 100 mg/kg/day.

Clinical Chemistry

Day 28 of dosing and day 24 of recovery. Clinical chemistry findings were only noted at the 50 or 100 mg/kg doses.

Findings included minimally to mildly higher total protein and globulin for animals given 50 or 100 mg/kg/day, mildly higher albumin for animals given 100 mg/kg/day, mildly higher cholesterol for females given 50 or 100 mg/kg/day, minimally lower aspartate aminotransferase activity for animals given 100 mg/kg/day, mildly higher calcium for animals given 100 mg/kg/day, and minimally lower chloride for females given 100 mg/kg/day. Albumin-to-globulin ratio was minimally lower for females given 50 or 100 mg/kg/day.

Higher urine volume and pH and lower urine specific gravity was noted for males and females given 100 mg/kg/day and females given 50 mg/kg/day.

All clinical pathology findings were reversible at the end of the recovery phase.

Gross Pathology

At sacrifice. No major findings noted.

Organ Weights

At sacrifice. Test article-related organ weight increases were noted in the liver of males and females given 50 or 100 mg/kg/day and in the thyroid/parathyroid of females given 100 mg/kg/day at the dosing phase final sacrifice. Liver weights remained slightly increased in males at 50 mg/kg/day and females at 50 and 100 mg/kg/day after a 4-week recovery phase.

Histopathology

Adequate Battery - yes

Peer Review – No.

Histological Findings
At sacrifice. Test article-related microscopic findings were noted in the liver of males and females given 100 mg/kg/day (slight hepatocellular hypertrophy) and the thyroid gland of males and females given 50 or 100 mg/kg/day (slight follicular cell hyperplasia/hypertrophy) after four weeks of dosing. No test article-related microscopic findings were noted in any male or female treatment group after a 4-week recovery phase.

The liver and thyroid effects could be attributed to adaptive changes which have been noted with microsomal enzyme inducers. However, without further data, monitoring for hepatic toxicities should be included in the clinical protocol.

Special Evaluation – CYP Activity

No changes in protein yield or total cytochrome P450 content were noted after exposure to GS-9350. Exposure to GS-9350 at 20, 50, and 100 mg/kg/day mildly increased activity of CYP3A (2.1 fold). Increases in CYP3A activity with no concurrent increase in protein yield was not explained in the sponsor's submission. This increase in activity is directly opposite the proposed MOA and is opposite from the CYP3A activity in the repeat dose study in the dog which could be due to species differences.

Table 80 - Four Week Repeat Dose Study in Rats: Cyp Activity

	Percent of Control							
Group:	2		3		4		5	
Dose Level (mg/kg/day):	10		20		50		100	
Parameter Sex:	M	F	M	F	M	F	M	F
Protein yield	92.5	101	88.5	91.3	91.6	96.6	75.8	86.9
Total cytochrome P450 content (CYP450)	115	101	111	96.3	121	110	131	116
Ethoxyresorufin O-deethylase (CYP1A)	128	108	130	110	150	157	174	116
Pentoxyresorufin O-dealkylase (CYP2B)	146	103	138	107	166	118	153	88.9
Testosterone 6β-hydroxylase (CYP3A)	133	148	175	**210**	**213**	**502**	151	**314**
Lauric acid 11-hydroxylase (CYP2E)	122	105	114	101	133	120	137	112
Lauric acid 12-hydroxylase (CYP4A)	140	108	99.3	94.7	153	111	124	89.3
Uridine diphosphoglucuronosyltransferase (UDPGT 4-MU)	94.1	122	105	128	128	140	146	163

M = Male.
F = Female.
Note: Values in bold indicate either a notable (2- to 4-fold) or marked (> 4-fold) change compared to the control value.

Table excerpted from Sponsor

Toxicokinetics

Increases in Cmax and AUC of GS-9350 were generally greater than dose proportional between 10 and 50 mg/kg/day, and slightly less than dose proportional between 50 and 100 mg/kg/day. In general, exposures were higher in females as compared to males. On Day 27, exposures were generally lower in animals administered 20, 50 and 100 mg/kg/day compared to Day 1 values.

Table 81 - Four Week Repeat Dose Study in Rats: Toxicokinetics

Dose Level		AUC_{0-t} (ng.hr/mL)		C_{max} (ng/mL)	
(mg/kg/day)	Sex	Day 1	Day 27	Day 1	Day 27
10	Male	639	1228	277	436
	Female	2975	3046	611	949
20	Male	6982	5758	1380	1319
	Female	15418	7483	2107	1663
50	Male	31004	21165	4007	3590
	Female	34667	24764	5030	5107
100	Male	67686	36973	6280	3790
	Female	51641	35741	5923	4690

Table excerpted from Sponsor

Dosing Solution Analysis

The stability of formulations prepared from 0.5 to 40 mg/mL was established after storage for 24 hours and for at least 8 and 15 days under room temperature conditions ((b) (4) Study 6511-334).

According to the sponsor, GS-9350 was a solution at the concentration range of the dose preparations used in this study, and homogeneity analysis was not required.

Study title: 26-Week Oral Gavage Toxicity and TK Study with GS-9350 in Rats with a 13-Week Recovery Period.

Study no.:	TX-216-2017
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 11, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-9350, Lot 3168-157-8, 50.5% pure (wt% in ethanol)

Key Study Findings

There were two drug related deaths (female only) at 100 mg/kg. Body weight loss and food consumption decreased at 100 mg/kg. Thyroid hormones (TSH, T4, or both) were altered somewhat at 30 mg/kg, and more pronounced at 100 mg/kg. Diuretic effects were noted at 100 mg/kg. Weak CYP1A inhibition was noted. Thyroid and liver weights increased at 30 and 100 mg/kg. Adrenal weights were increased at 100 mg/kg. Thyroid follicular cell hypertrophy was noted at 100 mg/kg. Liver cellular hypertrophy was noted at all doses, with increased occurrence in a dose-dependent manner.

A single male rat at the high dose (100 mg/kg) had a thyroid follicular cell carcinoma. Most of the animals at the 100 mg/kg dose had follicular cell hypertrophy in the thyroid and thyroid hormone changes. The thyroid changes were attributed to the adaptive changes in the liver caused by CYP inhibition. The thyroid and liver findings have been frequently reported in rodents exposed to microsomal enzyme inducers. No other carcinomas were noted in the other repeat dose toxicology studies.

NOAEL was 30 mg/kg based on the limited effects at 30 mg/kg and the more pronounced toxicity and drug-related deaths at 100 mg/kg.

Methods

Doses:	0, 10, 30, 100 mg/kg
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	2.5 mL/kg/day
Formulation/Vehicle:	95% propylene glycol, 5% ethanol with 1.5 eq. HCl, pH adjusted to 2.3 with HCl
Species/Strain:	SD Rats
Number/Sex/Group:	See table below
Age:	At least 7 wks old
Weight:	161-232 g (males) 133-187 g (females)
Satellite groups:	TK animals (see table below)
Unique study design:	None
Deviation from study protocol:	No significant deviations that would affect study outcome.

Table 82 - 26 Week Study in Rats - Overall Study Design

Group[a]	No. of Animals[b,c] Male	Female	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
Toxicity Animals				
1 (Control)	15	15	0	0
2 (Low)	10	10	10	4
3 (Mid)	10	10	30	12
4 (High)	15	15	100	40
Toxicokinetic Animals				
5 (Control)	5	5	0	0
6 (Low)	14	14	10	4
7 (Mid)	14	14	30	12
8 (High)	14	14	100	40

a Groups 1 and 5 received the formulation vehicle (95% propylene glycol, 5% ethanol with 1.5 eq. HCl, pH adjusted to 2.3 with HCl) only.
b Toxicity animals designated for recovery sacrifice (the last five animals/sex in Groups 1 and 4) underwent 13 weeks of recovery following dose administration.
c Two extra animals/sex in Groups 5 through 8 were assigned to study and used as replacement animals. One extra female was used to replace Group 7 female B36514 for blood collection and is included in the table for toxicokinetic purposes. Any replacement animal not used for blood collection was sacrificed and discarded with the surviving toxicokinetic animals after final blood collection.

Observations and Results

Mortality

Checked 2x daily. 5 deaths noted on study.

10 mg/kg:	1 female (not drug related)
30 mg/kg:	2 males (not drug related)
100 mg/kg:	2 females (likely related to drug treatment)

Clinical Signs

Checked 2x daily. Minor changes.

30 mg/kg:	Oral discharge (males only)
100 mg/kg:	Oral discharge (females and males)
	Rough haircoat (females and males)

Body Weights

Predose then weekly. Significant decreases in body weights in males at 100 mg/kg (a 16% decrease at week 26). This also correlated to a decrease (21%) in mean body weight gain during the dosing phase in males. The male recovery animals also had decreases (~7%) compared to controls. Females had similar decreases at 100 mg/kg during dosing and recovery, but the maximal decrease was about 7% of the control during both dosing and recovery.

No change in body weight at 10 or 30 mg/kg.

Figure 17 - Rat 26 Week Study – Mean Male Body Weights

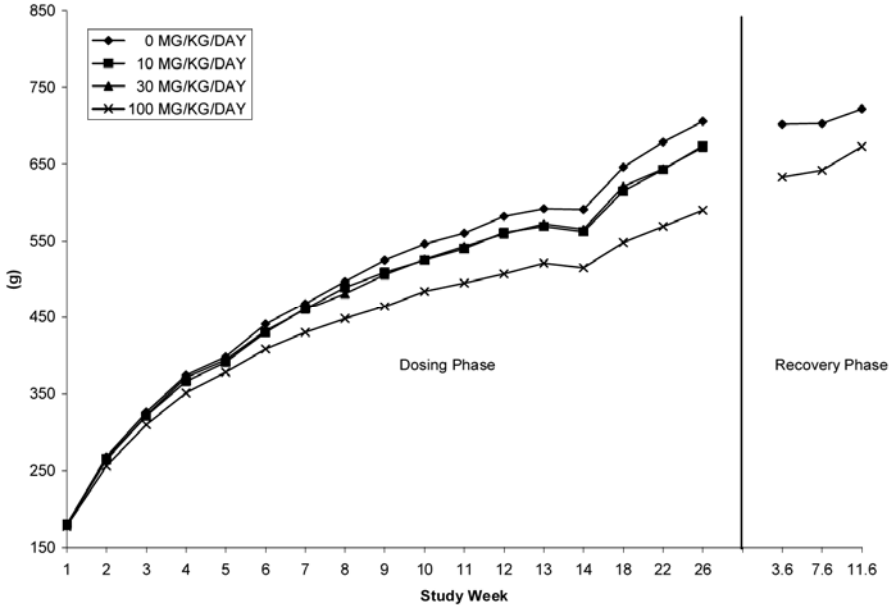
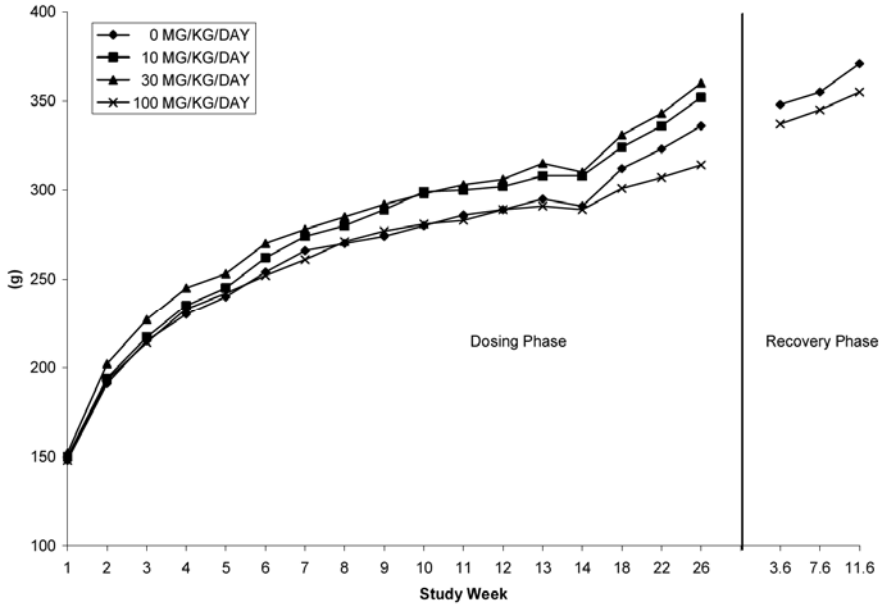


Figure 18 - Rat 26 Week Study – Mean Female Body Weights



Feed Consumption

Predose then weekly. Significant decreases (males only) at 100 mg/kg during dosing and recovery phases. Food consumption in females was not changed at any dose of GS-9350. No change at 10 or 30 mg/kg.

Ophthalmoscopy

Predose (all animals) as well as week 13 and 26 (toxicity animals only). No changes.

Hematology

Slightly lower mean values for erythrocyte count (RBC, males only), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) values in 100 mg/kg/day rats at Weeks 13 and 26. Slightly higher mean platelet counts in 30 mg/kg/day males at Week 13 and in 100 mg/kg/day males and females at Weeks 13 and 26.

The effects were reversible and were not noted after the recovery period

Clinical Chemistry

30 mg/kg:	Increase in total protein (females only), cholesterol (females only), and globulin (females only) Increase in TSH (1.6 to 2.4 fold) in females only
100 mg/kg:	Slightly higher GGT, cholesterol, total protein, albumin, globulin, and calcium in both sexes Decrease in T4 (decrease by roughly 1/4 to 1/2 of control) Increase in TSH (2 to 3.4 fold in males and 4.3 to 5.4 fold in females).

Urinalysis

GS-9350 appears to cause diuretic effects. Significantly higher urine volume and significantly lower urine osmolality (with lower urine specific gravity) were observed in 100 mg/kg/day rats at Weeks 4, 13, and 26. Similar changes, but of smaller magnitude, were also observed for these parameters in 30 mg/kg/day rats at Week 26 (significant for urine volume). There were no histological correlates to these findings.

Sodium levels were slightly elevated in the 100 mg/kg group. Inorganic phosphorus, calcium, potassium, and creatinine were slightly decreased in the 100 mg/kg group. These slight changes are consistent with higher urine volume and lower specific gravity in the urine.

By week 14 of recovery, all urinary parameters returned to normal.

Gross Pathology

At sacrifice. No gross findings were attributed to GS-9350.

Organ Weights

At sacrifice. Mean thyroid with parathyroid-to-body weights (males) and mean liver weights (absolute, organ-to-body weight percentages, and organ-to-brain ratios) in males and females increased at both 30 mg/kg and 100 mg/kg. The increased thyroid weights correlated with a microscopic diagnosis of follicular cell hypertrophy. The increased liver weights correlated with a microscopic diagnosis of centrilobular hepatocellular hypertrophy.

Adrenal (absolute and relative in males only) weights increased at 100 mg/kg but not at 30 mg/kg. Adrenal weight changes were not observed in females, or at the recovery sacrifice in males or females.

Pituitary, brain, pituitary, heart, testes, and spleen weights increased at 100 mg/kg but not at 30 mg/kg. Significantly increased organ weight changes at 100 mg/kg/day in the brain, pituitary, heart, testes, and spleen in dosing phase animals were attributed to decreased body weights.

These weight changes were without histopathologic correlates, were not considered adverse, and their relationship to treatment is unclear.

Histopathology

Adequate Battery – yes.

Peer Review - A pathology peer review was conducted of the spleen, thymus and lymph nodes from all Group 1 and Group 4 dosing and recovery phase animals, and from any unscheduled deaths.

Histological Findings: In the thyroid, histologic evaluation revealed a minimal to slight degree of follicular cell hypertrophy in the thyroid follicles of both sexes in association with increased thyroid weights. In the liver, the incidence and severity of centrilobular hepatocellular hypertrophy characterized by the presence of enlarged hepatocytes with poorly distinguishable cell borders and associated with pale to slightly eosinophilic cytoplasm were increased with dose.

A single male (#B36383) given 100 mg/kg/day had a thyroid follicular cell carcinoma. In general, the affected follicular cells in the follicular cell hypertrophy were enlarged and had vacuolated cytoplasm with decreased colloid in the follicle. These liver and thyroid effects are considered adaptive changes, which have been reported in rodents exposed to microsomal enzyme inducers and are likely secondary to the observed microsomal enzyme induction and increases in TSH levels. Further, as these morphological changes were completely resolved during the recovery phase, they were not considered adverse under the conditions of this study.

Table 83 – 26 Week Rat Study: Incidence of Notable Microscopic Observations in Thyroid and Liver

	Male				Female			
Dose Level, mg/kg/day	0	10	30	100	0	10	30	100
Thyroid Follicular Cell Hypertrophy								
Final Phase sacrifice								
Occurance/Number examined	0/10	0/10	0/9	10/10	0/10	1/8	1/10	7/8
Recovery sacrifice								
Occurance/Number examined	0/5	-	-	0/5	0/5	-	-	0/5
Thyroid Follicular Cell Carcinoma								
Final Phase sacrifice								
Occurance/Number examined	0/10	0/10	0/9	1/10	0/10	0/8	0/10	0/8
Recovery sacrifice								
Occurance/Number examined	0/5	-	-	0/5	0/5	-	-	0/5
Liver Hepatocellular Hypertrophy								
Final Phase sacrifice								
Occurance/Number examined	0/10	5/10	9/9	10/10	0/10	7/9	10/10	8/8
Recovery sacrifice								
Occurance/Number examined	0/3	-	-	0/4	0/5	-	-	0/5

Special Evaluation – Coagulation

Week 13 and prior to sacrifice. No effects noted.

Special Evaluation – Hepatic Microsomal Analysis

At sacrifice from first 5 toxicity animals/sex/group. The microsomal fractions were analyzed for microsomal protein, total cytochrome P450, CYP1A, CYP2B, CYP2B/C, CYP3A, CYP2E, CYP4A, and UDPGT activities.

No notable changes in protein yield, total CYP450 content, and CYP2B, CYP2E, CYP4A, and UDPGT activities. However, notable to marked increases in CYP1A and CYP3A activities were observed in females at 30 mg/kg/day, and in males and females at 100 mg/kg/day. Decreases in CYP2C activity were noted in males, with notable increases in females at 100 mg/kg/day. The maximum increase in CYP1A activity (3.5-fold increase in males and 2.4-fold in females) represents only a small fraction of that achievable by a positive control suggesting that GS-9350 is a weak inducer of CYP1A in this species.

Special Evaluation – Immunophenotyping

Prior to sacrifice. There was a slight increase in B cells at 30 and 100 mg/kg in both sexes. There was also a slight decrease in T cell subsets (CD4 and CD8) at 30 (females only) and 100 mg/kg (both sexes).

Special Evaluation – Hormone Analysis

Collected at week 13 (males only) and at sacrifice (both sexes). Test article effects in thyroid parameters included decreased thyroxine (T4) in 100 mg/kg/day males and dose-dependent increases in thyroid-stimulating hormone (TSH) in males and females. Mean TSH values were

consistently higher at each collection interval throughout the dosing phase (Weeks 4, 13, and 27) in 30 mg/kg/day females (ranging from 1.6- to 2.4-fold control) and 100 mg/kg/day rats (ranging from 2- to 3.4-fold in males and 4.3- to 5.4-fold control in females) and slightly higher at Week 27 in 10 mg/kg/day females (1.8-fold control) and Weeks 13 and 27 in 30 mg/kg/day males (ranging from 1.4- to 1.5-fold control). The TSH values decreased in 100 mg/kg/day rats after the 13-week recovery period at which time the mean values were slightly lower in males and slightly higher in females relative to controls. The T4 values were consistently lower in 100 mg/kg/day males at each collection interval throughout the dosing phase, but were similar to the control mean at the end of the recovery period.

Toxicokinetics

Day 1 and weeks 13 and 26. Exposure to GS-9350 increased with the increase in GS-9350 dose level from 10 to 100 mg/kg/day. Increases in C_{max} and AUC_{0-t} of GS-9350 were generally greater than dose proportional between 10 and 100 mg/kg/day. Exposures were higher in females as compared to males. In general, no accumulation of GS-9350 was observed after multiple dosing.

Table 84 - 26 Week Rat Study: Toxicokinetic Parameters for GS-9350 in Plasma on Day 1

Dose Group	GS-9350 Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	T_{last} (hr)	C_{last} (ng/mL)
6	10	M	132	1.00	382	8.00	17.9
		F	854	1.00	2053	8.00	10.2
7	30	M	1646	2.00	5662	12.0	8.42
		F	2830	1.00	13867	12.0	666
8	100	M	3720	1.00	32711	24.0	36.6
		F	4970	1.00	42886	24.0	813

Table excerpted from Sponsor's application

Table 85 - 26 Week Rat Study: Toxicokinetic Parameters for GS-9350 in Plasma on Week 13

Dose Group	GS-9350 Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	T_{last} (hr)	C_{last} (ng/mL)
6	10	M	161	1.00	651	8.00	24.3
		F	823	1.00	2003	8.00	26.9
7	30	M	1374	2.00	8226	12.0	6.61
		F	2090	2.00	10370	12.0	29.4
8	100	M	3807	2.00	29920	24.0	6.99
		F	5680	1.00	51558	24.0	5.77

Table excerpted from Sponsor's application

Table 86 - 26 Week Rat Study: Toxicokinetic Parameters for GS-9350 in Plasma on Week 26

Dose Group	GS-9350 Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	T_{last} (hr)	C_{last} (ng/mL)
6	10	M	797	2.00	2093	8.00	25.5
		F	1315	1.00	3516	8.00	90.2
7	30	M	1933	1.00	9916	12.0	9.26
		F	3993	1.00	13297	12.0	8.24
8	100	M	4400	4.00	47214	24.0	48.5
		F	7670	4.00	71448	24.0	74.6

Table excerpted from Sponsor's application

Dosing Solution Analysis

The stability of samples prepared at 0.9259 and 277.8 mg/mL was confirmed for 15 days at room temperature ((b) (4) Study 6511-334).

Samples prepared at 0.9259 and 277.8 mg/mL were confirmed to be solutions under (b) (4) Study 6511-334; therefore, homogeneity analysis was not necessary.

Study title: 4-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Dogs with a 4-Week Recovery Phase

Study no.:	TX-216-2005
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	30 Aug 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-9350, Lot# 3168-121-23, 92.9% pure

Key Study Findings

There was a decrease in body weights (and food consumption) at 45 mg/kg, which required food supplementation and dose reduction in the females. 1 male in the 15 mg/kg group also required supplemental food due to inappetance and body weight loss. Animals dosed at 15 and 45 mg/kg had GI distress (vomiting, loose stools). There were effects noted on the liver characterized by increased liver weight parameters and increased cytoplasmic vacuolation in males and females and minimal to mild increases (reversible) in clinical chemistry parameters (bilirubin, alanine aminotransferase, and alkaline phosphatase activities) in males given 45 mg/kg/day. No treatment-related liver changes were present in males or females at the end of the recovery phase sacrifice interval.

Exposure to GS-9350 was increased (greater than dose proportionally) between week 1 and 4. Accumulation of GS-9350 was evident between week 1 and week 4.

Sponsor proposed NOAEL in dogs at 15 mg/kg for 4 weeks. This reviewer considered the NOAEL to be 5 mg/kg, based on toxicities noted at both 15 and 45 mg/kg, but not at 5 mg/kg. Although the findings at 15 mg/kg were not as severe as 45 mg/kg, they were still considered adverse.

Methods

Doses:	0, 5, 15, 45 mg/kg (45 then 30 mg/kg for females – dose reduction required)
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	2 ml/kg
Formulation/Vehicle:	95% propylene glycol (PG), 5% ethanol (v/v), pH adjusted to 2.3 + 0.1 with hydrochloric acid
Species/Strain:	Beagle dog
Number/Sex/Group:	3 or 5 per sex per group (see table below)
Age:	6 to 7 months old
Weight:	6.4 to 9.3 kg for males; 6.6 to 9.3 kg for females
Satellite groups:	Recovery animals at 0, 15, and 45 mg/kg (2/sex/group) TK Samples were taken on Day 1 and Week 4 from all groups. The 45 mg/kg group was also bled on day 15.
Unique study design:	None
Deviation from study protocol:	

Group	No. of Animals[b] Male	Female	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)[c]
1 (Control)[a]	5	5	0	0
2 (Low)	3	3	5	2.5
3 (Mid)	5	5	15	7.5
4 (High)	5	-	45	22.5
4 (High)	-	5	45/30[d]	22.5/15[d]

a Group 1 received control article only.
b Animals designated for recovery phase sacrifice (one male and two females in Group 1, two animals/sex in Groups 3 and 4) underwent 4 weeks of recovery following dose administration.
c Dose volume was 2 mL/kg.
d Group 4 females were dosed at 45 mg/kg/day on Days 1 through 10, dosing was suspended on Days 11 through 13, and dosing was resumed at 30 mg/kg/day beginning on Day 14.

Observations and Results

Mortality

Checked 2x daily. 2 animals were sacrificed moribund (1 female at 15 mg/kg; 1 male at 0 mg/kg) due to dosing procedure complications. The female animal died immediately after dosing on day 1 and was replaced. Data for this animal is included in the raw data, but not the tabulated data.

Clinical Signs

Checked 2x daily. No effects were noted in animals dosed 5 mg/kg.

Dose-related emesis and salivation, observed within 1 hour of dosing, was noted in most animals at 15 and 45 mg/kg/day. Emesis occurred frequently in the 15 mg/kg and throughout the dosing

phase in the 45 mg/kg groups. Other effects noted in the 15 and 45 mg/kg group included: salivation, hypoactivity, and liquid/mucoid feces.

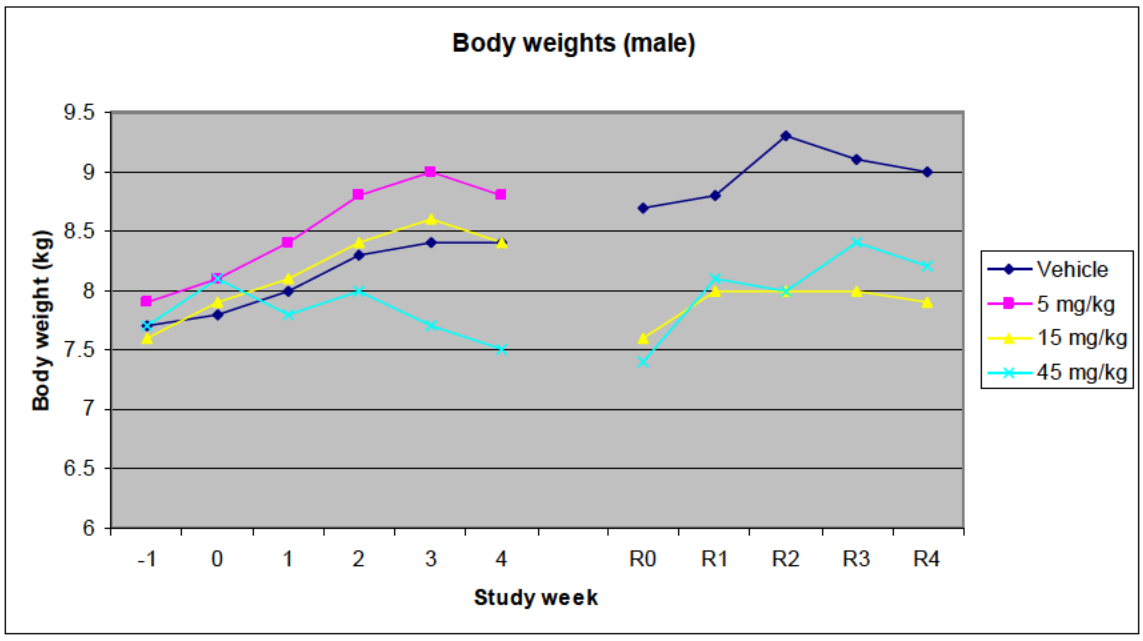
Animals dosed at 45 mg/kg exhibited a thin body condition and had cold muzzles.

One female (control group, day 11) and 1 male (45 mg/kg group, day 22) were diagnosed with giardia and were administered metronidazole and fenbendazole orally during the study period.

Body Weights

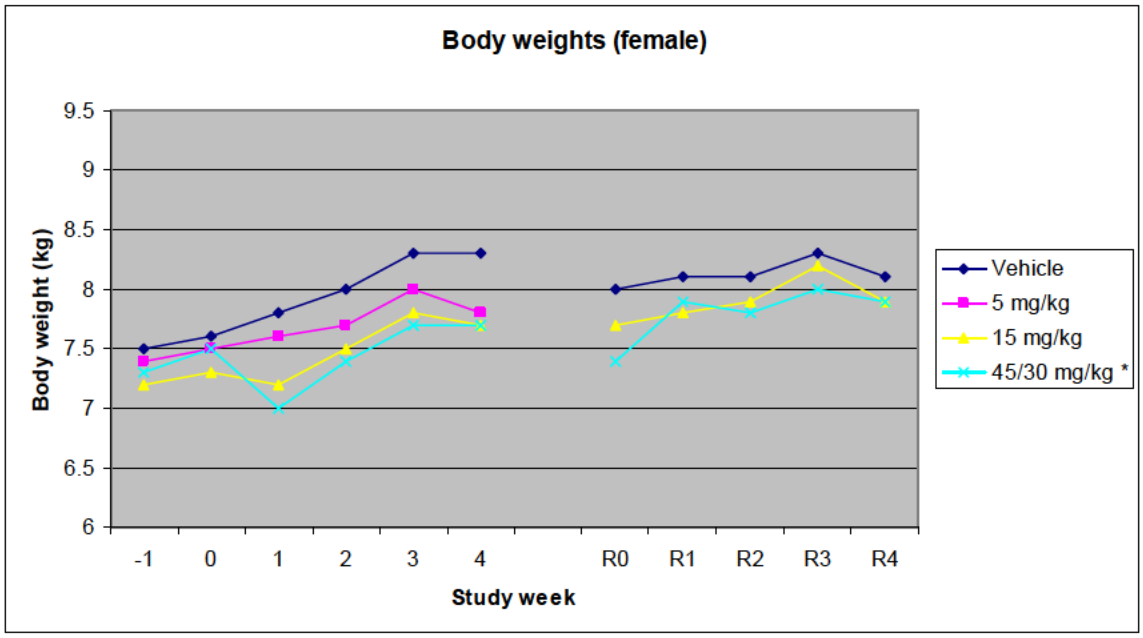
Predose then weekly. Males: Mean body weight change was significantly reduced for males at 45 mg/kg on weeks 3 and 4. Body weights recovered to normal levels in all groups after cessation of drug administration. Females: Mean body weights were reduced for females at 45 mg/kg on week 1 to such an extent that a modified diet (canned food) and reduction of dose (to 30 mg/kg) was required to prevent morbidity and mortality. Body weights increased to normal levels by week 3 and 4 at the 30 mg/kg dose. Body weights recovered to normal levels in all groups after cessation of drug administration.

Figure 19 - Four Week Repeat Dose Study in Dogs - Body Weights (Male)



Study weeks labeled with an "R" are the Recovery groups.

Figure 20 - Four Week Repeat Dose Study in Dogs - Body Weights (Female)



Study weeks labeled with an "R" are the Recovery groups.
** Note: Female dogs in the 45 mg/kg group had the dose reduced to 30 mg/kg after study week 1 accompanied by a modified (canned) diet.*

Feed Consumption

Predose then weekly. Food consumption was not altered for the 5 mg/kg group. The 15 mg/kg (1 male) and 45 mg/kg groups (all females; 2 males) were given supplemental canned food due to thin body condition or weight loss. Therefore the NOAEL based on food consumption (and loss of appetite) is 5 mg/kg.

Ophthalmoscopy

Predose then during week 4. No significant findings were noted.

ECG and Blood Pressure

Predose, week 1, week 4. Mild increase in PR interval on day 3 for for females dosed 15 and 45 mg/kg. Mild increase in RR interval on day 22 for males dosed 45 mg/kg. No arrhythmias found.

Blood pressure: No significant findings were noted.

Hematology

At sacrifice. No significant findings were noted.

Coagulation

At sacrifice. No significant findings were noted.

Clinical Chemistry

At sacrifice. No significant findings were noted for 5 or 15 mg/kg groups. Males dosed 45 mg/kg had mildly higher bilirubin, ALT, and ALP compared to controls. The increase in these parameters occurred at week 4 and resolved during recovery.

Urinalysis

No significant findings were noted.

Gross Pathology

At sacrifice. No significant findings were noted.

Organ Weights

At sacrifice. No significant findings were noted at 5 mg/kg.

Liver:

Liver weight parameters (liver-to-body and liver-to-brain ratio) were significantly increased (~ 1.4x control values) after 4 weeks of dosing for females given 45/30 mg/kg/day. The absolute mean liver weight for this group was also increased (~ 1.3x control value but not statistically significant). Liver weight parameters (liver-to-body and liver-to-brain ratio) were slightly higher (~ 1.2 to 1.3x control values) in males given 15 or 45 mg/kg/day, but the group differences were not statistically significant. The differences in liver weights returned to normal during the recovery phase.

Thymus:

Thymus weight parameters (absolute and/or thymus-to-brain ratio) were significantly decreased for females (15 mg/kg and 45 mg/kg). Other immune parameters were not affected, so it could be related to an indirect stress response after drug exposure.

Brain:

Brain weight (absolute value) was decreased at 15 and 45 mg/kg in females. A similar decrease (~ 1.1x decrease; not statistically significant) was noted in males dosed 45 mg/kg. The ratio of brain/body weight did not show significant changes between the groups.

Kidney:

Kidney weight (kidney-to-body ratio) was significantly decreased in females dosed 45 mg/kg. Although absolute weights were not statistically significant, males and females dosed 45 mg/kg had increases in absolute kidney weights (~1.3x and 1.2x control values).

Histopathology

Adequate Battery – Yes.

Peer Review – No.

Histological Findings: At sacrifice. No significant findings were noted. However, vacuolation of hepatocytes was slightly increased in severity in males and females given 45 or 45/30 mg/kg/day. These findings were undetectable in the recovery animals.

Table 87 - Four Week Repeat Dose Study in Dogs - Histological Findings

Sex:	Male				Female			
Dose (mg/kg/day):	0	5	15	45	0	5	15	45/30
No. Examined:	4	3	3	3	3	3	3	3
Vacuolation, Hepatocyte								
Minimal	2	1	0	0	3	0	2	1
Slight	1	2	3	1	0	3	1	0
Moderate	1	0	0	2	0	0	0	2

Table excerpted from Sponsor

Special Evaluation – CYP Activity

No changes in protein yield or total cytochrome P450 content were noted after exposure to GS-9350. Exposure to 5, 15, and 45 mg/kg/day decreased activity of CYP3A. This decrease in activity agrees with the proposed MOA and is opposite from the CYP3A activity reported in the repeat dose study in the rat.

Table 88 - Four Week Repeat Dose Study in Dogs - CYP Activity

		Percent of Control					
	Group:	2		3		4	
	Dose Level (mg/kg/day):	5		15		45/30[a]	
Parameter	Sex:	M	F	M	F	M	F
Terminal Sacrifice							
Protein yield		95.0	89.0	96.5	103	116	97.9
Total cytochrome P450 content (CYP450)		70.6	85.2	71.1	76.3	74.2	55.7
Ethoxyresorufin O-deethylase (CYP1A)		84.0	65.4	71.5	71.1	51.4	62.6
Pentoxyresorufin O-dealkylase (CYP2B)		106	133	121	121	73.3	90.0
Testosterone 6β-hydroxylase (CYP3A)		**44.1**	60.7	**44.0**	**44.3**	**20.0**	**30.8**
Lauric acid 11-hydroxylase (CYP2E)		87.2	82.7	84.2	76.9	71.5	63.0
Lauric acid 12-hydroxylase (CYP4A)		90.5	103	102	118	110	108
Uridine diphosphoglucuronosyltransferase (UDPGT)		106	98.1	112	94.5	109	98.4
Recovery Sacrifice							
Protein yield		NA	NA	125	110	112	96.0
Total cytochrome P450 content (CYP450)		NA	NA	96.5	95.5	82.6	96.4
Ethoxyresorufin O-deethylase (CYP1A)		NA	NA	110	150	66.2	179
Pentoxyresorufin O-dealkylase (CYP2B)		NA	NA	116	139	115	117
Testosterone 6β-hydroxylase (CYP3A)		NA	NA	105	103	106	111
Lauric acid 11-hydroxylase (CYP2E)		NA	NA	87.6	108	83.8	112
Lauric acid 12-hydroxylase (CYP4A)		NA	NA	95.9	93.0	94.1	98.9
Uridine diphosphoglucuronosyltransferase (UDPGT)		NA	NA	99.1	113	93.1	105

M = Male.
F = Female.
NA = Not applicable.
Note: Values in bold represent a change, which is less than half of the control values.
a Female animals were dosed at 45 mg/kg/day on Days 1 through 10, dosing was suspended on Days 11 through 13, and resumed at 30 mg/kg/day on Day 14. Male animals were dosed at 45 mg/kg/day.

Table excerpted from Sponsor

Toxicokinetics

Exposure to GS-9350 increased with the increase in dose. Accumulation of GS-9350 in dog plasma was observed after 4 weeks of daily dosing. Increases in exposure (C_{max} and AUC_{0-t}) were, in general, greater than dose proportional between 5 and 15 mg/kg/day doses, and dose proportional between 15 and 45/30 mg/kg/day doses. There didn't appear to be any sex differences in exposure.

Table 89 - Four Week Repeat Dose Study in Dogs - Toxicokinetics

Dose Level		AUC_{0-t} (ng·hr/mL)		C_{max} (ng/mL)	
(mg/kg/day)	Sex	Day 1	Week 4	Day 1	Week 4
5	M	457	4390	195	1140
	F	251	2175	119	739
15	M	17,185	22,859	3004	3520
	F	15,673	34,664	2569	4456
45	M	57,156	89,045	7162	7048
45[a]	F	61,608	-[b]	6436	-[b]
30[a]	F	53,805[c]	53,590[d]	6928[c]	6036[d]

a Females were dosed at 45 mg/kg/day on Days 1–10, and at 30 mg/kg/day on Days 14–28
b Not Applicable
c Day 15 (Day 2 at 30 mg/kg/day)
d Day 23 (after 10 days dosing at 30 mg/kg/day)

Table excerpted from Sponsor

Dosing Solution Analysis

For the concentration range of the GS-9350 dose preparations, the mixtures were solutions; therefore, no homogeneity analysis was necessary.

The stability of formulations prepared from 1.0 to 300 mg/mL was confirmed after storage for 24 hours, and for 8 and 15 days under room temperature conditions ([(b) (4)] Study 6511-334).

Study title: 39-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Dogs with a 13-Week Interim Necropsy and a 13-Week Recovery Period

Study no.:	TX-216-2016
Study report location:	EDR
Conducting laboratory and location:	[(b) (4)]
Date of study initiation:	10 March 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-9350, Lot 3168-157-8, 50.5% pure (wt%)

Key Study Findings

Salivation and vomiting were noted. Clinical signs (increased salivation, emesis, and fecal changes), decreased body weight and food consumption, and minimally adaptive changes in the liver (increased weights and hypertrophy) were noted at 20 mg/kg at 13 and 39 week sacrifice. Supplemental food was offered to 20 mg/kg groups beginning at week 10 due to significant body weight loss.

Changes in thymus (atrophy) and adrenal glands (increases with hypertrophy) was observed at 20 mg/kg at 13 and 39 weeks. These changes were not noted in recovery which support chemical stress rather than drug specific effects as the cause. Increased urine in females was at 20 mg/kg (weeks 13 and 39). Increased incidence of bilirubinuria noted in males at 20 mg/kg (13 and 39 weeks). Limited effects were noted at 10 mg/kg – minimal hepatocelluar hypertrophy in males and slightly increase liver weights in females.

NOAEL was 10 mg/kg based on the body weight loss and decreased food consumption (overt toxicity) at 20 mg/kg and lack of significant findings at 10 mg/kg.

At the NOAEL of 10 mg/kg/day at Week 39 the C_{max} and AUC_{0-t} corresponded to 2640 and 2418 ng/mL and 19589 and 16817 ng•h/mL in males and females, respectively.

Methods

Doses:	0, 5, 10, 20 (see table below)
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	95% propylene glycol (PG), 5% ethanol (EtOH) with 1.5 eq. HCl, pH adjusted to 2.3 with HCl.
Species/Strain:	Beagle dog, (b) (4)
Number/Sex/Group:	9 or 7 per sex per group (see table below)
Age:	~ 7 months old
Weight:	8.0 to 10.3 kg for males and 6.7 to 8.5 kg for females.
Satellite groups:	Animals designated for interim sacrifice (three/sex in Groups 1, 2, 3 and 4) were terminated after at least 13 weeks of dose administration. Four animals/sex/group were designated for terminal sacrifice after at least 39 weeks of dose administration. The remaining two animals/sex in Groups 1 and 4 underwent 13 weeks of recovery following at least 39 weeks of dose administration.
Unique study design:	None.
Deviation from study protocol:	Two minor deviations. Neither would affect the study interpretation or outcome.

Group[a]	No. of Animals[b] Male	Female	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
1 (Control)	9	9	0	0
2 (Low)	7	7	5	2.5
3 (Mid)	7	7	10	5
4 (High)	9	9	20	10

a Group 1 received control article only [95% propylene glycol and 5% ethanol with 1.5 eq. HCl (pH 2.3)].
b Animals designated for interim sacrifice (three/sex in Groups 1, 2, 3 and 4) were terminated after at least 13 weeks of dose administration. Four animals/sex/group were designated for terminal sacrifice after at least 39 weeks of dose administration. The remaining two animals/sex in Groups 1 and 4 underwent 13 weeks of recovery following at least 39 weeks of dose administration.

Observations and Results

Mortality

Twice daily. No deaths on study.

Clinical Signs

Twice daily. No adverse changes noted in at 5 mg/kg or 10 mg/kg. Excessive salivation occurred in all dose groups and was dose-dependent.

Dose-related clinical signs at 20 mg/kg also included, emesis (containing food, foamy, white and/or yellow), and fecal changes (discolored, liquid, mucoid and nonformed). Emesis and salivation were primarily observed within 1 hour of dosing.

Veterinarian treatments were mainly conducted for body weight loss, low food consumption and thin body condition. Supplemental food (canned food) was necessary on occasion.

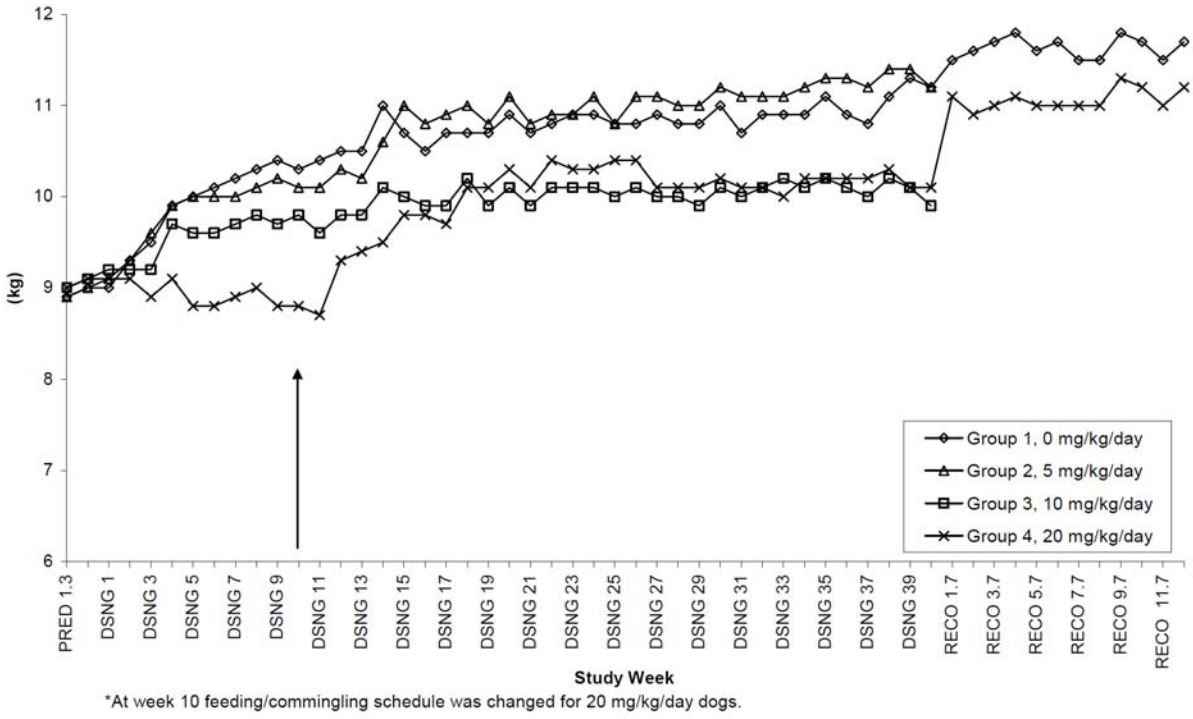
Body Weights

Predose then weekly. Significant, GS-9350 dose-related, decreases in mean body weight were observed at 20 mg/kg/day during Weeks 5-12 and 14, and Weeks 3-14 of the dosing phase for males and females, respectively, corresponding with significantly lower food consumption. Slight (not statistically significant) body weight decreases noted at 10 mg/kg after week 5.

The mean body weight for 20 mg/kg/day dogs remained slightly lower but similar to controls throughout the dosing phase after the commingling schedule for this group was altered to allow continuous access to food overnight with supplemental canned food for some dogs beginning at Week 10 of study.

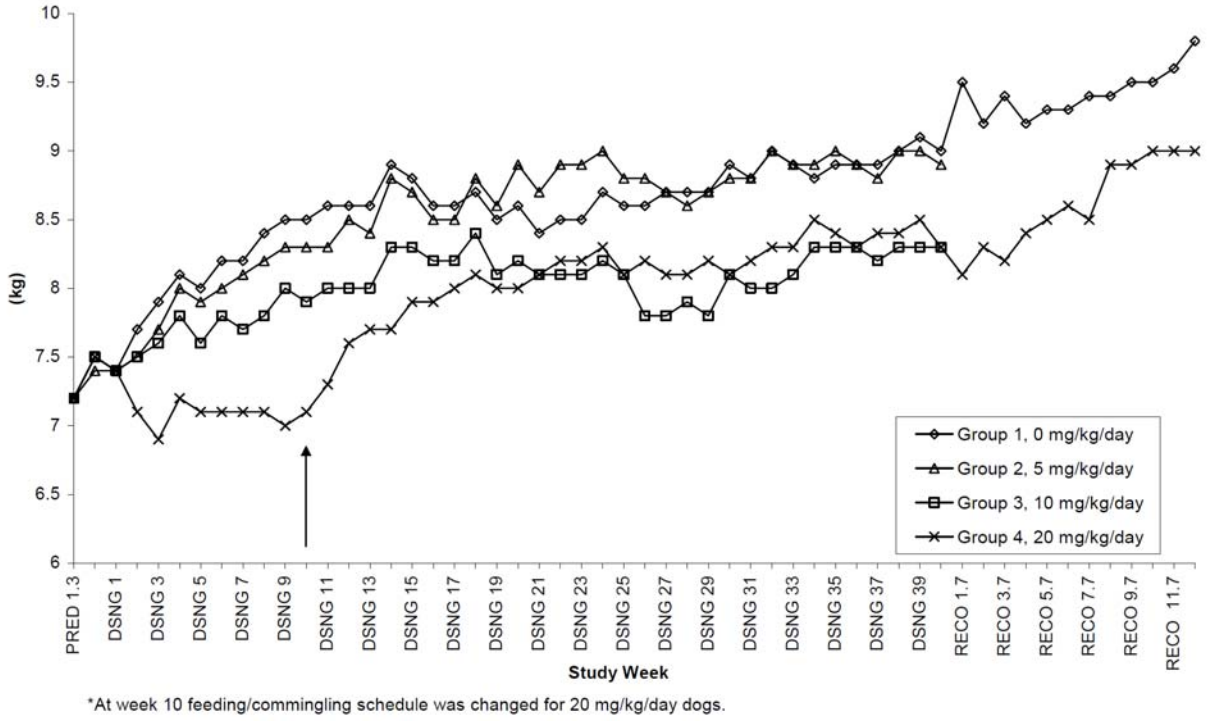
Significantly reduced mean terminal body weights (85% of control) were noted in terminal sacrifice males at 20 mg/kg/day. Slight, but not statistically significant, reductions (≥90% of control) in terminal body weights were also noted in males at 10 mg/kg/day and in females at 10 and 20 mg/kg/day.

Figure 21 - 39 Week Dog Study: Body Weights in Males



*At week 10 feeding/commingling schedule was changed for 20 mg/kg/day dogs.

Figure 22 -39 Week Dog Study: Body Weights in Females



*At week 10 feeding/commingling schedule was changed for 20 mg/kg/day dogs.

Feed Consumption

Weekly. Significant decreases in mean food consumption (not including canned supplemental food) were noted in both sexes at 20 mg/kg/day mainly during the first 14 weeks of the dosing phase; slight decreases in the 10 mg/kg/day males did not reach statistical significance except during Week 39, when the mean food consumption was significantly decreased for all male groups compared to the control group. In general, decreases in food consumption corresponded to the decreases in body weight.

Supplemental food was offered to 3 males and 5 females at 20 mg/kg, which corresponded to overt toxicity and decreased body weights.

Ophthalmoscopy

Predose then week 13 and week 29. No changes noted.

ECG and Blood Pressure

Predose then week 13 and week 39. All the electrocardiograms through Week 39 evaluated in this study were qualitatively and quantitatively considered normal for the canine. No arrhythmias were found.

No changes in blood pressure were noted.

Hematology

Weeks 13, 26 (interim – no sacrifice), and at terminal and recovery sacrifices. Slight (not significant) drug-related changes in hematology included slightly higher mean platelet counts in 20 mg/kg/day dogs at Weeks 13, 26 and 39 of the dosing phase, and in 10 mg/kg/day females at Week 26.

Clinical Chemistry

Weeks 13, 26 (interim – no sacrifice), and at terminal and recovery sacrifices. No significant changes. Slightly higher platelet counts and ALP as well as slightly lower total protein and albumin at 20 mg/kg (not significant). No changes in thyroid hormone parameters.

Urinalysis

Weeks 13, 26 (interim – no sacrifice), and at terminal and recovery sacrifices.. Diuretic effect noted. Slightly higher urine volume, lower urine osmolality and lower urine specific gravity, observed in a few females at 20 mg/kg/day during the dosing phase (primarily at Weeks 13 and 26) resulted in slightly to moderately lower urinary sodium, potassium, chloride, calcium, and inorganic phosphorus concentrations and excretion rates. There was also a greater incidence of bilirubinuria in males at 20 mg/kg/day throughout dosing.

Gross Pathology

At sacrifice. No significant effects.

Organ Weights

At sacrifice. Significant decrease (approximately 45% of control at week 13) in mean thymus weight and a minimal increase (approximately 129% of control at week 13) in mean adrenal weight in males at 20 mg/kg/day. At the terminal sacrifice (week 39), minimally adaptive changes in the liver (increased weights - 113% and 124% in males and females with associated hypertrophy) were noted at 20 mg/kg.

Limited effects at 10 mg/kg – minimal hepatocelluar hypertrophy in males and slightly increase liver weights in females.

Histopathology

Adequate Battery – yes.

Peer Review – No.

Histological Findings: At sacrifice (weeks 13, 39, and recovery).

- At the interim (13 week) sacrifice, microscopic changes occurred in the thymus, and adrenal cortex of all males at 20 mg/kg/day, and in the adrenal cortex of one female at 20 mg/kg/day.
- At the terminal sacrifice (week 29), minimal diffuse hepatocellular hypertrophy was observed in three of four males at 10 or 20 mg/kg/day and in all four females at 20 mg/kg/day. There was no evidence of degeneration and was not considered adverse.
- The thymus, adrenal, and liver findings were not detectible at the recovery sacrifice.

Special Evaluation - Immunophenotyping

At week 26 (interim with no sacrifice). No changes in total T cells, helper T cells, cytotoxic T cells, and B cells) compared with controls.

Toxicokinetics

Blood samples (approximately 2 mL) were collected via a jugular vein predose and at approximately 0.5, 1, 2, 4, 8, 12 and 24 hours postdose on Day 1, and during Weeks 13 and 39 of the dosing phase. Exposure to GS-9350 (COBI) increased with the increase in GS-9350 dose level from 5 to 20 mg/kg/day. Increases in mean C_{max} and AUC_{0-t} were generally greater than dose proportional. In general, no marked (> 2-fold) sex differences were observed in GS-9350 C_{max} and AUC_{0-t} values. Accumulation of GS-9350 was observed in dog plasma after multiple dosing of GS-9350.

Exposure to metabolite GS-9612 generally increased with the increase in GS-9350 dose level from 5 to 20 mg/kg/day. No consistent sex differences were observed in GS-9612 C_{max} and AUC_{0-t} values. Accumulation of GS-9612 was observed in dog plasma after multiple dosing of GS-9350. The percent metabolite to parent ratios for C_{max} and AUC_{0-t} ranged from 1.3 to 9.7%, indicating that GS-9350 is not extensively converted to GS-9612 in dogs following oral gavage administration of GS-9350.

Table 90 - 39 Week Study in Dogs: Toxicokinetic Analysis of GS-9350

Dose Group	GS-9350 Dose Level (mg/kg/day)	Gender	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)
			Day 1		
2	5	M	112	2.00	248
		F	42.8	1.67	116
3	10	M	1115	2.43	5251
		F	311	2.29	1142
4	20	M	3650	2.44	26382
		F	3697	3.89	30763
			Week 13		
2	5	M	1258	1.21	5894
		F	724	2.14	2984
3	10	M	3404	1.86	22174
		F	2471	2.14	15882
4	20	M	4459	1.50	39667
		F	6448	3.83	75928
			Week 39		
2	5	M	1029	2.00	5270
		F	1738	0.875	9123
3	10	M	2640	3.50	19589
		F	2418	3.00	16817
4	20	M	7090	3.67	71269
		F	8405	1.00	99671

Table 91 - 39 Week Study in Dogs: Toxicokinetic Analysis of GS-9612 (Metabolite)

Dose Group	GS-9350 Dose Level (mg/kg/day)	Gender	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	M/P Ratio[1] (%) C_{max}	M/P Ratio[1] (%) AUC_{0-t}
			Day 1				
2	5	M	9.07	1.57	20.9	8.1	9.7
		F	3.95	2.00	9.15	9.2	8.5
3	10	M	44.8	1.71	181	4.0	3.7
		F	15.0	2.14	51.9	4.8	5.7
4	20	M	174	2.33	1084	4.8	4.1
		F	101	2.67	822	2.7	2.9
			Week 39				
2	5	M	24.6	2.00	118	2.4	2.5
		F	42.9	2.50	239	2.5	2.6
3	10	M	33.1	4.00	257	1.3	1.3
		F	42.3	4.00	296	1.8	1.7
4	20	M	114	3.92	1534	1.6	1.8
		F	285	1.67	2796	3.4	2.5

[1] Metabolite (GS-9612) to parent (GS-9350) ratio

Dosing Solution Analysis

Samples prepared at 0.9259 mg/mL to 277.8 mg/mL were confirmed to be solutions under (b) (4) Study 6511-334; therefore homogeneity analysis is not necessary.

The stability of samples prepared at 0.9259 and 277.8 mg/mL was confirmed for 15 days at room temperature ((b) (4) Study 6511-334).

Dose formulations were solutions, and routine concentration analyses were within 7.9% of target and acceptable for use on study ((b) (4) Study 6511-334).

6.3 Combination Toxicity Studies with Cobicistat

Study title: Five Day Oral Toxicity and TK Study of GS-9350 and Atazanavir in Female SD Rats

Study no.:	TX-216-2027 and TX-216-2027-TK
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	23 July 2008
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	The pre-formulated dosing formulations were used as received from the Sponsor and no formulation preparation was required (b) (4) See table below for further information.

Formulation	Lot Number	Group	Dose Level mg/kg[1]	Target Concentration mg/mL[1]
Placebo, blank vehicle [2]	QN-3160-154-D	1	0	0
GS-9350	QN-3160-154-1	2 / 6	50	10
Atazanavir (ATV, atazanavir bisulfate)	QN-3160-154-2	3 / 7	150	30
GS-9350 / ATV	QN-3160-154-3	4 / 8	30 / 100	6 / 20
GS-9350 / ATV	QN-3160-154-4	5 / 9	50 / 150	10 / 30

[1] ATV doses and concentrations based on ATV free base equivalents
[2] Placebo is the blank vehicle as was 5% ethanol / 95% propylene glycol (v/v)

Key Study Findings

The purpose of this range finding combination toxicology study was to evaluate the toxicity and toxicokinetics of the test articles [GS-9350 and atazanavir (ATV)] when administered alone or in combination daily by oral gavage to rats for five days.

There were no adverse effects noted in female rats administered 50 mg/kg/day GS-9350 or ATV 30/100 mg/kg/day by oral gavage for five days. Increases in liver transaminases and/or bilirubin levels were noted in animals administered 150 mg/kg/day ATV or GS-9350/ATV 50/150 mg/kg/day. Increased liver weights were noted in animals administered 50 mg/kg/day GS-9350, and GS-9350/ATV 30/100 and 50/150 mg/kg/day.

Plasma exposure of ATV increased when co-administered with GS-9350, compared to ATV administered alone. GS-9350 plasma exposure decreased approximately 30-50% when co-administered with ATVcompared to GS-9350 administered alone.

The decreases in COBI (GS-9350) in rats could be due to autoinduction of CYP3A in rats as well as activation of PXR (which did not appear to occur in other species).

Methods

Doses:	See table below
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	5 ml/kg
Formulation/Vehicle:	Vehicle unidentified. "clear colorless liquid" provided by Gilead Sciences to CRO.
Species/Strain:	SD rats
Number/Sex/Group:	See table below. Females only.
Age:	13.5 wks at study start
Weight:	228 to 269 g at study start
Satellite groups:	See below
Unique study design:	Combination toxicology study
Deviation from study protocol:	Not noted.

Group Assignments

Group	Treatment	Dose Level (mg/kg/day)	Number of Animals Female
1	Vehicle	0	5
2	GS-9350	50	5
3	ATV	150	5
4	GS-9350 + ATV	30 + 100	5
5	GS-9350 + ATV	50 + 150	5
6[a]	GS-9350	50	9 + 1[b]
7[a]	ATV	150	9 + 1[b]
8[a]	GS-9350 + ATV	30 + 100	9 + 1[b]
9[a]	GS-9350 + ATV	50 + 150	9 + 1[b]

[a]Designated TK animals
[b]One extra animal per group was dosed to serve as a possible replacement animal.

Observations and Results

Toxicokinetics

Plasma exposure of ATV increased when co-administered with GS-9350 (30 or 50 mg/kg/day), compared to ATV administered alone at 150 mg/kg/day. When co-administered with GS-9350, ATV plasma exposure also increased with dose in the range of 100 to 150 mg/kg/day. The systemic exposure of ATV in rat plasma decreased approximately 30% after 5 days of oral dosing with or without GS-9350. Co-administration of ATV at 150 mg/kg/day with 50 mg/kg/day GS-9350, resulted in 2.6-fold and 2.7-fold increases in exposure (AUC_{last}) on Day 1 and Day 5, respectively, compared to ATV administration at 150 mg/kg/day alone.

GS-9350 plasma exposure decreased approximately 40-50% when co-administered with ATV (150 mg/kg/day) compared to GS-9350 administered alone at 50 mg/kg/day. After 5 days of co-administration of GS-9350 and ATV at 30/100 or 50/150 mg/kg/day, GS-9350 exposure (AUC_{last}) decreased approximately 30-50% compared to Day 1 exposures.

Table 92 - 5 Day Rat Combination Toxicology and TK Study: ATZ Toxicokinetics

Dose Level (mg/kg/day)	AUC_{last} (µg•hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	C_{last} (µg/mL)	T_{last} (hr)
Day 1					
*Gr.7, 150	44.2	5.07	2.0	0.07	24.0
Gr.8, 100 (+GS-9350, 30)	64.2	9.40	2.0	0.84	24.0
Gr.9, 150 (+GS-9350, 50)	104	11.7	4.0	1.80	24.0
Day 5					
*Gr.7, 150	29.8	3.27	2.0	0.14	24.0
Gr.8, 100 (+GS-9350, 30)	45.8	3.37	2.0	1.08	24.0
Gr.9, 150 (+GS-9350, 50)	71.8	5.93	1.0	5.20	24.0

*Group 7 was administered ATV only

Table 93 - 5 Day Rat Combination Toxicology and TK Study: COBI Toxicokinetics

Dose Level (mg/kg/day)	AUC_{last} (µg•hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	C_{last} (µg/mL)	T_{last} (hr)
Day 1					
*Gr.6, 50	51.3	6.42	2.0	0.003	24.0
Gr.8, 30 (+ATV, 100)	7.99	1.66	1.0	0.04	24.0
Gr.9, 50 (+ATV, 150)	31.8	2.87	4.0	0.40	24.0
Day 5					
*Gr.6, 50	34.0	3.71	2.0	0.02	24.0
Gr.8, 30 (+ATV, 100)	5.82	0.61	2.0	0.03	24.0
Gr.9, 50 (+ATV, 150)	16.6	2.16	12.0	0.50	24.0

*Group 6 was dosed with GS-9350 only

Study title: 14-Day Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 and GS-017415 in Rats

Study no.:	TX-216-2001 and TX-216-2001-TK
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	1 May 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	See tables below

Code numbers: GS-9350 = Cobi. GS-017415 = Ritonavir.

Test Article A	Lot No.	Storage	Expiration Date	For Use
GS-9350 (as a concentrate in 100% SEDDS) 20 mg/mL	3073-RGS-184-1	At room temperature	31 May 2007	Group 3/9
GS-9350 (as a concentrate in 100% SEDDS) 66.67 mg/mL	3073-RGS-184-2	At room temperature	31 May 2007	Group 4/10
GS-9350 (as a concentrate in 100% SEDDS) 200 mg/mL	3073-RGS-185-3	At room temperature	31 May 2007	Group 5/11/13/14

Test Article B	Lot No.	Storage	Expiration Date	For Use
GS-017415	3073-RGS-185-5	At room temperature	31 May 2007	Group 6/12

Key Study Findings

The purpose of this range finding study was to evaluate the toxicity and determine the toxicokinetics of GS-9350 (COBI) and GS-017415 (Ritonavir) when administered daily via oral gavage to rats for at least 14 days *(range finding).*

ATZ (alone) Findings:
No deaths occurred. Clinical signs were minimal at 100 mg/kg. Body weights and food consumption were not significantly different after ATZ administration at 100 mg/kg. No clinical pathology effects noted. Liver weights were increased after exposure to 100 mg/kg of ATZ. In the ATZ animals, CYP3A activity was decreased, whereas CYP4A was unaffected.

COBI Findings:
Multiple animals were sacrificed moribund at higher doses of Cobi (300 mg/kg). Clinical signs noted at 300 mg/kg on Days 4, 5, and 6 included discolored and/or rough haircoat, hypoactivity, clear oral discharge, few and liquid feces, red discharge from the eyes, cold to the touch, thinness, and general debilitation.

Decreases in mean body weight and mean food consumption were noted over the same period at 300 mg/kg of GS-9350. At necropsy, multiple raised areas were observed on the nonglandular region of the stomach, with minimal to marked erosion/ulceration and associated microscopic

observations, including edema, exudate, hemorrhage, acute inflammation, and chronic active inflammation. All other groups survived to scheduled sacrifice.

Analysis of liver microsomal enzymes showed no notable changes in total cytochrome P450 content and CYP1A, CYP2B, CYP2E, and UDPGT activities in GS-9350-treated animals up to 300 mg/kg/day. No notable change in protein yield and CYP4A and CYP3A activities were observed in animals dosed at 30 or 100 mg/kg/day GS-9350. However, in animals dosed at 175 or 300 mg/kg/day GS-9350, notable decreases in protein yield, notable to marked decreases in CYP4A activity, and marked decreases in CYP3A activity were observed.

Increased liver weights with slight hepatocellular hypertrophy was noted at 100 and 175 mg/kg of GS-9350, consistent with liver adaptation to a CYP inhibitor. Distended urinary bladders was also noted at 3/5 animals at 175 mg/kg. This is consistent with the urine dilution and increased water intake noted in other studies.

Following daily oral administration of GS-9350 for 8 to 14 days, the plasma exposure of GS-9350 decreased slightly in all dosing groups ranging from 30 mg/kg/day to 175 mg/kg/day. As a comparison group, the plasma exposure of GS-017415 at 100 mg/kg/day decreased about 2-fold following daily oral administration for 14 days.

The NOAEL for oral gavage administration of COBI to rats was 30 mg/kg/day for 14 days, and 175 mg/kg/day for 8 days. The NOAEL for oral gavage administration of RTV was 100 mg/kg/day, the highest dose tested.

Methods

Doses:	See table below
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	Animals in Groups 1 through 12 were dosed at a dose volume of 10 mL/kg. Animals in Groups 13 and 14 were dosed at a dose volume of 6.8 mL/kg.
Formulation/Vehicle:	ethanol : propylene glycol: water::5:50:45 (v/v).
Species/Strain:	SD rats
Number/Sex/Group:	See table below
Age:	56 to 68 days old
Weight:	288 to 418 g at initiation of treatment
Satellite groups:	See below
Unique study design:	Combination study – cobi + RTV
Deviation from study protocol:	None that would affect study outcomes or interpretation.

Table 94 – 14 Day Rat Study - Overall Design

Group	No. of Animals Male	Dose Volume (mL/kg)	Dose Level[a] (mg/kg/day)	Dose Concentration[a] (mg/mL)
Toxicity Animals				
1 (Control A)[b]	5	10	0	0
2 (Control B)[c]	5	10	0	0
3 (GS-9350-Low)	5	10	30	3
4 (GS-9350-Mid)	5	10	100	10
5 (GS-9350-High)	5	10	300	30
6 (GS-017415)	5	10	100	10
Toxicokinetic Animals				
7 (Control A)	3	10	0	0
8 (Control B)	3	10	0	0
9 (GS-9350-Low)	6	10	30	3
10 (GS-9350-Mid)	6	10	100	10
11 (GS-9350-High)	6	10	300	30
12 (GS-017415)	6	10	100	10
Toxicity Animals				
13 (GS-9350)	5	6.8	175	25.7
Toxicokinetic Animals				
14 (GS-9350)	6	6.8	175	25.7

a Concentration verification analysis of formulations for Groups 4/10 and 5/11, intended to be 10 mg/mL and 30 mg/ml, respectively, were, in fact, 9.4 mg/mL and 25.6 mg/mL, making the respective doses 94 and 256 mg/kg/day, rather than the nominal 100 and 300 mg/kg/day (see Appendix 2.)
b 15% SEDDS
c Ethanol:propylene glycol:water::5:50:45 (by volume)

Observations and Results

Toxicokinetics

Following daily oral administration of GS-9350 for 8 to 14 days, the plasma exposure of GS-9350 decreased slightly in all dosing groups ranging from 30 mg/kg/day to 175 mg/kg/day. As a comparison group, the plasma exposure of GS-017415 at 100 mg/kg/day decreased about 2-fold following daily oral administration for 14 days.

Table 95 - 14 Day Study in Rats: COBI TK on Day 1

Dosage (mg/kg)	Dosing Day	TK Parameter				
		AUC_{last} (nM•hr)	C_{max} (µg/mL)	T_{max} (hr)	C_{last} (µg/mL)	T_{last} (hr)
0	1	—	BLQ	—	—	—
30	1	11.7	1.84	2.0	1.13	8
100	1	64.7	5.50	2.0	0.01	24
175	1	62.6	4.01	1.0	1.61	24
300	1	93.6	7.16	1.0	2.49	24

Table 96 - 14 Day Study in Rats: COBI TK on Day 14

Dosage (mg/kg)	Dosing Day	TK Parameter				
		AUC_{last} (nM•hr)	C_{max} (µg/mL)	T_{max} (hr)	C_{last} (µg/mL)	T_{last} (hr)
0	14	—	BLQ	—	—	—
30	14	8.79	1.68	2.0	0.002	12
100	14	53.5	6.07	1.0	0.003	24
175	8	56.9	5.91	1.0	1.58	24

Table 97 - Table 41 - 14 Day Study in Rats: Ritonavir TK on Day 1 and Day 14

Dosage (mg/kg)	Dosing Day	TK Parameter				
		AUC_{last} (nM•hr)	C_{max} (µg/mL)	T_{max} (hr)	C_{last} (µg/mL)	T_{last} (hr)
0	1	—	BLQ	—	—	—
100	1	100	7.41	2.0	0.99	24
0	14	—	BLQ	—	—	—
100	14	43.0	5.13	2.0	0.01	24

Study title: 7-Day Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 and GS-017415 in Dogs

Study no.:	TX-216-2002 and TX-216-2002-TK
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11 July 2007
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	See tables below.

Test Article	Lot/Batch No.	Storage	Purity	Expiration Date	Reserve (Archive) Sample
GS-9350	3201-142-35	Room temperature	93.8%	16 August 2007	Not collected
GS-017415 (Norvir)	3180-JK-132	Room temperature	100%	20 Oct 2007	Not collected

Control Article	Manufacturer	Lot/Batch No.	Storage	Purity	SOP-Assigned Expiration Date	Reserve (Archive) Sample
Propylene Glycol, USP	(b) (4)	C42615	Room temperature	NA	25 Jan 2009	Not collected
Ethyl Alcohol, USP		06120GA	Room temperature	NA	26 Sep 2009	Not collected
Methanesulfonic acid, 99% (MSA)		A0210256001	Room temperature	NA	13 Sep 2008	Not collected

NA Not applicable. Product met supplier manufacturing and testing standards prior to release.

Key Study Findings

The purpose of this range finding combination toxicology study was to evaluate the effects of Cobi compared to ritonavir in beagle dogs for 7 days.

Dogs were moribund at dose levels of 125 and 250 mg/kg/day GS-9350. Clinical observations of salivation and emesis, decreases in body weight and food consumption in dogs occurred at all doses of Cobi as well as the single dose of Ritonaivr. Due to adverse events at all dose groups, a NOAEL could not be determined for either compound in this study.

Methods

Doses:	0, 50, 125, 200 mg/kg of Cobi or 125 mg/kg Ritonavir
Frequency of dosing:	Daily
Route of administration:	Oral
Dose volume:	2 ml/kg
Formulation/Vehicle:	95% propylene glycol, 5% ethanol with 1.5M equivalents of methane sulfonic acid
Species/Strain:	Beagle dog
Number/Sex/Group:	3 males/group (see table below)
Age:	"at least 6 months old"
Weight:	8.0 to 9.7 kg
Satellite groups:	See below
Unique study design:	See below
Deviation from study protocol:	None noted.

Group	No. of Animals Male	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)
1 Control A[a]	3	0	2	0
2 Control B[b]	3	0	2	0
3 GS-9350-Low	3	50	2	25
4 GS-9350-Mid	3	125	2	62.5
5 GS-9350-High	3	250	2	125
6 GS-017415	3	125	2	62.5

a Animals in Group 1 received Control Article A (94.4% PG, 5% ethanol, and 0.6% MSA)
b Animals in Group 2 received Control Article B (46.6% propylene glycol, 34.6% ethanol, 18% water, and 0.8% Cremophor EL).

Observations and Results

Toxicokinetics

Due to overt toxicity at day 5 in all dose groups, those data are likely not relevant. However, the day 1 data are still valid.

Following daily oral doses of GS-9350 in dogs, the increase in plasma exposure of GS-9350 was less than proportional to the increase in dose on the range of 50 to 250 mg/kg/day.

Table 98 - 7 Day Dog Study - COBI Toxicokinetics

Dosage (mg/kg)	TK Parameter				
	AUC_{last} (nM•hr)	T_{max} (µg/mL)	C_{max} (µg/mL)	$t_{½}$ (hr)[a]	C_{24hr} (µg/mL)
0	NC	NC	BLQ	NC	BLQ
50	86.2 ± 23.7	2.2 ± 1.8	9.98 ± 1.51	1.5 ± 0.2	0.01 ± 0.01
125	77.3 ± 45.1	1.2 ± 0.8	10.6 ± 3.21	1.7	0.80 ± 1.38
250	156 ± 96.7	3.3 ± 1.2	11.5 ± 3.89	1.9	2.21 ± 3.78

Table 99 - 7 Day Dog Study - Ritonavir Toxicokinetics

Dosage (mg/kg)	Dosing Day	TK Parameter				
		AUC_{last} (nM•hr)	T_{max} (µg/mL)	C_{max} (µg/mL)	$t_{½}$ (hr)[a]	C_{24hr} (µg/mL)
0	1	NC	NC	BLQ	NC	BLQ
125	1	148 ± 95.1	3.3 ± 1.2	23.6 ± 14.0	1.11 ± 0.04	0.001
0	14	NC	NC	BLQ	NC	BLQ
125	14	199 ± 93.3	2.2 ± 1.8	24.0 ± 3.16	1.78 ± 0.52	0.07 ± 0.10

BLQ: below the limit of quantification (1 ng/mL)

NC: not calculated due to insufficient data.

Study title: 90-Day Oral Gavage Bridging Study with GS-9350 and Atazanavir in Rats with a 1-Month Recovery Period

Study no.:	TX-216-2024
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	6 Aug 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	See table below for COBI and Atazanavir purity information

Test Article	Lot/Batch No.	Storage	Purity	Retest Date	Reserve (Archive) Sample
GS-9350	3168-157-8	REF, PFL	50.5%[a]	31 July 2009	1.0 mL
Atazanavir (ATV sulfate salt, GS-9270-01)	3168-179-10	RT	100.9%[a]	31 July 2009	0.5 g

REF Refrigerator set to maintain 2 to 8°C
PFL Protected from light
RT Room temperature (15 to 30°C)
a See Test Article Analytical Report

Key Study Findings

This study was performed to evaluate the effect of combinbing atazanavir and COBI in relation to the single drug toxicity. There were no major alterations in the COBI or atazanavir toxicity profile in combination with each other. The majority of the findings in the Cobi or Cobi+ATZ group were mild liver compensation (increased liver weights) and increased urine volume. These findings are consistent with other COBI studies.

The NOAEL for COBI with or without atazanavir was 30 mg/kg in rats (90 day study). The NOAEL for atazanavir was 50 mg/kg with or without COBI.

At the 30/50 mg/kg/day GS-9350/ATV dose, systemic exposures on Day 90 were: GS-9350 C_{max} and AUC_{0-t}: 1231 and 1661 ng/mL, and 6100 and 6336 ng·h/mL in males and females, respectively; ATV C_{max} and AUC_{0-t}: 6753 and 7787 ng/mL and 44339 and 45904 ng·h/mL in males and females, respectively.

Methods

Doses:	See table below
Frequency of dosing:	Daily
Route of administration:	Oral avage
Dose volume:	2 ml/kg
Formulation/Vehicle:	95% propylene glycol (PG) and 5% ethanol (EtOH)
Species/Strain:	Sprague Dawley rats (Charles River)
Number/Sex/Group:	10 or 15/sex in tox group and 3 or 6/sex in TK group (see table below)
Age:	~ 7 wks old at start of study
Weight:	182 to 233 g for males and 158 to 195 g for females
Satellite groups:	TK animals – see table below
Unique study design:	Bridging toxicology study
Deviation from study protocol:	No major deviations that would affect study outcome or interpretation

Group	No. of Animals Male	Female	GS-9350 Dose Level (mg/kg/day)	Atazanavir Dose Level[b] (mg/kg/day)	GS-9350 Concentration (mg/mL)	Atazanavir Concentration[b] (mg/mL)
Toxicity Animals[a]						
1 (Vehicle Control)[c]	15	15	0	0	0	0
2 (GS-9350 – high)	15	15	30	0	15	0
3 (Atazanavir – low)	10	10	0	20	0	10
4 (Atazanavir – high)	15	15	0	50	0	25
5 (Atazanavir and GS-9350 –low)	10	10	30	20	15	10
6 (Atazanavir and GS-9350 – high)	15	15	30	50	15	25
Toxicokinetic Animals						
7 (Vehicle Control)[c]	3	3	0	0	0	0
8 (GS-9350 – high)	6	6	30	0	15	0
9 (Atazanavir – low)	6	6	0	20	0	10
10 (Atazanavir – high)	6	6	0	50	0	25
11(Atazanavir and GS-9350 – low)	6	6	30	20	15	10
12 (Atazanavir and GS-9350 – high)	6	6	30	50	15	25

a Toxicity animals designated for recovery (the last 5 animals/sex in Groups 1, 2, 4, and 6) underwent at least 28 days of recovery following dose administration.
b Atazanavir dose levels and concentrations expressed as free base equivalents.
c Goups 1 and 7 received the vehicle [95% propylene glycol (PG) and 5% ethanol (EtOH)], only.

Observations and Results

Toxicokinetics

Slight (≥ 2-fold) accumulation of GS-9350 was observed after multiple dosing at 30 mg/kg/day. Co-administration of 30 mg/kg/day GS-9350 with ATV at 20 and 50 mg/kg/day, resulted in decreases in GS-9350 exposures (AUC_{0-t}) of up to 70% on Day 1, and up to 60% on Day 90.

Exposure to ATV increased with the increase in ATV dose level from 20 to 50 mg/kg/day. Slight accumulation (2.3-2.7-fold) of ATV was observed after multiple dosing at 20 mg/kg/day. Co-administration of ATV at 20 mg/kg/day with 30 mg/kg/day GS-9350, resulted in 5.4 to 11.3-fold

and 2.6 to 3.9-fold increases in exposure (AUC_{0-t}) on Day 1 and Day 90, respectively, compared to ATV administration at 20 mg/kg/day alone. Co-administration of ATV at 50 mg/kg/day with 30 mg/kg/day GS-9350, resulted in 2.7 to 4.2-fold and 1.5 to 2.9-fold increases in exposure on Day 1 and Day 90, respectively, compared to ATV administration at 50 mg/kg/day alone.

Table 100 - 90 Day Bridging Study with COBI and Atazanavir: COBI Toxicokinetic Analysis

Day 1

Dose Group	GS-9350 Dose Level (mg/kg/day)	Atazanavir Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	T_{last} (hr)	C_{last} (ng/mL)	Dose Group 11/8 Ratio C_{max}	Dose Group 11/8 Ratio AUC_{0-t}	Dose Group 12/8 Ratio C_{max}	Dose Group 12/8 Ratio AUC_{0-t}
8	30	0	M	1152	1.00	6918	8.00	853				
			F	1949	2.00	12866	12.0	117				
11	30	20	M	806	4.00	4158	12.0	26.1	0.7	0.6		
			F	795	2.00	6410	12.0	502	0.4	0.5		
12	30	50	M	478	2.00	2584	12.0	18.8			0.4	0.4
			F	373	4.00	3748	12.0	363			0.2	0.3

Day 90

Dose Group	GS-9350 Dose Level (mg/kg/day)	Atazanavir Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	T_{last} (hr)	C_{last} (ng/mL)	Dose Group 11/8 Ratio C_{max}	Dose Group 11/8 Ratio AUC_{0-t}	Dose Group 12/8 Ratio C_{max}	Dose Group 12/8 Ratio AUC_{0-t}
8	30	0	M	2987	2.00	10788	12.0	26.5				
			F	2593	1.00	12799	12.0	16.7				
11	30	20	M	862	2.00	4580	24.0	88.4	0.3	0.4		
			F	2113	1.00	11229	12.0	8.36	0.8	0.9		
12	30	50	M	1231	4.00	6100	12.0	22.6			0.4	0.6
			F	1661	1.00	6336	12.0	18.1			0.6	0.5

Table 101 - 90 Day Bridging Study with COBI and Atazanavir: ATZ Toxicokinetic Analysis

Day 1

Dose Group	Atazanavir Dose Level (mg/kg/day)	GS-9350 Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	T_{last} (hr)	C_{last} (ng/mL)	Dose Group 11/9 Ratio C_{max}	Dose Group 11/9 Ratio AUC_{0-t}	Dose Group 12/10 Ratio C_{max}	Dose Group 12/10 Ratio AUC_{0-t}
9	20	0	M	1134	1.00	2918	12.0	11.5				
			F	990	1.00	4967	12.0	55.1				
10	50	0	M	2457	0.500	12007	12.0	16.0				
			F	3170	0.500	17615	24.0	11.4				
11	20	30	M	4060	4.00	32875	24.0	20.7	3.6	11.3		
			F	2533	2.00	27018	24.0	36.0	2.6	5.4		
12	50	30	M	5793	4.00	50394	24.0	47.2			2.4	4.2
			F	3580	2.00	48005	24.0	63.0			1.1	2.7

Day 90

Dose Group	Atazanavir Dose Level (mg/kg/day)	GS-9350 Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	T_{last} (hr)	C_{last} (ng/mL)	Dose Group 11/9 Ratio C_{max}	Dose Group 11/9 Ratio AUC_{0-t}	Dose Group 12/10 Ratio C_{max}	Dose Group 12/10 Ratio AUC_{0-t}
9	20	0	M	1314	0.500	7942	12.0	159				
			F	2400	1.00	11655	12.0	80.2				
10	50	0	M	3327	2.00	15360	12.0	52.6				
			F	5233	1.00	31616	24.0	26.4				
11	20	30	M	2990	2.00	30790	24.0	1300	2.3	3.9		
			F	3927	1.00	30564	12.0	116	1.6	2.6		
12	50	30	M	6753	4.00	44339	12.0	581			2.0	2.9
			F	7787	1.00	45904	24.0	152			1.5	1.5

Dosing Solution Analysis

The stability of GS-9350, ATV, and GS-9350/ATV coformulated samples in 95% PG and 5% EtOH were confirmed for at least 24 hours at room temperature and for at least 8 and 15 days under both room temperature and refrigerated conditions using the samples prepared for formulation method validation.

Homogeneity analysis was not required since both formulations for dosing were solutions.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: GS-9350 *Salmonella-Escherichia Coli*/Mammalian-Microsome Reverse Mutation Assay

Study no.:	TX-216-2010
Study report location:	EDR
Conducting laboratory and location:	(b) (4) (conducted the study) and (b) (4) (only the dose formulation analysis)
Date of study initiation:	Week of 17 Sept 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-9350, Lot 3168-121-23, 97.0% pure

Key Study Findings

No substantial increases in the revertant colony counts were obtained with any strain following exposure to the test article in either the plate incorporation or pre-incubation assay in the absence or presence of S9 mix. It is concluded that GS-9350 did not show any evidence of genotoxic activity in this *in vitro* mutagenicity assay when tested in accordance with regulatory guidelines.

Methods

Strains:	*S. typhimurium* TA1535 *his*G46 *rfa* Δ*uvr*B *S. typhimurium* TA1537 *his*C3076 *rfa* Δ*uvr*B *S. typhimurium* TA98 *his*D3052 *rfa* Δ*uvr*B pKM101 *S. typhimurium* TA100 *his*G46 *rfa* Δ*uvr*B pKM101 *E. coli* WP2 *trp uvrA*
Concentrations in definitive study:	See below. Up to 5000 µg/plate.
Basis of concentration selection:	Maximum dose limit recommended by regulatory guidelines
Negative control:	Vehicle
Positive control:	Sodium azide, 9-aminoacridine, 2-nictroflourine, 4-nitroquinoline N-oxide With S9: 2-aminoanthracene, benzo[a]pyrene
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Incubated in S9 for 30 minutes. Plates were incubated at 37°C for 48 to 72 hrs.

Table 102 - Ames assay: Study Design

Material	Formulation conc. (µg/mL)	Final conc. (µg/plate)	Number of replicates 0S9	Number of replicates +S9	Number of strains
Vehicle	-	-	3	3	5
Test article	15.8	1.58	3	3	5
	50	5.0	3	3	5
	158	15.8	3	3	5
	500	50	3	3	5
	1581	158	3	3	5
	5000	500	3	3	5
	15811	1581	3	3	5
	50000	5000†	3	3	5
Positive control	‡	‡	3	3	5

‡ Depends on the test organism, positive control agent and methodology used.
† Test article was tested at levels up to 5000 µg/plate, which is the standard limit dose recommended by regulatory guidelines.

Study Validity

The study was valid.

Results

Negative with or without S9.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: GS-9350 L5178Y+/- Mouse Lymphoma Forward Mutation Assay

Study no.:	TX-216-2011
Study report location:	EDR
Conducting laboratory and location:	(b) (4) (conducted the study) and (b) (4) (only the dose formulation analysis)
Date of study initiation:	Week of 17 Sept 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-9350, Lot 3168-121-23, 97.0% pure

Key Study Findings

No substantial increases in mutation frequency (neither in small or large colonies) were observed after treatment of cells with GS-9350 at dose levels up to the limit of toxicity. It is concluded that GS-9350 did not show any evidence of genotoxicity in this *in vitro* test when evaluated in accordance with regulatory guidelines.

Methods

Cell line:	Mouse lymphoma L5178Y TK+/- cells
Concentrations in definitive study:	Up to 80 µg/mL
Basis of concentration selection:	Cell survival based on a preliminary study
Negative control:	Vehicle
Positive control:	Without S9: 4-Nitroquinoline N-oxide With S9: Benzo[a]pyrene
Formulation/Vehicle:	DMSO
Incubation & sampling time:	See figure on next page.

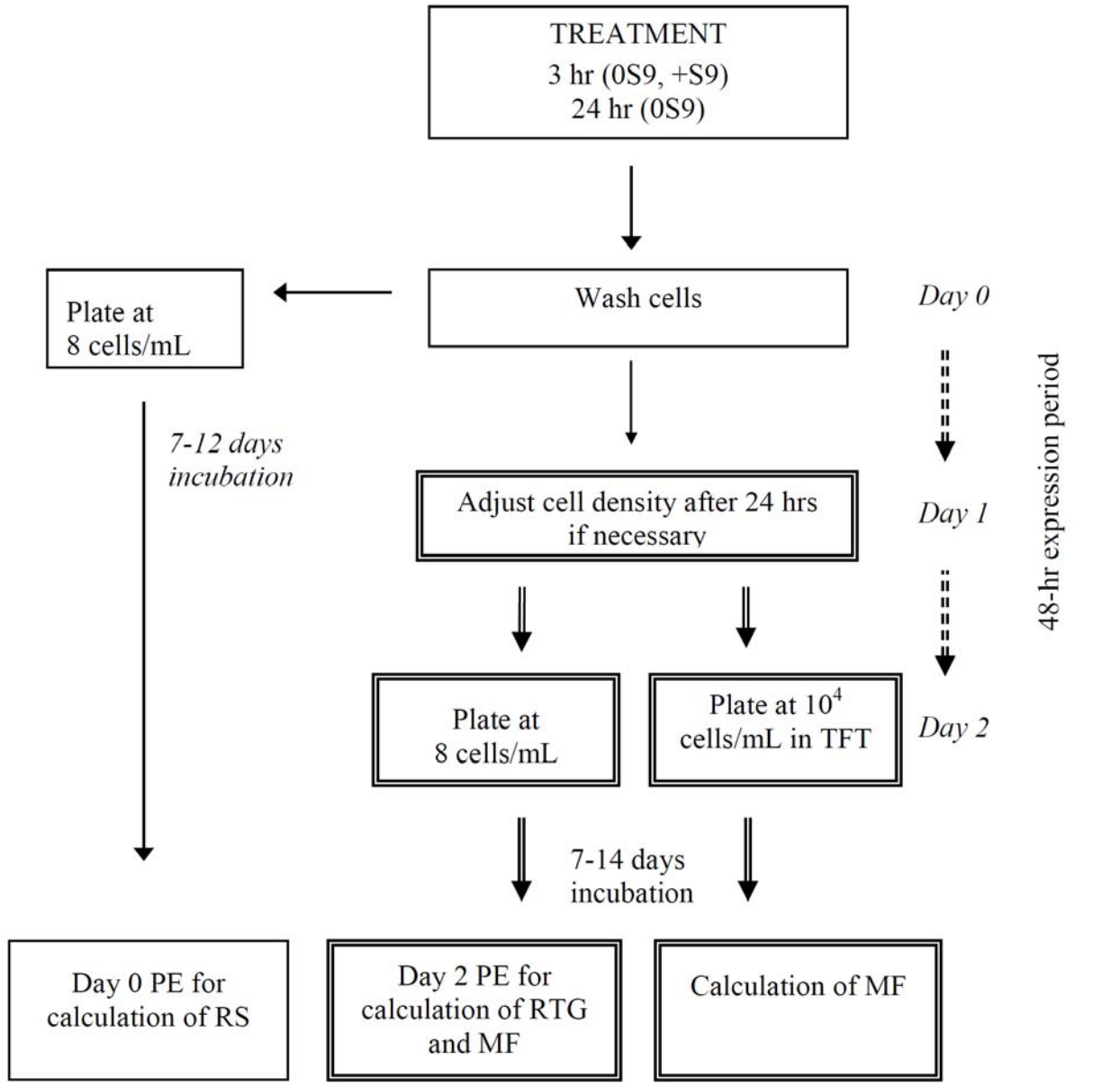
Table 103 -Mouse Lymphoma Assay: Doses and Design

Material	Formulation conc. (µg/mL)	Final conc. (µg/mL)	Number of cultures		
			3 Hours (0S9) †	3 Hours (+S9) †	24 Hours (0S9) †
Vehicle	-	-	4	4	4
Test article	687	6.87	-	-	2
	859	8.59	-	-	2
	1074	10.7	-	-	2
	1342	13.4	2	2	2
	1678	16.8	2	2	2
	2097	21.0	2	2	2
	2621	26.2	2	2	2
	3277	32.8	2	2	2
	4096	41.0	2	2	2
	5120	51.2	2	2	-
	6400	64.0	2	2	-
	8000	80.0	2	2	-
Positive controls	*	*	4	4	4

† High level for each regime = the concentration level which is expected to produce a reduction in cell survival by at least 80-90% based on the results of the preliminary study.

* Depends on the positive control and regime.

Table 104 - Mouse Lymphoma Assay: Overall Study Design



Study Validity

The study was valid.

Results

GS-9350 was negative in the *in vitro* mouse lymphoma assay.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: GS-9350 Rat Micronucleus Test

Study no:	TX-216-2012
Study report location:	EDR
Conducting laboratory and location:	(b) (4) (conducted the study) and (b) (4) (only the dose formulation analysis)
Date of study initiation:	Week of 17 Sept 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-9350, Lot 3168-121-23, 92.9% pure

Key Study Findings

GS-9350 did not show any evidence of genotoxic activity in this *in vivo* test for induction of chromosome damage when tested in accordance with regulatory guidelines.

Methods

Doses in definitive study:	See table below. MTD was lower for females vs males.
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Dose volume:	10 ml/kg
Formulation/Vehicle:	Propylene glycol/ethanol (95%/5%)
Species/Strain:	Sprague Dawley Rats (Harlan)
Number/Sex/Group:	See below
Satellite groups:	None. See below
Basis of dose selection:	Maximum tolerated dose
Negative control:	Vehicle control
Positive control:	Cyclophosphamide (see below)

Table 105 - Rat Micronucleus Assay: Study Design

Group: Material	Dosage (mg/kg) Male/Female	Dose Volume (mL/kg)	Sampling Time (Hours)	Number of Main Animals		Number of Bioanalysis Animals [a]	
				Male	Female	Male	Female
1/ Vehicle control	-	10	24	5	5	3	3
			48	5	5		
2/ GS-9350	212.5/125	10	24	5	5	3	3
3/ GS-9350	425/250	10	24	5	5	3	3
4/ GS-9350	850/500	10	24	5	5	3	3
			48	5	5		
5/ Cyclophosphamide	20	10	24	3	3	NP	

Where 850 and 500 mg/kg is the maximum tolerable dose for males and females, respectively, as determined independently for each sex in a preliminary toxicity test
a = Bioanalysis animals were sampled 1 hour following dosing on Day 1
NP: Not performed

Study Validity

The study was valid.

Results

GS-9350 was negative in the *in vitro* rat micronucleus assay.

8 Carcinogenicity

Final carcinogenicity study reports were not included with this NDA submission.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Oral Gavage Study of Fertility and Early Embryonic Development to Implantation with GS-9350 in Rats

Study no.:	TX-216-2023
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	23 June 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	See table below

Test Article	Lot/Batch No.	Storage	Purity	Retest Date	Reserve (Archive) Sample
GS-9350	3168-157-8	REF, PFL	50.5%	31 January 2009	a

REF Refrigerated (2 to 8°C).
PFL Protected from light.
a Per protocol, a reserve sample was to be taken under Covance study number 6511-355.

Key Study Findings

The NOAEL for parental toxicity for GS-9350 when administered daily via oral gavage to male and female rats prior to mating and until termination (males) or through early gestation (females) is 30 mg/kg/day for both sexes based on significant decreases in body weight gain and food consumption at 100 mg/kg/day. The NOEL for male and female reproductive performance and competency (fertility), and embryo/fetal viability is 100 mg/kg/day.

Note: TK was not performed as part of this study.

Methods

Doses:	0, 10, 30, 100 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	2.5 mL/kg.
Route of administration:	Oral
Formulation/Vehicle:	95% propylene glycol (PG), 5% ethanol (EtOH) with 1.5 eq. HCl, pH adjusted to 2.3 with HCl.
Species/Strain:	SD rats (Charles River)
Number/Sex/Group:	4 groups (22/sex/group): 0 (vehicle), 10, 30, and 100 mg/kg/day.
Satellite groups:	None
Study design:	All males were dosed for at least 28 days prior to mating and throughout the mating phase (minimum of 10 weeks). Females were dosed for at least 14 days prior to mating (premating phase), throughout the mating phase and through Gestation Day 7 (GD 7). Treated males were paired with treated females during the mating phase. On GD 13, females were necropsied and the uterus of each was examined for the number of live and dead fetuses and resorptions.
Deviation from study protocol:	No significant deviations that would alter study outcome or study interpretation.

Table 106 - GS-9350 Seg I Study Design

Group[a]	No. of Animals Male	No. of Animals Female	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
1 (Control)	22	22	0	0
2 (Low)	22	22	10	4
3 (Mid)	22	22	30	12
4 (High)	22	22	100	40

a Group 1 received control article only.

Observations and Results

Mortality

Evaluated 2x daily. No unscheduled deaths. All animals survived to scheduled sacrifice.

Clinical Signs

Evaluated 2x daily. Treatment-related clinical observations for the males and females during the premating phase included clear, oral discharge and alopecia in the 30 and 100 mg/kg/day groups. Clear oral discharge was also observed immediately after dosing in the 100 mg/kg/day females during the gestation phase.

Body Weight

Prior to treatment, then twice weekly.

In males, significantly decreased mean body weight was observed at 100 mg/kg/day beginning on Day 49 and continued through the remainder of the dosing phase. Significant decreases in mean body weight gain were noted at the initiation of dosing (Days 0-3) and over the entire dosing phase (Days 0-71; 86% of control mean weight gain) at 100 mg/kg/day.

In females, mean body weights were generally similar across treatment groups over the premating phase (Days 0-14), although a slight decrease in mean body weight change was observed at 100 mg/kg/day.

A transient but significant decrease in mean body weight gain was observed during GD 0-3 for the 100 mg/kg/day group, resulting in slightly lower mean maternal body weights (albeit nonsignificant) during the gestation phase.

Figure 23 - Seg I Study in Rats: Male Body Weights

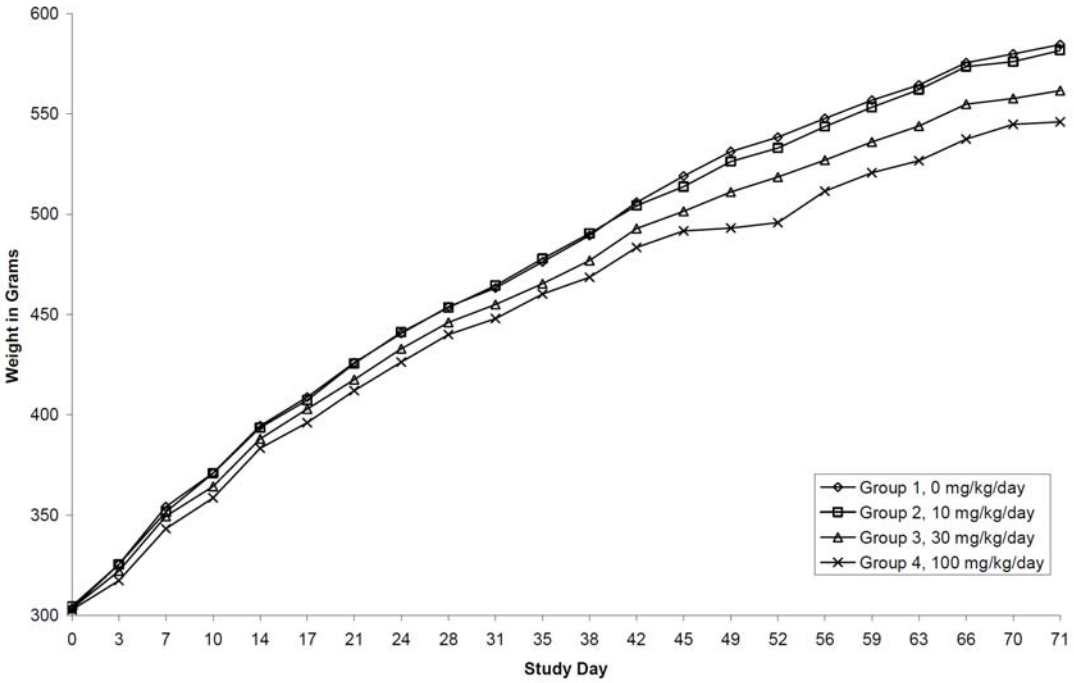


Figure 24 - Seg I Study in Rats: Female Body Weights

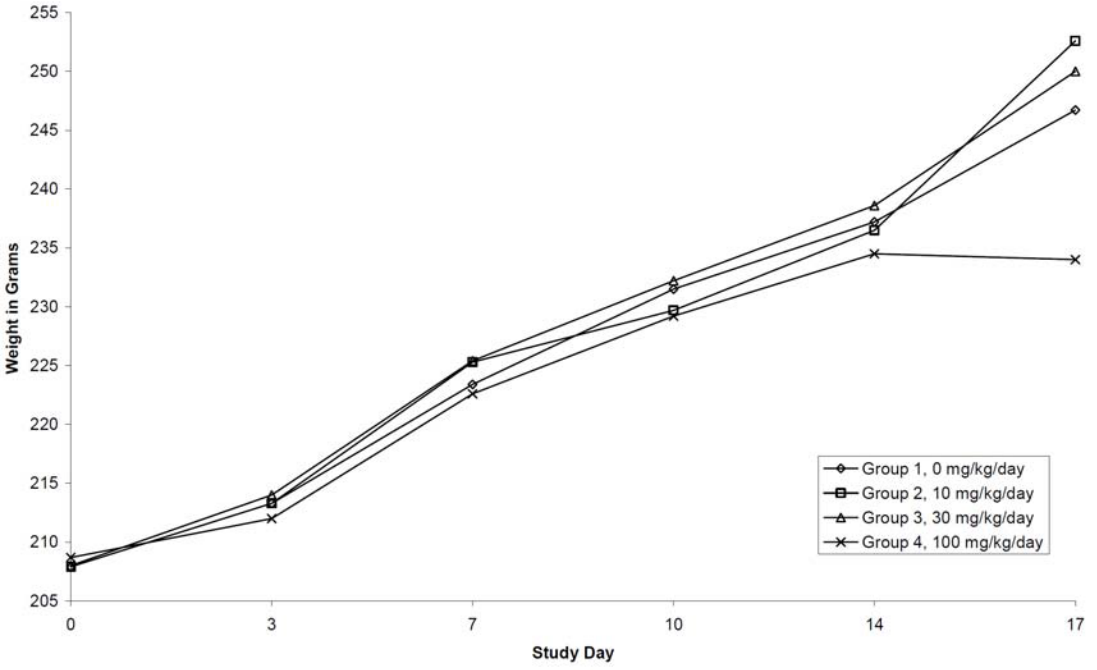
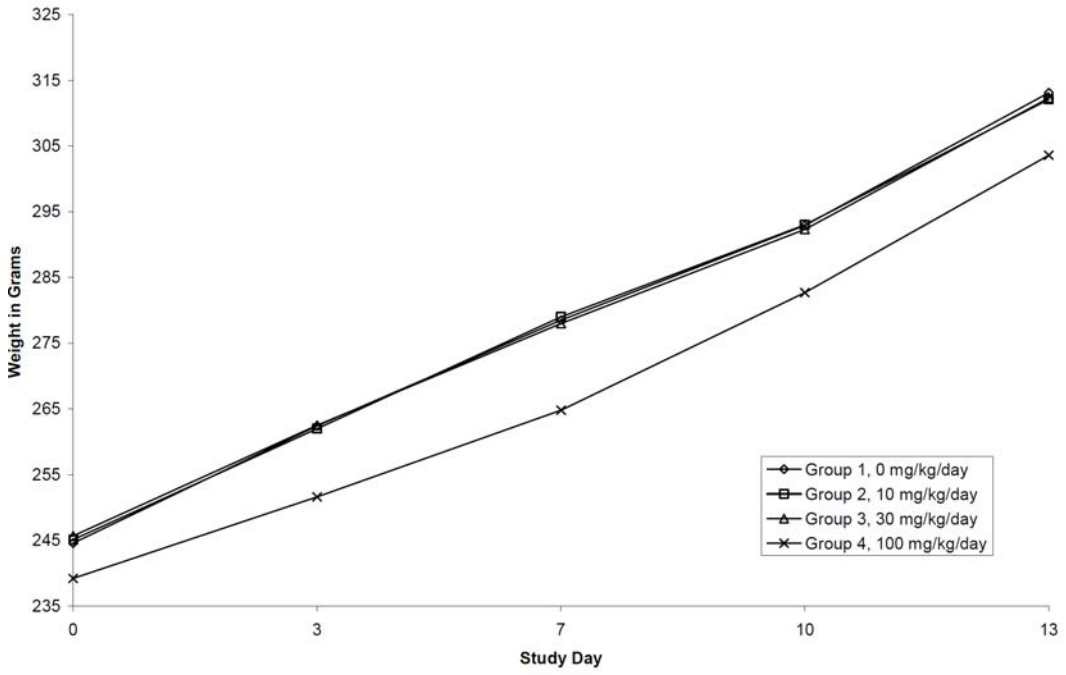


Figure 25 - Seg I Study in Rats: Female Body Weights During Gestation



Feed Consumption

Weekly during premating phase. Not measured during mating for any rats or after mating for males. Measured in mated females on GD 0, 3, 7, 10, 13. Significantly decreased mean food consumption was observed in the 100 mg/kg/day males and females and correlated with the observed effects on body weight.

Toxicokinetics

No toxicokinetics were evaluated during the study.

Dosing Solution Analysis

The stability of samples prepared at 0.9259 and 277.8 mg/mL was confirmed after storage for up to 15 days at room temperature ((b) (4) Study 6511-334).

Samples prepared between 0.9259 and 277.8 mg/mL were confirmed to be solutions under (b) (4) Study 6511-334; therefore, homogeneity was not required.

Necropsy

Although there were no parental necropsy findings attributed to treatment, liver and thyroid weights were significantly increased in the 100 mg/kg/day males and females. This is likely attributable to compensatory mechanisms in the liver.

No biologically relevant effects were noted on the estrous cycle, and the majority of females were confirmed to have mated within the first 4 days of mating. Male/female copulation and fertility were unaffected by treatment. Cesarean section data were unremarkable. Mean percent

preimplantation losses were similar across groups, indicating no treatment-related effects on the females. Mean postimplantation loss and mean percent live fetuses were similar across groups, indicating that GS-9350 had no effect on embryo/fetal viability.

Mean sperm assessment parameter values were generally similar across groups, although a slight decrease in total sperm count was observed for the 100 mg/kg/day males. The apparent decrease was due to a single male, not the result of a general trend across multiple animals in the group, and therefore was not attributed to treatment.

9.2 Embryonic Fetal Development

Study title: Oral Gavage Dose Range-finding Developmental Toxicity and Toxicokinetic Study with GS-9350 in Rats

Study no.:	TX-216-2018
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	31 Mar 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	See below

Test Article	Lot/ No.	Storage	Purity	Retest Date	Reserve (Archive) Sample
GS-9350	3168-157-8	REF, PFL	50.5%	31 August 2008	NC

REF Refrigerated (2 to 8°C).
PFL Protected From Light.
NC Not collected.

Key Study Findings

This range-finding study was performed to determine the maternal and in-utero toxicity and toxicokinetics of GS-9350 when administered daily via oral gavage to pregnant rats during the period of organogenesis to set the dosage levels for a definitive developmental study. The study design is outlined below:

Group[a]	No. of Animals Mated Female	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)	Dosing Schedule Days
Main Study Animals				
1 (Control)	8	0	0	6-17
2 (Low)	8	25	5	6-17
3 (Mid)	8	75	15	6-17
4 (High)	8	200	40	6-17
Toxicokinetic Animals				
5 (Control)	3	0	0	6-17
6 (Low)	9	25	5	6-17
7 (Mid)	9	75	15	6-17
8 (High)	9	200	40	6-17

a Groups 1 and 5 received control article only.

Mortality, clinical signs, and effects on mean body weight and food consumption were noted at 200 mg/kg/day. There were no maternal effects noted at 75 mg/kg/day. Slight increases in the mean percentage of early resorptions and a corresponding slight decrease in the mean percent of live fetuses at 25 and 75 mg/kg/day suggest a possible effect on embryo/fetal viability. Mean fetal weights were similar across all groups. There were no external malformations. Therefore, a dose of greater than 75 mg/kg/day but less than 200 mg/kg/day was recommended as the high dose for a definitive developmental toxicity study in rats.

Study title: Oral Gavage Study for Effects on Embryo-fetal Development with GS-9350 in Rats

Study no.:	TX-216-2020
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	8 May 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	See below

Test Article	Lot/Batch No.	Storage	Purity	Retest Date	Reserve (Archive) Sample
GS-9350	3168-157-8	REF, PFL	50.5%	31 August 2008	0.5 g

REF Refrigerator set to maintain 2 to 8°C
PFL Protected from light

Key Study Findings

This study determined the maternal and embryo/fetal toxicity, teratogenic potential and toxicokinetics of GS-9350 when administered daily via oral gavage to pregnant rats during the period of organogenesis

The NOAEL for maternal toxicity for GS-9350 when administered by oral gavage to pregnant rats during the period of organogenesis is 50 mg/kg/day. The no-observable-effect level (NOEL) for embryo/fetal viability and growth and developmental toxicity is 50 mg/kg/day (GD17 C_{max} and AUC_{0-t}: 1650 ng/mL and 14797 ng·hr/mL, respectively), based on increased postimplantation loss and decreased fetal weights associated with significant decreases in maternal body weights at 125 mg/kg/day.

Methods

Doses:	0, 25, 50, 125 mg/kg (see below)
Frequency of dosing:	Daily
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	95% propylene glycol (PG), 5% ethanol (EtOH) with 1.5 eq. HCl, pH adjusted to 2.3 with HCl
Species/Strain:	Sprague Dawley Rats
Number/Sex/Group:	Groups of 25 time-mated female Crl:CD(SD) rats were administered 0 (vehicle control: propylene glycol/ethanol 95/5 with 1.5 eq. HCl [pH 2.3]), 25,

50 or 125 mg/kg/day GS-9350.

Satellite groups: See table below

Study design: Doses were administered on Gestation Days (GD) 6 through 17. Additional time-mated rats (3-9/group) were used for TK analysis to determine maternal GS-9350 plasma concentrations at selected timepoints on GD 6 and 17.

Deviation from study protocol: Gestation Day 0 body weights and clinical signs were received from the supplier and used for randomization of animals prior to study initiation. These data were collected by a non-GLP facility; however, the integrity of the study was not affected.

Group[a]	No. of Animals Mated Female	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)	Dosing Schedule Days
Main Study Animals				
1 (Control)	25	0	0	6-17
2 (Low)	25	25	5	6-17
3 (Mid)	25	50	10	6-17
4 (High)	25	125	25	6-17
Toxicokinetic Animals				
5 (Control)	3	0	0	6-17
6 (Low)	9	25	5	6-17
7 (Mid)	9	50	10	6-17
8 (High)	9	125	25	6-17

a Groups 1 and 5 received control article only.

Observations and Results

Mortality

2x daily. All animals survived to scheduled sacrifice.

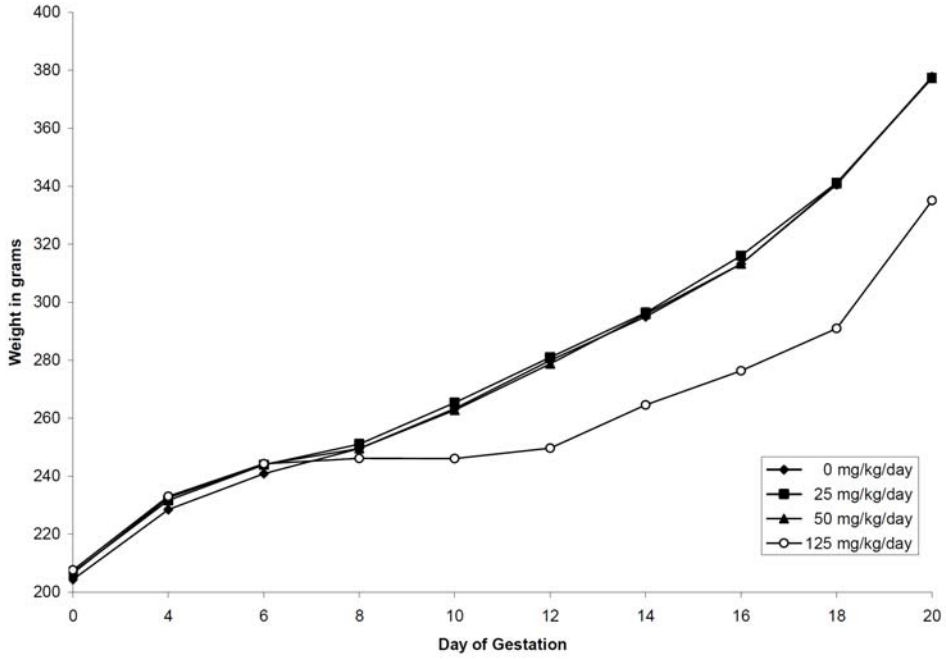
Clinical Signs

2x daily. A dose-related increase in the incidence of clear oral discharge was observed in all treated groups during the dosing phase. Treatment-related clinical signs of thin appearance, hypoactivity, audible and/or irregular respiration, alopecia, rough haircoat, and few or no feces were limited to the 125 mg/kg/day group.

Body Weight

GD 0, 4, 6, 8, 10, 12, 14, 16, 20. A decrease in mean maternal body weight and body eight gain was noted at 125 mg/kg/day. A transient, but statistically significant, decrease in mean body weight gain was noted for the 50 mg/kg/day group during the initial dosing interval correlated with significantly decreased mean food consumption.

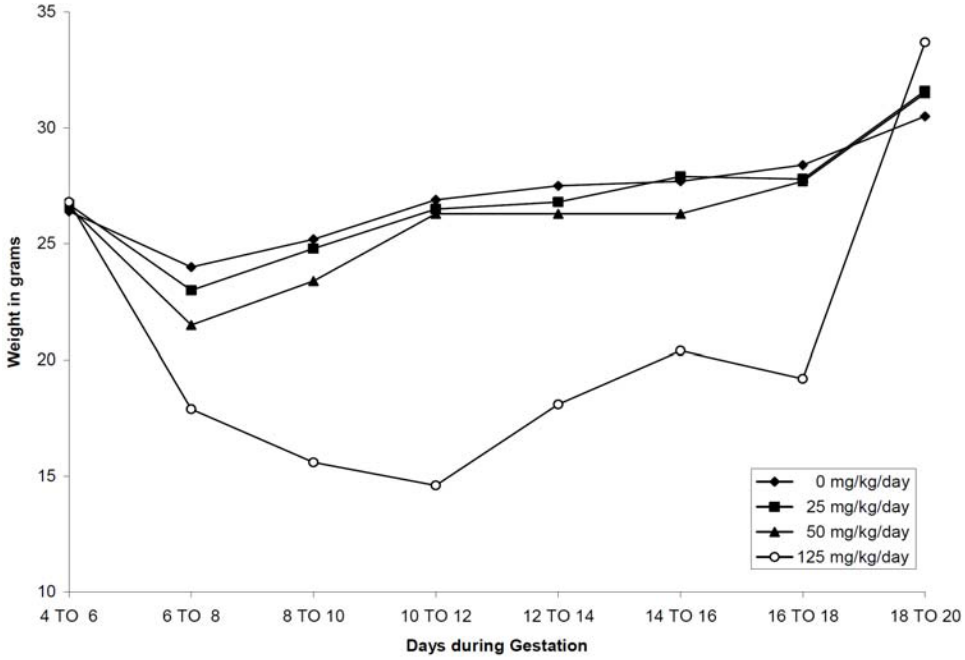
Figure 26 - Seg II Study in Rats: Body Weights



Feed Consumption

GD 4, 6, 8, 10, 12, 14, 16, 18, 20. A decrease in mean maternal food consumption was noted at 125 mg/kg/day. A transient, but statistically significant, decrease in mean food consumption was noted in the 50 mg/kg/day group during the initial dosing interval.

Figure 27 - Seg II Study in Rats: Food Consumption



Toxicokinetics

Approx 0.5 ml collected on GD 6 and 17. Exposure to GS-9350 increased with the increase in GS-9350 dose level from 25 to 125 mg/kg/day. The increases in C_{max} and AUC_{0-t} were generally dose proportional. No accumulation of GS-9350 was observed after multiple dosing of GS-9350 in pregnant rats.

Table 107 - Seg II Study in Rats: TK in Pregnant Rat Plasma

Dose Group	Dose Level (mg/kg/day)	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg/day)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	DN AUC_{0-t} [(ng•hr/mL)/(mg/kg/day)]	AUC_{0-24} (ng•hr/mL)	$t_{1/2}$ (hr)
				Gestation Day 6				
6	25	1434	57.4	8.00	9945	398	10279	NC
7	50	2687	53.7	1.00	27137	543	27137	1.50
8	125	5063	40.5	1.00	57916	463	57916	NC
				Gestation Day 17				
6	25	922	36.9	4.00	6660	266	6898	NC
7	50	1650	33.0	4.00	14797	296	14797	1.83
8	125	4193	33.5	4.00	61379	491	61379	9.27

Table 108 - Seg II Study in Rats: Dose Proportionality Ratios in Pregnant Rat Plasma

Interval	Dose Level Increase	C_{max} Ratio	AUC_{0-t} Ratio
Gestation Day 6	1.0-fold	1.0-fold	1.0-fold
	2.0-fold	1.9-fold	2.7-fold
	5.0-fold	3.5-fold	5.8-fold
Gestation Day 17	1.0-fold	1.0-fold	1.0-fold
	2.0-fold	1.8-fold	2.2-fold
	5.0-fold	4.5-fold	9.2-fold

Dosing Solution Analysis

Dose solutions at concentrations of 0, 5, 10 and 25 mg/mL were mixed weekly (Mixes 1 and 2), apportioned for daily use, and stored at room temperature until used for dosing. Concentration verification results for Mixes 1 and 2 were within 7% of target concentration.

Necropsy

No significant findings noted.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

The mean gravid uterine weight and corrected maternal body weights were significantly decreased in the 125 mg/kg/day group.

The cesarean section data were unremarkable with the exception of a slight increase in the mean percent of postimplantation loss at 125 mg/kg/day that corresponded to a slight decrease in mean percent of live fetuses. The covariate-adjusted mean fetal weight was significantly decreased for the 125 mg/kg/day fetuses, corresponding to the decrease in mean gravid uterine weight and was likely an indirect effect from the treatment-related decrease in mean maternal body weights.

Offspring (Malformations, Variations, etc.)

There were no test article-related malformations. Treatment-related fetal anomalies were limited to skeletal variations related to ossification in the spinal column and sternebra in the 125 mg/kg/day fetuses. The increase in these skeletal variations correlated with significantly decreased mean fetal weights for this high-dose group. However, these variations had no effect on body conformity or the well-being of the animals and are not considered adverse.

Study title: Oral Gavage Dose Range-finding Developmental Toxicity and Toxicokinetic Study with GS-9350 in Rabbits

Study no.:	TX-216-2019
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	22 May 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	See below

Test Article	Lot/Batch No.	Storage	Purity	Retest Date	Reserve (Archive) Sample
GS-9350	3168-171-16	REF	96.1%	30 March 2009	1 g

REF Refrigerated (2 to 8°C) with desiccant under nitrogen

Key Study Findings

The range-finding study was performed to determine the maternal and in-utero toxicity and toxicokinetics (TK) of GS-9350 when administered daily via oral gavage to pregnant NZ White rabbits during the period of organogenesis for the purpose of setting dosage levels for a definitive developmental study.

In summary, the doses of 175 and 300 mg/kg/day were not tolerated. At 100 mg/kg/day, mean body weight losses and reduced food consumption were noted during the dosing phase, with one rabbit aborting on GD26. There were no other adverse maternal or fetal (viability or growth) effects from GS-9350 treatment at doses up to 100 mg/kg/day (C_{max} of 9493 ng/mL and $AUC_{0\text{-}tlast}$ of 76136 ng•hr/mL on GD 20). Based on these results, doses of 25, 50 and 100 mg/kg/day GS-9350 were recommended for the definitive developmental toxicity study in rabbits.

Study title: Oral Gavage Study for Effects on Embryo-fetal Development and Toxicokinetics with GS-9350 in Rabbits

Study no.:	TX-216-2021
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9 July 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	See below

Test Article	Lot/Batch No.	Storage	Purity	Retest Date	Reserve (Archive) Sample
GS-9350	3168-171-16	REF	96.1%	30 March 2009	1 g

REF Refrigerated (2 to 8°C) with desiccant.

Key Study Findings

This study evaluated the maternal and embryo/fetal toxicity and teratogenic potential and determined the toxicokinetics of GS-9350 when administered daily by oral gavage to pregnant rabbits during the period of organogenesis. The dose of 100 mg/kg as a high dose was chosen based on MTD at 175 mg/kg in the range finding study.

The NOAEL for maternal effects of GS-9350 when administered by oral gavage to pregnant NZ White rabbits during the period of organogenesis is 100 mg/kg/day (corresponding to a GD 20 AUC_{0-t} and C_{max} of 35662 ng.hr/mL and 3020 ng/mL, respectively). There were no treatment-related effects on embryo/fetal viability and growth and no fetal anomalies; therefore, the NOEL for developmental toxicity is 100 mg/kg/day.

Methods

Doses:	0, 20, 50, 100 mg/kg (see below)
Frequency of dosing:	Daily
Dose volume:	1 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	95% propylene glycol (PG), 5% ethanol (EtOH) with 1.5 eq. HCl, pH adjusted to 2.3 with HCl
Species/Strain:	HRA:(NZW)SPF rabbits (b) (4)
Number/Sex/Group:	See table below
Satellite groups:	TK animals (see below)
Study design:	Groups of 20 time-mated female Hra:(NZW)SPF rabbits were randomly assigned to 4 main groups and administered 0 (vehicle), 25, 50, or 100 mg/kg/day GS-9350. Doses were administered on Gestation Days (GD) 7 through 20. Additional time-mated rabbits (three/group) were used for toxicokinetic analysis to determine maternal GS-9350 plasma concentrations at selected timepoints on GD 7 and 20.

Deviation from study protocol: Gestation Day 0 body weights and clinical signs were received from the supplier and used for randomization of animals prior to study initiation. These data were collected by a non-GLP facility; however, the integrity of the study was not affected.

Group[a]	No. of Females	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)	Dosing Schedule Days of Gestation
Main Study Animals				
1 (Control)	20	0	0	7-20
2 (Low)	20	25	25	7-20
3 (Mid)	20	50	50	7-20
4 (High)	20	100	100	7-20
Toxicokinetic Animals				
5 (Control)	3	0	0	7-20
6 (Low)	3	25	25	7-20
7 (Mid)	3	50	50	7-20
8 (High)	3	100	100	7-20

a Groups 1 and 5 received control article only.

Observations and Results

Mortality

2x daily. No unscheduled deaths noted.

Clinical Signs

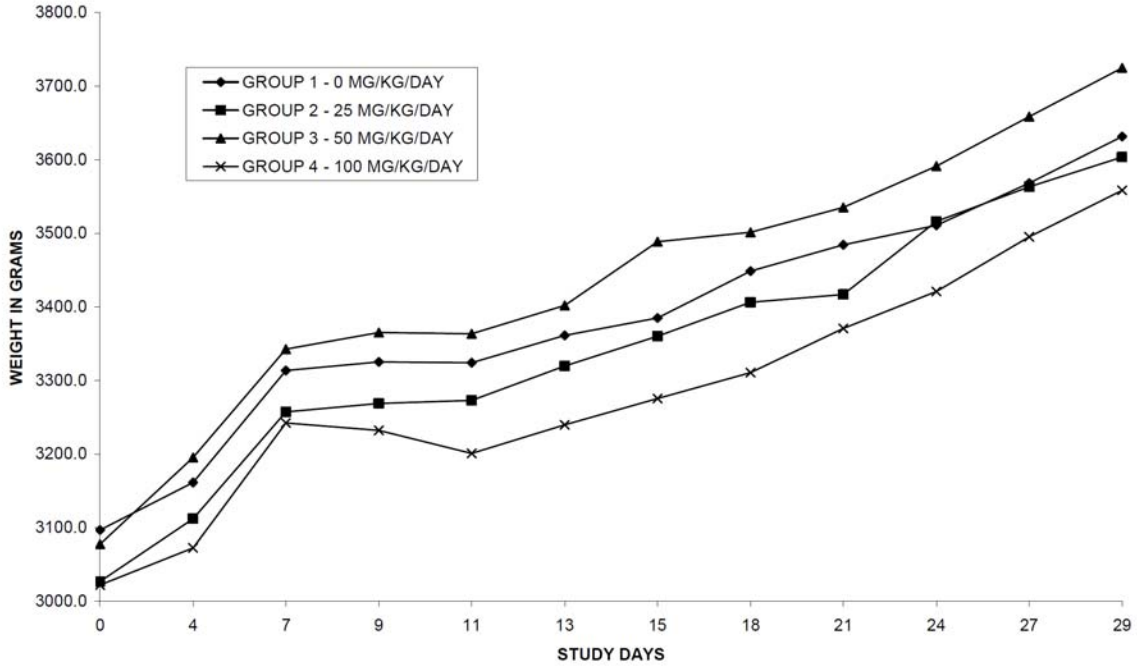
2x daily. No significant clinical signs noted.

Body Weight

Recorded on GD 0, 4, 7, 9, 11, 13, 15, 18, 21, 24, 27, and 29. At initiation of treatment, the animals were 5 months old, and their body weights ranged from 2841 to 3979 g for the main study females and 2905 to 3600 g for the toxicokinetic females.

During the dosing phase, total mean body weight gain (GD 7-21) was decreased for the 100 mg/kg/day group and corresponded to decreased mean food consumption. However, these effects were not considered adverse since overall mean body weight was similar to control throughout the dosing phase, and the effects on mean body weight gain and food consumption were reversed during the postdose phase (GD 21-29).

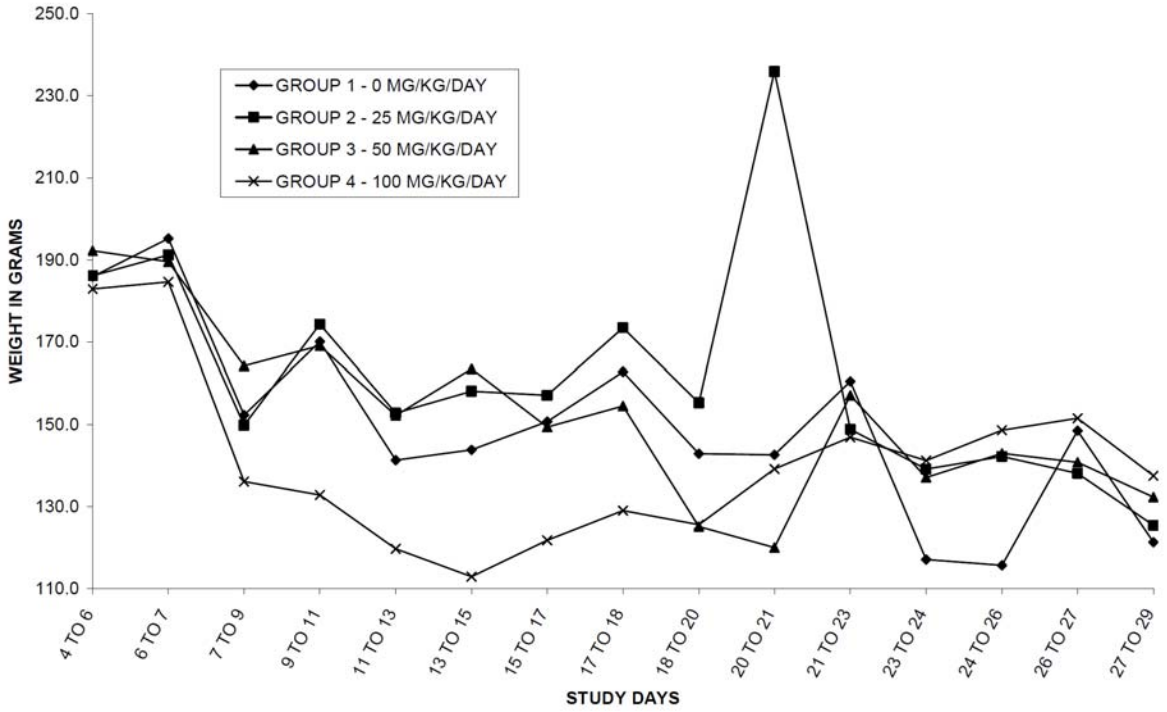
Figure 28 - Seg II Study in Rabbits: Body Weights



Feed Consumption

Measured on GD 4, 6, 7, 9, 11, 13, 15, 17, 18, 20, 21, 23, 24, 26, 27, and 29. Decreased food consumption at 100 mg/kg (more commonly in the early phases of dosing, and then sporadically during the latter phase of dosing). Even though the food consumption decreased and the body weights trended downward at 100 mg/kg, the overall mean body weight did not decrease, so it was not considered adverse.

Figure 29 - Seg II Study in Rabbits: Food Consumption



Toxicokinetics

Exposure to GS-9350 increased with the increase in dose level from 25 to 100 mg/kg/day. The increases in mean C_{max} and AUC_{0-t} were greater than dose proportional. Accumulation of GS-9350 was observed after multiple dosing in pregnant rabbits.

Table 109 - Seg II Study in Rabbits: TK on GD 7

Dose Group	Dose Level (mg/kg/day)	Animal Number	C_{max} (ng/mL)	DN C_{max} (ng/mL)/(mg/kg/day)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	DN AUC_{0-t} (ng•hr/mL)/(mg/kg/day)	AUC_{0-24} (ng•hr/mL)	$t_{1/2}$ (hr)
6	25	Mean	36.8	1.47	1.25	42.3	1.69	58.7	NC
		SD	13.3	NA	1.06	20.8	NA	5.3	NC
		N	2	NA	2	2	NA	2	0
7	50	Mean	190	3.80	2.00	237	4.74	313	NC
		SD	241	NA	1.73	234	NA	243	NC
		N	3	NA	3	3	NA	3	0
8	100	Mean	2750	27.5	2.00	10629	106	11133	NC
		SD	NA	NA	NA	NA	NA	NA	NC
		N	1	NA	1	1	NA	1	0

Table 110 - Seg II Study in Rabbits: TK on GD 20

Dose Group	Dose Level (mg/kg/day)	Animal Number	C_{max} (ng/mL)	DN C_{max} (ng/mL)/(mg/kg/day)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	DN AUC_{0-t} (ng•hr/mL)/(mg/kg/day)	AUC_{0-24} (ng•hr/mL)	$t_{1/2}$ (hr)
6	25	Mean	717	28.7	1.33	1591	63.6	1668	NC
		SD	192	NA	0.58	550	NA	608	NC
		N	3	NA	3	3	NA	3	0
7	50	Mean	4593	91.9	1.67	19189	384	19251	0.901
		SD	1071	NA	0.58	5055	NA	5118	NA
		N	3	NA	3	3	NA	3	1
8	100	Mean	3020	30.2	2.00	35662	357	35662	3.38
		SD	NA	NA	NA	NA	NA	NA	NA
		N	1	NA	1	1	NA	1	1

Table 111 - Seg II Study in Rabbits: Dose Proportionality Ratios (fold increases) of Cobi in Pregnant Rabbit Plasma

Interval	Actual Dose Level Increase	C_{max}	AUC_{0-t}
GD 7	1-fold	1.0-fold	1.0-fold
	2-fold	5.2-fold	5.6-fold
	4-fold	75-fold	251-fold
GD 20	1-fold	1.0-fold	1.0-fold
	2-fold	6.4-fold	12-fold
	4-fold	4.2-fold	22-fold

Dosing Solution Analysis

The stability of samples prepared at 0.9259 and 277.8 mg/mL was confirmed after storage for up to 15 days at room temperature ((b) (4) Study 6511-334).

GS-9350 was a solution in the formulation and concentration range used on this study. Therefore, homogeneity analysis was not required.

Necropsy

No effects noted.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

The cesarean section data were unremarkable and indicated GS-9350 had no effect on embryo/fetal viability.

There were no effects on the covariate-adjusted mean fetal weights in any group or any treatment-related fetal anomalies.

Offspring (Malformations, Variations, etc.)

No effects noted.

9.3 Prenatal and Postnatal Development

Study title: An Oral Pre and Postnatal Development Study, Including a 28-day Juvenile Toxicity Evaluation, of GS-9350 in the Rat

Study no.:	TX-216-2033
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Study director's signature on 14Dec2010. Study dosing started 20Dec2010.
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-9350, Lot 3471-189-36, 98.3% pure (100% pure assumed)

Key Study Findings

In the development and peri-natal/postnatal reproduction phase of the study, including a postnatal behavioral/functional evaluation, daily oral gavage administration of GS-9350 resulted in lower body weights and food intake at 75 mg/kg/day during gestation and lactation, with no effects on maternal performance. In the untreated offspring (F_1 generation), there were no effects on survival, growth, physical and behavioral development, reflex responses, visual function, gross pathology or reproductive parameters. Based on these effects, the NOAEL for maternal toxicity for the F_0 generation was considered to be 30 mg/kg/day and the NOAEL for the reproduction in the dams and viability, growth and development of the offspring was considered to be 75 mg/kg/day (PND 10 C_{max} and AUC_{0-t}: 1854 ng/mL and 9927 ng.hr/mL, respectively).

In the juvenile toxicity phase of the study, daily oral gavage administration of GS-9350 to F_1 generation pups from PND 22 to 49 resulted in slight decreases in body weight and food consumption, non-adverse clinical chemistry changes, and adaptive changes in liver and thyroid. The adaptive changes in the liver and thyroid are secondary changes due to liver CYP inhibition and have been documented in rodents with other CYP inhibitors.

Based on these results, the NOAEL for the juvenile males and females was considered to be 75 mg/kg/day (PND 49 C_{max} and AUC_{0-t}: 3157 and 3083 ng/mL, and 20578 and 21158 ng.hr/mL, in males and females, respectively).

Methods

Doses:	See below
Frequency of dosing:	Daily
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	10% (v/v) PG in 90% (v/v) 40 mM acetate buffer (pH 4.0)
Species/Strain:	SD Rats (time mate females) – Charles River, (b) (4)
Number/Sex/Group:	See tables below.
Satellite groups:	See tables below.
Study design:	During Phase I, GS-9350 was administered to time-mated F_0 generation female rats (24 per group) at 0 (vehicle), 10, 30 and 75 mg/kg/day. One or 2 weanlings/sex/litter were randomly selected to form the F_1 generation (Behavioral/Reproductive Phase, 24 animals/sex/group) and were not directly treated. Ten (10) weanlings/sex/group were selected from the F_1 generation and were dosed 0, 10, 30 and 75 mg/kg/day from PND 22 to PND 49.
Deviation from study protocol:	No deviations were noted that would affect study outcomes or study interpretation.

Table 112 - Seg III Study: Experimental Design - Phase I (F_0 Generation)

Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Main Study	Toxicokinetic Study[a]
		Females	Females
1/ Control	0	24	4
2/ GS-9350	10	24	8
3/ GS-9350	30	24	8
4/ GS-9350	75	24	8

[a] Toxicokinetic animals were used only for determination of drug levels in milk and maternal blood drug levels at the corresponding timepoint.

Table 113 - Seg III Study: Experimental Design - Phase I Adult F_1 Generation (Behavioral/Reproductive Phase)

Group Number	Dose Level (mg/kg/day)[b]	Targeted Maximum Number of Animals	
		Behavioral/Reproductive Phase[c]	
		Males	Females
1/ Control	0	24	24
2/ GS-9350	10	24	24
3/ GS-9350	30	24	24
4/ GS-9350	75	24	24

[b] The F_1 adult generation animals remained in the same dose group as their respective dam, but not directly treated.

[c] 1 or 2 weanling/sex/litter was randomly selected to form the F_1 adult generation

Table 114 - Seg III Study: Experimental Design - Phase II (Juvenile Toxicity Phase)

Group Number	Dose Level (mg/kg/day)	Number of Animals			
		Juvenile Toxicity Study			
		Main Study[d]		Toxicokinetic Study[e]	
		Males	Females	Males	Females
5/ Control	0	10	10	6	6
6/ GS-9350	10	10	10	24	24
7/ GS-9350	30	10	10	24	24
8/ GS-9350	75	10	10	24	24

[d] At each dose level, 1 weanling/sex from 10 Phase I litters were selected for the main study. The F_1 adult generation animals remained at the same dose level as their respective dam.

[e] 6 pups/sex were selected from control litters and 24 pups/sex were selected from each GS-9350 group for juvenile toxicokinetic evaluation. Pups were selected and allocated in a manner that allowed for no more than one male and female from the same litter to be sampled at the same time point. The F_1 adult generation animals remained at the same dose level as their respective dam.

Observations and Results

F_0 Dams

Survival:	No effects noted. Animals survived to scheduled sacrifice.
Clinical signs:	Higher incidence of salivation, and wet fur of the muzzle and/or lower jaw during the gestation and post natal (PND 0 to PND 21) periods in dams administered 30 and 75 mg/kg/day.
Body weight:	Decreases in body weight gains noted from GD 6 through GD 11 at 75 mg/kg/day. These differences resulted in significantly decreased body gain during the gestation period (decreased 15% versus control) and significantly lower mean body weight (from PND 0 to 12) during the lactation period at 75 mg/kg/day. At 10 and 30 mg/kg/day, transient decreases in body weight gain were noted during GD 6 through 8, only. Body weight gains over the entire post natal period (PND 0 to 21) were unaffected by treatment at any dose level.
Feed consumption:	Decreases food consumption noted from GD 6 through GD 11 at 75 mg/kg/day.
Uterine content:	GS-9350 had no effect on pregnancy rate, gestation index, length of gestation, duration of parturition, number of live, dead and malformed pups, sex ratio, number of implantation scars and the live birth index.
Necropsy observation:	No gross findings noted in the dams or pups.
Toxicokinetics:	Exposure to GS-9350 increased with the increase in dose level from 10 to 75 mg/kg/day. Increases in C_{max} and AUC_{0-t} were approximately proportional between the 10 to 30 mg/kg/day dose levels and less than proportional between 30 and 75 mg/kg/day. GS-9350 was present in milk samples 2 hours post-dose on PND 10 with mean plasma to milk ratios ranging from 0.56 to 0.82. See tables below.

Dosing Solution Analysis: The stability of GS-9350 samples prepared at 0.5 to 24 mg/mL in the diluent was confirmed for 15 days at room temperature ((b) (4) Study No. 8203827, Gilead Sciences Ref. No. TX-216-2030). Samples prepared from 0.5 mg/mL to 24 mg/mL were confirmed to be solutions ((b) (4) Study No. 8203827, Gilead Sciences Ref. No. TX-216-2030); therefore, homogeneity analysis was not required.

Table 115 - Seg III Study: TK in Female Rats (PND 10)

Dose Group	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	T_{last} (hr)	C_{last} (ng/mL)
2	10	F	389	0.500	1922	8.00	50.1
3	30	F	1144	4.00	6411	8.00	478
4	75	F	1854	0.500	9927	8.00	1200

Table 116 - Seg III Study: Mean Plasma to Milk Ratio in Female Rats (PND 10)

Dose Group		Plasma:Milk Ratio Time After Dose (hr) 0	2
10 mg/kg	Mean	NA	0.819
	SD	NA	0.166
	N	0	4
30 mg/kg	Mean	0	0.560
	SD	NA	0.167
	N	1	3
75 mg/kg	Mean	0	0.627
	SD	NA	0.216
	N	2	4

NA Not applicable.

F_1 Generation (Behavioral/Repro Assessment)

Survival: No effects noted. Animals survived to scheduled sacrifice.
Clinical signs: No effects noted.
Body weight: No effects noted.
Feed consumption: No effects noted.
Physical development: There were no effects on visual function and no effects on physical and behavioral development.
Neurological assessment: No effects noted.
Reproduction: There were no effects on estrous cycles, mating and fertility indices, conception rate, mean day to mating, and cesarean section parameters.
Other: There were no gross pathologic findings in the Phase I F_1 generation adults attributed to GS-9350.

F_1 Generation (Juvenile Toxicity)

Survival:	No effects noted. Animals survived to scheduled sacrifice.
Clinical signs:	At 30 and 75 mg/kg/day, males and females exhibited a dose-related, but non-adverse, increase in post-dose salivation and red staining of the fur of the lower jaw.
Body weight and Food consumption:	Slight, but not statistically significant, decreases in mean body weight, mean body weight gain and mean food consumption were noted for males at 75 mg/kg/day during the dosing period. There were no notable effects on body weight or food consumption in males at 10 and 30 mg/kg/day, or in females at any dose level.
Clinical Chemistry:	There were no treatment-related effects on hematology, coagulation or urinalysis parameters. Clinical chemistry changes noted at 30 and 75 mg/kg/day were not considered adverse. These changes included minimal, but statistically significant, decreases in alkaline phosphatase (ALP) levels in males at ≥ 30 mg/kg/day and in females at 75 mg/kg/day, and significant increases in globulin, with decreases in the A/G ratio, in both sexes at ≥ 30 mg/kg/day.
Hormone Analysis:	GS-9350-related increases in thyroid stimulating hormone (TSH), decreases in thyroxine (T4) and/or triiodothyronine (T3) were noted in both sexes at all dose levels. These decreases reached statistical significance for mean T4 and T3 in males at 75 mg/kg/day, and mean T3 in females at 30 and 75 mg/kg/day.
Pathological Assessment:	There were no GS-9350-related macroscopic findings. Significantly increased liver weights (males at 75 mg/kg/day and females at ≥ 30 mg/kg/day) and increased thyroid weights (females at all dose levels) were associated with hepatocellular hypertrophy (in females at 75 mg/kg/day) and thyroid follicular cell hypertrophy (in males at 75 mg/kg/day and in females at ≥ 30 mg/kg/day), respectively. In addition, there was an increased incidence of hepatocellular vacuolation in females at ≥ 30 mg/kg/day. These liver and thyroid effects are considered to be adaptive changes commonly seen in rodents with microsomal enzyme inducers and not considered adverse under the conditions of this study.
Toxicokinetics:	Exposure to GS-9350 increased with the increase in dose level from 10 to 75 mg/kg/day. Increases in C_{max} and AUC_{0-t} were greater than proportional between the 10 and 75 mg/kg/day. C_{max} and AUC_{0-t} values were generally similar on PNDs 22 and 49, except for higher values in females at the 10 mg/kg/day dose level, indicating no accumulation of GS-9350 after multiple dosing to juvenile rats. Sex differences were generally less than 2-fold in C_{max} and AUC_{0-t} values, except at 10 mg/kg/day on PND 49, where females had 3- to 6-fold higher C_{max} and AUC_{0-t} values, respectively, than males. See table below.

Table 117 - Seg III Study: TK in Juvenile Rats (PNDs 22 and 49)

PND	Dose Group	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	T_{last} (hr)	C_{last} (ng/mL)
22	6	10	M	119	4.00	327	4.00	119
			F	183	2.00	483	4.00	134
	7	30	M	1677	4.00	9129	8.00	307
			F	1703	2.00	6533	8.00	250
	8	75	M	3653	2.00	32138	12.0	77.2
			F	3083	1.00	18628	12.0	122
49	6	10	M	70.5	2.00	303	8.00	21.8
			F	349	4.00	1836	8.00	73.8
	7	30	M	1104	4.00	4992	8.00	184
			F	1890	1.00	8494	8.00	567
	8	75	M	3157	4.00	20578	12.0	13.4
			F	3083	2.00	21158	12.0	11.7

10 Special Toxicology Studies

10.1 Local Tolerance

Study title: GS-9350: Skin Irritation to the Rabbit

Study no.:	TX-216-2044
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Protocol signed on 3/23/2011.
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-9350, Lot 4646-065-18, 99.4% pure
Deviation from study protocol:	No deviations were noted that would affect study outcomes or study interpretation.

Key Study Findings

The study was performed to assess the skin irritation potential of GS-9350 in the rabbit.

Three female rabbits received a single four hour, semi-occlusive, dermal administration of approximately 0.5 g of GS-9350 as supplied and were observed for up to 13 days.

Very-slight erythema was evident at the test-site of each animal during the first seven days after bandage removal, persisting in two animals through Day 8 and in one animal through Day 12. Observations of 'loss of elasticity' and 'sticky to touch' were noted at the test site of one animal throughout the 13 days of observations and were attributed to test material residue.

The means of scores for these reactions at approximately 24, 48 and 72 hours after administration, calculated separately for each animal, are summarized below:

Table 118 - Skin Irritation Potential of GS-9350 (Means of scores at approximately 24, 48 and 72 hours)

Animal number	Erythema	Oedema
10	1.0	0.0
128	1.0	0.0
129	1.0	0.0
EC trigger values*	≥2.3	≥2.3

*Classification according to regulation (EC) 1272/2008 is triggered if means of scores for either effect are ≥ 2.3 for two or three animals (or if effects persist to Day 14 in at least two animals).

The Primary Irritation Index was calculated as 1.0; GS-9350 was classified as "mildly-irritating".

Study title: GS-9350: The Bovine Corneal Opacity and Permeability Assay

Study no.:	TX-216-2043
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Protocol signed on 2/25/2011.
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-9350, Lot 4646-065-18, 99.4% pure
Deviation from study protocol:	No deviations were noted that would affect study outcomes or study interpretation.

Key Study Findings

The Bovine Corneal Opacity and Permeability Assay was performed to assess the *in vitro* ocular irritancy potential of GS-9350. Imidazole was tested in parallel as a positive control.

Isolated bovine corneas were obtained and evaluated for ocular corrosivity and severe irritancy potential. Two endpoints, corneal opacity and permeability, were measured and combined to give an In Vitro Irritancy Score which were used to classify and rank test substances as potential eye irritants.

GS-9350 (20% w/w) elicited an In Vitro Irritancy Score of -1.6 ± 0.0 and is predicted to be a non-corrosive/non-severe eye irritant. The positive control was appropriate and was detected as positive (161.8 ± 25.5)

Study title: GS-9350: Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (Individual animal approach)

Study no.:	TX-216-2042
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Protocol signed on 3/1/2011.
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-9350, Lot 4646-065-18, 99.4% pure
Deviation from study protocol:	No deviations were noted that would affect study outcomes or study interpretation.

Key Study Findings

This study was performed to assess the skin sensitization potential of GS-9350 using the local lymph node assay (LLNA).

Mice were dosed dermally with GS-9350 in AOO (4:1 acetone olive oil) at concentrations of 2.5, 5, or 10 % w/v. Positive control was HCA (25% v/v hexyl cinnamic aldehyde). Five days post-dosing, mice were challenged with tritiated thymidine. Draining lymph nodes were removed and tritium was measured and was evaluated relative to the positive control as a SI (stimulation index).

All animals survived to the terminal sacrifice. No GS-9350 – related adverse clinical observations, dermal reactions, ear thickness or body weight changes were noted during the preliminary or main phases of the study.

The SI obtained for 2.5, 5 and 10% w/v were 2.2, 2.3 and 2.9 respectively, which indicates that GS-9350 did not show the potential to induce skin sensitization. The SI for the positive control substance HCA was 14.8, which demonstrates the validity of this study.

10.2 Immunotoxicity Assessment

Study title: 4-Week Oral Gavage T-Cell Dependent Antibody Assay with GS-9350 in Rats

Study no.:	TX-216-2022
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	5 May 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	See below.

Test Article	Lot No.	Storage	Purity	Retest Date	Reserve (Archive) Sample
GS-9350	3168-157-8	Protected from light in a refrigerator, set to maintain 2 to 8°C	97.5%	31 Jan 2009	Collected

Key Study Findings

Administration of GS-9350 to rats by oral gavage for at least 4 weeks produced immunosuppressive effects in females at 50 and 150 mg/kg/day, based on decreased response to KLH immunization (lower anti-KLH IgG antibody titers). Decreased anti-KLH IgG responses in males did not reach statistical significance at 150 mg/kg/day. No GS-9350-related changes in the anti-KLH IgM response in males and females were noted. In addition, clinical signs, decreases in body weight gain and/or food consumption, increases in liver and thyroids weights, and lymphoid depletion of germinal centers in the spleen were observed at 50 and/or 150 mg/kg/day.

The NOEL for the T-cell dependent antibody response (TDAR) is considered 20 mg/kg/day in females (Week 4: C_{max} and AUC_{0-t} of 1416 ng/mL and 5694 ng•h/mL, respectively), and 50 mg/kg/day in males (Week 4: C_{max} and AUC0-t of 2197 ng/mL and 11232 ng•h/mL, respectively).

Based on the observed changes in the spleen, the NOAEL for the study is considered to be 20 mg/kg/day in males and females (Week 4 AUC_{0-t} of 2325 and 5694 ng•h/mL, respectively).

Although there were decreases in IgG, it was not likely immune specific. The decrease in IgG antibody production to KLH antigen only occurred in the presence of overt toxicity. At doses that did not produce decreases in body weight gain and food consumption (≤20 mg/kg), the anti-KLH response was considered normal. Therefore, GS-9350 does not appear to have selective immunotoxic effects.

Methods

Doses:	0, 20, 50, 150 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	3.75 ml/kg
Route of administration:	Oral gavage.
Formulation/Vehicle:	95% (v/v) propylene glycol (PG), 5% (v/v) ethanol, pH adjusted to 2.3 + 0.1 with 12N HCl.
Positive Control:	Cyclosporin A in a vehicle of 5% (v/v) of ethaniol in olive oil
Species/Strain:	SD Rats (Charles River (b) (4))
Number/Sex/Group:	See table below
Satellite groups:	TK animals. See table below.
Study design:	TDAR. All toxicity animals received one dose of keyhole limpet hemocyanin (KLH) by intravenous injection into a tail vein on Day 5 of the dosing phase.
Deviation from study protocol:	No deviations were noted that would affect study outcomes or study interpretation.

Group	No. of Animals Males	Females	Dose Level (mg/kg/day)[a]	Dose Concentration (mg/mL)[a]
T-Cell Dependent Antibody Animals (Toxicity)[c]				
1 GS-9350 (Control)[b]	8	8	0	0
2 GS-9350 (Low)	8	8	20	5.3
3 GS-9350 (Mid)	8	8	50	13.3
4 GS-9350 (High)	8	8	150	40
5 (Cyclosporin A)[d]	5	5	30	6
Toxicokinetic Animals[e]				
6 (Control)[b]	3	3	0	0
7 GS-9350 (Low)	6	6	20	5.3
8 GS-9350 (Mid)	6	6	50	13.3
9 GS-9350 (High)	6	6	150	40

a The test and control articles were administered at a dose volume of 3.75 mL/kg.
b Animals in Groups 1 and 6 were dosed with the control article only [95% (v/v) propylene glycol (PG), 5% (v/v) 200 proof ethanol, pH adjusted to 2.3 + 0.1 with 12N HCl].
c Animals in Groups 1 through 5 received one dose of KLH (0.2 mg/animal administered at a constant volume of 0.5 mL/animal) by intravenous injection into a tail vein on Day 5 of the dosing phase.
d Animals received cyclosporin A at a dose volume of 5 mL/kg via oral gavage once daily for at least 4 weeks.
e Toxicokinetic animals were included solely for the purpose of blood sample collections.

11 Integrated Summary and Safety Evaluation

All nonclinical studies required to support chronic use have been performed and submitted as a part of the nonclinical assessment for COBI. The only exception is the carcinogenicity studies. The Division agreed with the Sponsor that these studies can be submitted post-marketing.

No significant effects were noted that would preclude approval of COBI as a pharmacoenhancer in an HIV combination drug product.

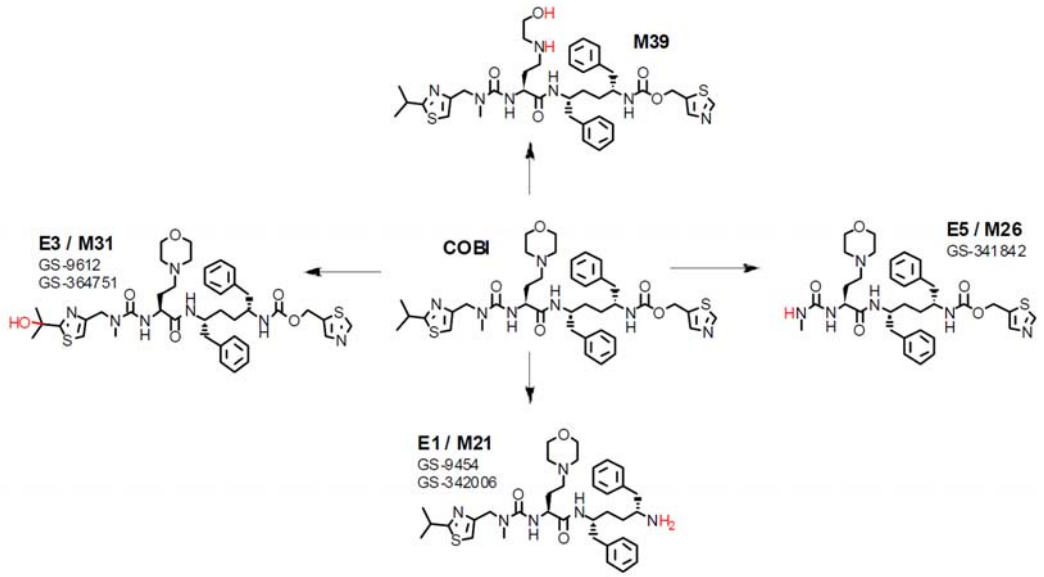
Safety pharmacology was evaluated for cardiotoxicity, CNS toxicity, and respiratory effects. Initially, a safety pharmacology signal was detected for potential cardiac effects in the hERG assay. COBI inhibited hERG potassium current at a low concentration (IC_{50} = 1.8 μM). Furthermore, COBI inhibited the hCav1.2 L-type calcium channel at a moderately low concentration (IC_{50} = 6 μM). During further characterization *in vitro* utilizing rabbit hearts, COBI caused negative inotropic effects and shortening of the APD at ≥ 1 μM in rabbit Purkinje fibers. In a subsequent study in rabbit hearts, similar effects were noted as COBI produced negative inotropic effects (PR interval prolongation, and produced decreases in left ventricular [LV] function) at concentrations ≥ 1.5 μM. To address the *in vitro* cardiac concerns, *in vivo* studies in beagle dogs was performed. COBI caused no adverse effects up the highest dose administered (45 mg/kg). However, mild prolongation in PR intervals (primarily from 1 to 6 hours postdose), but it was within upper limits of normal. These findings correlated with human effects in a study of healthy volunteers up to 250 mg and 400 mg. At 250 mg dose and higher, there were no effects on QT interval, but there was evidence of PR interval prolongation. Five subjects at 400 mg and 2 in the 250 mg arm had asymptomatic absolute PR>200 ms post-baseline. Due to the significant concern for cardiac effects at doses of 250 mg and above, the

Sponsor chose the 150 mg dose for the Phase 2 and 3 clinical trials. At the 150 mg dose (the proposed clinical dose), there were no cardiac effects noted.

COBI had limited central nervous system effects in rats at 150 mg/kg and higher (increased salivation, decreases in arousal, decreases in locomotor and motor activities, and decreases in body temperature).

COBI was highly protein bound (98-99%) and was widely distributed with most of the drug in the GI and lesser amounts in the liver, adrenal, kidney, and pituitary. After oral dosing, bioavailability was low or low/moderate, likely due to high first-pass elimination. COBI had multiple metabolites (>50 to >80) in rats, mice, and dogs as well as in human hepatic microsomal fractions. All species tested had the major human metabolites (M31, M26, M21, M39). The parent drug as well as the M31 and M21 metabolites were excreted in the feces (79-89%) in all species tested. In rats and dogs, roughly 63-69% of the drug was recovered in the bile. Less than 2% of the drug was recovered in the urine.

Figure 30 - Proposed Metabolic Pathway of COBI (*in vivo*)



COBI has multiple predicted drug-drug interactions based on inhibition of CYP3A enzymes as well as inhibition of several renal transporters. COBI data from the rat indicated that CYP3A caused slight induction, whereas in the dog it caused inhibition of CYP3A. However, in humans, it appears that CYP3A is inhibited, which supports the primary mechanism of action of CYP3A inhibition. A clinical concern for COBI was the inhibition of renal transporters (mainly MATE1) which may increase serum creatinine (without affecting aGFR) as well as inhibition of p-gp may increase tenofovir renal exposure in humans. The Sponsor plans to evaluate the effects of COBI on tenofovir in humans to address these concerns. The Agency may request additional studies to further evaluate a potential COBI/TDF interaction.

Single dose toxicology was assessed in rats and mice. COBI was well tolerated in rats up to 500 mg/kg after a single dose with no adverse effects (NOAEL = 500 mg/kg). Mice, however did

not tolerate COBI and were euthanized moribund at 300 mg/kg after a single dose. The maximum tolerated single dose in mice was 100 mg/kg.

Repeat dose toxicology was assessed in mice, rats, and dogs. In repeat-dose studies (up to 13 weeks in mice, 26 weeks in rats, 39 weeks in dogs), the target organs were liver (mouse, rat, and dog) and thyroid (rat). Slight hematological changes were noted in rats. Slight clinical chemistry changes were noted in all three species. Urine changes (mainly dilution due to diuretic effects caused by increased water consumption) were noted in rats and dogs and were dose-dependent, but not considered adverse.

In the 13-week mouse study, mild-to-marked elevations in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were noted in males at 15 and 50 mg/kg/day. These changes were associated with microsomal enzyme induction, increases in liver weight and minimal hepatocellular hypertrophy at 50 mg/kg/day. Female mice were notably less sensitive; a marked elevation in ALT and AST was noted in only one high-dose female (50 mg/kg/day). The NOAELs were considered to be 5 mg/kg/day in males and 50 mg/kg/day in females. In a 4-week non-pivotal toxicity study using wild type mice, that was conducted to assess the feasibility of the transgenic CB6F1-non Tg(HRAS) for the 6-month transgenic carcinogenicity study, the NOAEL is considered to be 100 mg/kg/day. Mild increases (2-3-fold) in ALT and AST correlated with increased liver weights at 100 mg/kg/day in both sexes, and with minimal to slight hepatocellular hypertrophy in males at 100 mg/kg/day.

In the 4-week and 26-week oral dose rat studies, increases in liver and thyroid weights were associated with CYP3A enzyme induction, hepatocellular hypertrophy, thyroid hormone changes (decreased thyroxine [T4]; increased thyroid stimulating hormone [TSH]) and thyroid follicular cell hyperplasia/hypertrophy. These findings were reversible, and were not considered adverse. However, one high-dose male animal had a follicular cell carcinoma in the thyroid in the 26-week study. The liver and thyroid effects are considered adaptive changes, are commonly seen in rodents with microsomal enzyme inducers, and are considered secondary to microsomal enzyme induction and thyroid hormone imbalance (decreases in T4 and increases in TSH). Hematological and clinical chemistry changes were not considered adverse. Hematological changes (not exceeding 10% versus controls) included slightly lower mean values for erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin, and slightly higher mean platelet counts. Serum chemistry changes observed after 13 and/or 26 weeks of dosing included slightly higher mean gamma glutamyltransferase (GGT), cholesterol, total protein, albumin, globulin, and calcium. After a 13-week recovery period, cholesterol and total protein values remained slightly higher in high-dose females, whereas other values were generally comparable to control values, indicating reversibility. The NOAEL for COBI in the 26-week rat study was considered to be 30 mg/kg/day, based on significant decreases in body weight and food consumption, slight changes in hematological parameters, and increases in urine volume at 100 mg/kg/day.

In dogs, apart from salivation and vomiting associated with dosing, treatment with COBI was well tolerated at doses up to 15 mg/kg/day in the 4-week study, and up to 10 mg/kg/day in the 39-week study. Changes in the thymus and adrenal gland in dogs observed in high-dose animals after 13 weeks of dosing were absent after 39 weeks of dosing, and were considered stress-

related, and not a direct effect of COBI. In dogs administered 20 mg/kg/day for 39 weeks, clinical signs (salivation, emesis, fecal changes), decreases in body weight and food consumption, nonadverse changes in clinical pathology parameters, and minimal, adaptive changes in the liver (increased weights, hypertrophy) were noted. After 39 weeks of dosing at 10 mg/kg/day, effects were limited to minimal hepatocellular hypertrophy in males, and slightly increased liver weights in females. Clinical pathology changes in the 28-day study included minimal-to-mild increases in bilirubin, ALT, and alkaline phosphatase activities. In the 39-week study, slightly higher platelets counts, slightly higher alkaline phosphatase, and slightly lower total protein and albumin were observed; these changes were reversible following cessation of dosing. Based on these findings, the NOAEL for COBI when administered daily by oral gavage to dogs for up to 39 weeks is 10 mg/kg/day.

Urinalysis and urine chemistry changes, noted primarily in high-dose rats at 100 mg/kg/day and in female dogs at 20 mg/kg/day, included slightly higher electrolyte excretion and slightly lower electrolyte concentrations, consistent with findings of lower urine osmolality, higher urine volume and/or pH. These changes showed no progression after long term dosing, were reversible, were not associated with remarkable clinical chemistry changes, including serum creatinine and blood urea nitrogen (BUN), and were without histopathological correlates in the kidney. In dogs, a greater incidence of bilirubinuria was noted in males at 20 mg/kg/day during the 39-week study; no changes were observed at recovery. Clinically, there were reports of similar effects (increased water consumption and increased/diluted urine) without any adverse effects.

There were no significant adverse effects and no evidence of additive or synergistic effects in 90-day rat toxicity studies conducted with COBI in combination with ATV. The NOAEL was 30 mg/kg/day COBI and 50 mg/kg/day ATV, either alone or in combination.

The exposures based on plasma AUC values at the NOAEL doses in the longest duration studies were approximately 0.1- to 7.2-fold (mice), 1.2- to 1.6-fold (rats), and 2.1 to 2.4-fold (dogs) higher than the AUC in patients treated once daily with COBI at 150 mg in the EVG/COBI/FTC/TDF fixed dose tablet.

Table 119 – Safety Margins for COBI 150 mg Based on Exposure (AUC) at NOAELs in the Repeat Dose Toxicology Studies

Species Gender	Study Type	NOAEL Dose (mg/kg/day)	AUC_{0-t} (µg•h/mL)	Safety Margin[a]
Mouse				
Male - Female	13-week Toxicity	5 - 50	0.93 – 60.1	0.1 – 7.2 X
Rat				
Male - Female	26-week Toxicity	30	9.9 – 13.3	1.2 – 1.6 X
Rat + 50 mg/kg ATV				
Male - Female	13-week Combination Toxicity	30	6.1 – 6.3	0.7 – 0.8 X
Dog				
Male - Female	39-week Toxicity	10	19.6 – 16.8	2.4 – 2.1 X

[a] *Safety margins based on human exposure of 8.3 µg*h/mL at 150 mg of COBI.*

COBI was not genotoxic as evaluated by the Ames assay, chromosomal aberration assay, as well as an *in vivo* rat micronucleus assay. Carcinogenicity studies were not submitted with this NDA. The Division agreed with the Sponsor that the Carcinogenicity studies would be submitted post-marketing.

No adverse effects were observed in a rat fertility study; the NOEL for reproductive parameters was 100 mg/kg/day at exposures approximately 4-fold higher than human therapeutic exposures. No teratogenic effects were observed in rat and rabbit developmental toxicity studies. In rats at 125 mg/kg/day, increases in postimplantation loss and decreased fetal weights were associated with significant maternal toxicity (adverse clinical signs, decreased body weight and food consumption). The NOEL/NOAELs in the rat and rabbit studies were 50 and 100 mg/kg/day, respectively, where exposures were approximately 1.8- and 4.3-fold higher, respectively, than human therapeutic exposures. In the pre/postnatal study, the maternal NOAEL for general toxicity was 30 mg/kg/day, and the NOAEL for reproduction in the dams and viability and growth of the offspring was 75 mg/kg/day, the highest dose tested (exposures on lactation Day 10 were 1.2-fold higher than human therapeutic exposures). Cobicistat was secreted in the milk of nursing rats in the pre/postnatal study, with COBI milk to plasma ratios of 1.3 to 1.9.

In the juvenile toxicity phase of the pre/postnatal study in rats, daily oral gavage administration of COBI to F_1 generation pups from PND 22 to 49 was well tolerated at doses up to 75 mg/kg/day, with adaptive liver and thyroid changes observed at similar dose levels and exposures to adult animals. The NOAEL for toxicity of COBI is 75 mg/kg/day for juvenile rats where exposures were 2.5-fold higher than therapeutic human exposures at the 150 mg dose.

Table 120 - Estimated Safety Margins for COBI 150 mg Based on Exposure (AUC) at NOAELs in the Reproductive Toxicology Studies

Reproductive Toxicology Study	NOAEL (mg/kg/day)	AUC_{0-t} (µg*hr/ml)	Safety Margin [a]
Fertility and General Reproduction in Rat			
Male	100	32.7 [b]	3.9
Female	100	42.9 [b]	5.1
Embryo-Fetal Development in Rat			
Maternal	50	14.8	1.7
Fetal	50		
Embryo-Fetal Developmental in Rabbit			
Maternal	100	35.7	4.3
Fetal	100		
Perinatal Postnatal Toxicity in Rat			
Maternal General Toxicity			
Reproduction in Dams: Reproduction, viability, growth and development	75	9.93	1.2
Juvenile Toxicity:			
Male	75	20.6	2.5
Female	75	21.2	2.6

[a] *Safety margins based on human exposure of 8.3 µg*h/mL at 150 mg of COBI.*
[b] *AUC values were not collected in the study. The values are excerpted from the 26-week repeat-dose toxicology study in rats (Study TX-216-2004).*

The Sponsor completed several local tolerance studies (ocular irriation, dermal irritation/sensitization, phototoxicity) with mild skin irritation noted. Immunotoxicity was assessed by a TDAR with potentially positive findings. However these findings were only noted at doses that caused overt toxicity. With no other immunological findings noted in the repeat dose toxicology studies, it was unlikely to be a true finding for immunotoxicity.

The proposed specifications for most impurities in the cobicistat drug substance were deemed acceptable based on repeat dose general toxicology studies, in silico evaluation of mutagenic potential, and the serious nature of the clinical indication. However, (b) (4) were considered potentially mutagenic (b) (4) In addition, (b) (4) are known to be mutagenic in the Ames assay. Exposures to the expected or known mutagenic impurities will be controlled to appropriate levels as described in the FDA Draft Impurity Guidance. Please see Dr. Powley's review (Appendix C of the E/C/F/T NDA review) for that information.

The overall nonclinical program of COBI was considered adequate to support the safety of COBI as part of the E/C/F/T.

12 Appendix/Attachments

None.

EPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	203-100 (Appendix C)
Supporting document/s:	1, 15, 32, 34
Applicant's letter date:	October 26, 2011; January 31, 2012; April 27, 2012; May 21, 2012
CDER stamp date:	October 27, 2011; January 31, 2012; April 27, 2012; May 21, 2012
Product:	Elvitegravir 150 mg/Cobicistat 150 mg/Emtricitabine 200 mg/Tenofovir DF 300 mg Tablet (E/C/F/T) Appendix C: Impurities, Degradants, and Excipients
Indication:	Treatment of HIV-1 Infection
Applicant:	Gilead Sciences, Inc. 333 Lakeside Dr. Foster City, CA 94404
Review Division:	DAVP
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1 Executive Summary

1.1 Introduction

Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Disproxil Fumarate (E/C/F/T) is a combination drug product consisting of an HIV-1 integrase strand transfer inhibitor (elvitegravir), a pharmacoenhancer designed to inhibit CYP3A (cobicistat), an HIV-1 reverse transcriptase inhibitor (emtricitabine), and an HIV-1 reverse transcriptase inhibitor (tenofovir disproxil fumarate). Gilead has submitted an NDA in support of E/C/F/T combination therapy to treat HIV-1 infection in adults ≥ 18 years of age who are antiretroviral treatment naïve or have no known substitutions associated with resistance to the individual components. The proposed daily clinical dose is 150 mg elvitegravir, 150 mg cobicistat, 200 mg emtricitabine, and 300 mg tenofovir disproxil fumarate.

This review focuses on qualification of impurities, degradants, and excipients. Regulatory recommendations in regards to impurities are provided by ICH Q3A(R2) – "Impurities in New Drug Substance", ICH Q3B(R2) – "Impurities in New Drug Products", ICH Q3C(R5) – "Impurities: Guideline for Residual Solvents", and Draft FDA Guidance for Industry – "Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches" while recommendations addressing excipients are detailed in FDA Guidance for Industry – "Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients".

Note that the draft FDA impurity guidance recommends no further action for impurities below the ICH Q3A(R2) qualification threshold (i.e., < 0.15% for maximum daily dose ≤ 2 g/day) that lack structural alerts. Although several impurities have specifications greater than the ICH qualification threshold, a negative in silico assessment was considered acceptable for genotoxic qualification due to the serious nature of the indication.

Elvitegravir

The proposed specifications for impurities in the elvitegravir drug substance were deemed acceptable based on repeat dose general toxicology studies, in silico evaluation of mutagenic potential, and the serious nature of the clinical indication. The proposed specifications for residual solvents were also deemed acceptable based on calculated PDE values or those listed in ICH Q3C(R5). A compound specific risk assessment was made for (b) (4) *a potentially genotoxic impurity. Using carcinogenicity data for this impurity, an acceptable TTC* (b) (4) *was calculated.*

Cobicistat

The proposed specifications for most impurities in the cobicistat drug substance were deemed acceptable based on repeat dose general toxicology studies, in silico evaluation of mutagenic potential, and the serious nature of the clinical indication. However, (b) (4) *and* (b) (4) *were considered potentially mutagenic* (b) (4) *. In addition,* (b) (4) *and* (b) (4) *are known to be mutagenic in the Ames assay. Exposures to the expected or known mutagenic impurities will be controlled to appropriate levels as described in the FDA Draft Impurity Guidance.*

Drug Product
Proposed specifications for degradant levels in cobicistat, emtricitabine, and tenofovir disproxil fumarate were qualified by repeat dose general toxicology studies, in silico evaluation of mutagenic potential for cobicistat, or previous consideration of genotoxicity for the marketed products (i.e., emtricitabine and tenofovir disproxil fumarate).

2 Elvitegravir Impurities (DMF 25187)

Note: Elvitegravir is also known as GS-9137.

2.1 Routine Impurities

2.1.1 General Toxicology

The Sponsor conducted two general toxicology studies to evaluate elvitegravir impurities (Study nos. TX-183-2010 and TX-183-2010 summarized in Appendix C2). In each study the effects of GS-9137 spiked with impurities vs. GS-9137 alone were compared after 28 days of dosing in rats. Based on NOEL/NOAELs established in these studies, the qualified impurity levels detailed in Table 121 (taken from Sponsor submission) are accurate and exceed the proposed specifications.

Table 121. Summary of elvitegravir impurities qualification

Impurity	Maximum Observed in Toxicological Studies (%)	NOAEL from Study (mg/kg/day)	Qualified Level [a] (mg/kg/day)	Qualified Level [a] for 60 kg Human and 150 mg/day Dose	Lot Number	Toxicology Study Number
(b) (4)					112002	TX-183-2023
					062091	TX-183-2010
					062091	TX-183-2010
					062091	TX-183-2010
					062091	TX-183-2010
					112002	TX-183-2023
					112002	TX-183-2023
					112002	TX-183-2023
					112002	TX-183-2023
					062091	TX-183-2010
					112002	TX-183-2023
					062091	TX-183-2010
					112002	TX-183-2023
					112002	TX-183-2023
					112002	TX-183-2023
					112002	TX-183-2023
					112002	TX-183-2023
					112002	TX-183-2023
(b) (4)						

Note - qualified level = (% impurity x NOAEL) / (body surface area conversion factor x maximum clinical dose)

2.1.2 Genetic Toxicology

The draft FDA impurity guidance recommends no further action for impurities below the ICH Q3A(R2) qualification threshold (i.e., < 0.15% for maximum daily dose ≤ 2 g/day) that lack structural alerts. Although several impurities have specifications greater than the ICH qualification threshold, a negative in silico assessment was considered acceptable for qualification due to the serious nature of the indication. A review of the in silico evaluations

conducted by the Sponsor (Study no. TX-183-2024) and the CDER Computational Toxicology Group is provided in Appendix C3. A majority of the impurities were predicted positive for mutagenic potential (b) (4) *This weak alert had questionable human relevance and was also present in the Ames negative parent compound. As such, the positive predictions were considered unreliable and the impurities should be considered non-mutagenic.*

2.2 (b) (4)

Although the genotoxic potential (b) (4) *is not fully understood, this impurity is a rodent carcinogen and considered possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC, 1999). Because there is rodent carcinogenicity data* (b) (4)*, a chemical specific risk assessment was performed. The threshold of toxicological concern (TTC; exposure yielding an excess cancer risk of 1 in 1x10^5) is calculated using the following formula:*

$$TTC = TD_{50}/50{,}000 \text{ corrected for body weight}$$

Using the most conservative TD_{50} (b) (4) *for the formation of hematopoietic system tumors in male rats* (b) (4)*, the TTC* (b) (4) *is* (b) (4)*. Therefore, the proposed specification* (b) (4) *is overly conservative.*

2.3 Residual Solvents

The Sponsor's proposed acceptance limits for residual solvents are summarized in Table 122 (taken from Sponsor submission). According to the ICH Q3(R5) Option 2 calculation, the proposed specifications for ICH Class 2 solvents are acceptable. The proposed limit (b) (4) *for Class 3 solvents is acceptable for drugs administered at doses ≤ 10 g/day (i.e., using the Option 1 calculation). The limit* (b) (4) *is based on the Option 2 calculation. While this calculation is not routinely applied to Class 3 solvents, there is minimal toxicological concern.*

(b) (4) *is not mutagenic (Mortelmans et al., 1986) and appears to be relatively non-toxic in mice and rats* (b) (4) *The Sponsor has calculated a PDE for this solvent assuming the worst case scenario (i.e., teratogenicity) and an NOAEL of* (b) (4) *established in the long-term mouse study. Based on these conservative assumptions, the proposed specification* (b) (4) *is acceptable.*

In all cases, the Sponsor should make a reasonable effort to reduce residual solvent levels.

Table 122. Proposed acceptance limits for residual solvents in elvitegravir

Solvent	ICH Class	PDE (mg/day)	Qualified Level[a] (at 150 mg/day)	Proposed Acceptance limit
(b) (4)				

Note - The PDE value (b) (4) *should be* (b) (4)

References

(b) (4)

IARC (1999) Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide. *IARC Monogr. Eval. Carcinog. Risks Hum*. 71:1211-1221

Mortelmans K., Haworth S., Lawlor T., Speck W., Tainer B., Zeiger E. (1986) Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. *Environ. Mutagen.* 8:1-119

3 Cobicistat Impurities (DMF 25188)

Note: Cobicistat is also known as GS-9350.

3.1 Routine Impurities

3.1.1 General Toxicology

The Sponsor conducted two general toxicology studies to evaluate cobicistat impurities (Study nos. TX-216-2005 and TX-216-2045 summarized in Appendix C2). The GS-9350 lot used in a 4-week dog study contained a significant quantity of (b) (4) *impurity. Other impurities were evaluated in a 28-day rat study comparing GS-9350 spiked with impurities vs. GS-9350 alone. Based on NOEL/NOAELs established in these studies, the qualified impurity levels detailed in Table 123 (taken from Sponsor submission) are accurate and exceed the proposed specifications.*

Table 123. Summary of cobicistat impurities qualification

Impurity	Maximum Observed in Toxicological Studies (%)	NOEL or NOAEL from Study (mg/kg/day)	Qualified level [a] (mg/kg/day)	Qualified Level [a] for 60 kg Human and 150 mg/day Dose	Lot Number	Toxicology Study Number
(b) (4)					3793-143-22	TX-216-2005
					4646-065-15	TX-216-2045
					4646-065-15	TX-216-2045
					4646-065-15	TX-216-2045
					4646-065-15	TX-216-2045
					4646-065-15	TX-216-2045
					4646-065-15	TX-216-2045
					4646-065-15	TX-216-2045
					4646-065-15	TX-216-2045
					4646-065-15	TX-216-2045
					4646-065-15	TX-216-2045
					4646-065-15	TX-216-2045
					4646-065-15	TX-216-2045
					4646-065-15	TX-216-2045
					4646-065-15	TX-216-2045
					4646-065-15	TX-216-2045
					4646-065-15	TX-216-2045
(b) (4)						

Note - qualified level = (% impurity x NOAEL) / (body surface area conversion factor x maximum clinical dose)

3.1.2 Genetic Toxicology

The draft FDA impurity guidance recommends no further action for impurities below the ICH Q3A(R2) qualification threshold (i.e., < 0.15% for maximum daily dose ≤ 2 g/day) that lack structural alerts. Although several impurities have specifications greater than the ICH qualification threshold, a negative in silico assessment was considered acceptable for qualification due to the serious nature of the indication. A review of the in silico evaluations conducted by the Sponsor (Study nos. TX-216-2046 and TX-216-2054) and the CDER Computational Toxicology Group is provided in Appendix C3. With the exception of (b) (4) *all other cobicistat impurities were predicted negative for mutagenic potential.* (b) (4) *were considered potentially mutagenic* (b) (4) *(Figure 31). In addition to being identified by in silico methodology, the mutagenic potential of these functional groups has been described in the public literature* (b) (4) *Exposure to these impurities should be controlled to appropriate levels as described in the FDA Draft Impurity Guidance. The positive prediction* (b) (4) *was deemed unreliable and, therefore, this impurity should be considered non-mutagenic.*

(b) (4)

Figure 31. Structural alerts for potentially mutagenic cobicistat impurities

There is publicly available information indicating that (b) (4) *(see MSDS in Appendix 4) and* (b) (4) *(see TOXNET information in Appendix 5) are mutagenic in the Ames assay. Therefore, exposure to these impurities should be controlled to appropriate levels as described in the FDA Draft Impurity Guidance.*

(b) (4) *has been shown to be Ames negative* (b) (4) *and should be treated as non-mutagenic.*

References

(b) (4)

(b) (4)

4 Drug Product (NDA 203-100)

4.1 Drug Formulation

Table 124. Drug formulation components

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 (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			NF, Ph. Eur., JP	
(b) (4)			USP, Ph. Eur.	
Film Coat				
		(b) (4)	In-House (GSPEC-183-00)	
			USP, Ph. Eur.	
				(b) (4)

Table 125. Film coating components

Ingredient	Quantity % w/w	Reference to Standard
Polyvinyl Alcohol	(b) (4)	USP, Ph. Eur., JP
Indigo Carmine (FD&C Blue #2) Aluminum Lake (b) (4)		In-House
Polyethylene Glycol (b) (4)		NF, Ph. Eur., JP
Yellow Iron Oxide (b) (4)		NF, JP
Titanium Dioxide (b) (4)		USP, Ph. Eur., JP
Talc (b) (4)		USP, Ph. Eur., JP

4.2 Excipients

With the exception of silicon dioxide, excipients detailed in Tables 124 and 125 (taken from Sponsor submission) are present at levels less than those listed for marketed oral products in the FDA's Inactive Ingredient Database (IID). Per the CMC reviewer, the levels of silicon dioxide may be up to 180 mg/day. This exposure is slightly higher than the maximum of 170 mg listed in the IID. Although there is a report of clinical sarcoidosis associated with ingested colloidal silicon dioxide (Sola et al., 2009), this excipient is generally considered to be relatively non-toxic (WHO, 1974; FDA, 1979). Given the indication and lack of overt toxicity associated with silicon dioxide, the proposed levels are considered acceptable.

4.3 (b) (4)

The proposed specification for (b) (4) a known human carcinogen (b) (4), is (b) (4) in colloidal silicon dioxide. This is equivalent to (b) (4) at the proposed silicon dioxide exposure (b) (4). Given the indication, the low level exposure to (b) (4) is considered acceptable.

4.4 Impurities/Degradants

4.4.1 Cobicistat

Based on the NOEL/NOAEL established in the 28-day rat study described in Section 2.1.1, the qualified degradant levels detailed in Table 127 (taken from Sponsor submission) are accurate and exceed the proposed specifications. These degradation products are also considered qualified for genotoxicity based on the in silico evaluation described in Section 2.1.2.

Table 126. Summary of cobicistat degradation products qualification

Impurity	Level Observed in Toxicological Study	NOEL or NOAEL (mg/kg/day)	Qualified Level[b] (mg/kg/day)	Qualified Level for 60 kg Human and with 150 mg/day dose	Toxicology Study Number	Lot Number
(b) (4)					TX-216-2045	4646-065-15
					TX-216-2045	4646-065-15
					TX-216-2045	4646-065-15
					TX-216-2045	4646-065-15
					TX-216-2045	4646-065-15
					TX-216-2045	4646-065-15
						(b) (4)

Note - qualified level = (% impurity x NOAEL) / (body surface area conversion factor x maximum clinical dose)

4.4.2 Emtricitabine

The Sponsor conducted a 14-day rat study comparing degraded and undegraded test article to evaluate emtricitabine degradants (Study no. TX-164-2005 summarized in Appendix C2). Based on the NOEL/NOAEL established in this study, the qualified degradant levels detailed in Table 127 (taken from Sponsor submission) are accurate and exceed the proposed specifications. Because emtricitabine is a marketed drug, the genotoxic potential of degradants was previously considered.

Table 127. Summary of emtricitabine degradation products qualification

Impurity	Maximum observed in toxicological studies	NOEL or NOAEL (mg/kg/day)	Qualified Level[a] (mg/kg/day)	Qualified Level for 60 kg Human and with 200 mg/day dose	Toxicology Study Number	Lot Number
(b) (4)					TX-164-2005	JH-2199-21
					TX-164-2005	JH-2199-21
					TX-164-2005	JH-2199-21
					TX-164-2005	JH-2199-21
						(b) (4)

Note - qualified level = (% impurity x NOAEL) / (body surface area conversion factor x maximum clinical dose)

4.4.3 Tenofovir DF

Tenofovir disproxil fumarate degradation products were evaluated in three general toxicology studies (Study no. TX-164-2005 summarized in Appendix C2; Study nos. R2000081 and 97-TX-4331-002 previously reviewed under NDA#21-356). N^6-carbamate was present in the tenofovir disproxil fumarate used in a 13/42-week rat toxicity study. Other degradants were evaluated in 14-day studies in rat comparing undegraded and degraded test article. Based on NOEL/NOAELs established in these studies, the qualified degradant levels detailed in Table 128 (taken from Sponsor submission) appear accurate and exceed the proposed specifications. Because tenofovir disproxil fumarate is a marketed drug, the genotoxic potential of degradants was previously considered.

Table 128. Summary of tenofovir disproxil fumarate degradation products qualification

Impurity	Maximum observed in toxicological studies	NOEL or NOAEL (mg/kg/day)	Qualified level[a] (mg/kg/day)	Qualified level for 60 kg human and with 300 mg/day dose	Toxicology Study Number	Lot Number
(b) (4)					TX-164-2005	JH-2199-21
					R2000081	1616-31A
					R2000081	1616-31A
					R2000081	1616-31A
					R2000081	1616-31A
					R2000081	1616-31A
					97-TOX-4331-002	4331-05-XA-1
					TX-164-2005	JH-2199-21
					TX-164-2005	JH-2199-21
(b) (4)						

Note - qualified level = (% impurity x NOAEL) / (body surface area conversion factor x maximum clinical dose)

References

FDA (1979) Silicon dioxides. Database of Select Committee on GRAS Substances Report No. 61

(b) (4)

Sola R., Boj M., Hernandez-Flix S. Camprubi M. (2009) Silica in oral drugs as a possible sarcoidosis-inducing antigen. *Lancet* 373:1943-1944

World Health Organization (1974) Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents. WHO Food Additive Series No. 5, 17th Report of the Joint FAO/WHO Expert Committee on Food Additives

5 Appendix/Attachments

Appendix C1 – Impurity and Degradant Structures

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Appendix C2 – General Toxicology Study Reviews

Elvitegravir

Title: 28-Day Oral (Gavage) Toxicity Bridging Study with GS-9137 in Female Sprague Dawley Rat (Study no. TX-183-2010)

Summary – *Mortality, clinical signs, body weights, food consumption, ophthalmoscopy, hematology, coagulation, clinical chemistry, urinalysis, gross pathology, organ weights, histopathology, and toxicokinetics were evaluated in female Sprague-Dawley rats after oral administration of 300 and 2000 mg/kg/day GS-9137 spiked with impurities for 28 days. For comparison, a group was also given 2000 mg/kg/day of GS-9137 alone. The vehicle/control article was 0.5% (w/v) methylcellulose in water. Effects were limited to increased cecum weights in the high-dose of GS-9137 with or without impurities. Toxicokinetic analysis verified exposure to GS-9137.*

Overall, administration of GS-9137 spiked with impurities was consistent with effects noted in the concurrent GS-9137 alone group. Based on the absence of adverse effects at any dose level, the NOAEL was 2000 mg/kg/day of GS-9137 spiked with impurities or GS-9137 alone.

Title: 28-Day Oral Gavage Qualification Study with GS-9137 in Rats (Study no. TX-183-2023)

Summary – *Mortality, clinical signs, body weights, food consumption, hematology, coagulation, clinical chemistry, urinalysis, gross pathology, organ weights, histopathology, and toxicokinetics were evaluated in female Sprague-Dawley rats after oral administration of 300 and 2000 mg/kg/day GS-9137 spiked with impurities for 28 days. For comparison, a group was also given 2000 mg/kg/day of GS-9137 alone. There were no drug-related effects. Toxicokinetic analysis verified exposure to GS-9137.*

Overall, administration of GS-9137 spiked with impurities was consistent with effects noted in the concurrent GS-9137 alone group. Based on the absence of adverse effects at any dose level, the NOAEL was 2000 mg/kg/day of GS-9137 spiked with impurities or GS-9137 alone.

Cobicistat

Title: 28-Day Oral Gavage Qualification Toxicity and Toxicokinetic Study with GS-9350 in Rats (Study no. TX-216-2045)

Summary – *Mortality, clinical signs, body weights, food consumption, hematology, coagulation, clinical chemistry, urinalysis, gross pathology, organ weights, histopathology, and toxicokinetics were evaluated in female Sprague-Dawley rats after oral administration of 30 and 100 mg/kg/day GS-9350 spiked with impurities for 28 days. For comparison, a group was also given 100 mg/kg/day of GS-9350 alone. The vehicle/control article was 5% ethanol (v/v) and 95% propylene glycol (v/v). Minor effects on hematology included decreased red blood cells, hemoglobin, and hematocrit with increased platelets and several white blood cell types. Decreased A:G ratio, urea nitrogen, and chloride with increased aminotransferases, globulin,*

total protein, glucose, cholesterol, and calcium. Urinalysis changes included decreased specific gravity as well as increased volume and pH. An increase in liver weight parameters was consistent with clinical chemistry changes and histopathological lesion (hepatocellular hypertrophy). Increased thyroid/parathyroid weights correlated with microscopic change (follicular cell hypertrophy/hyperplasia). Changes noted in the liver and thyroid occurred in all dose groups and are likely adaptive responses to enzyme induction. An increase in ovary weights was also observed but was not accompanied by microscopic effects. With the exception of the liver and thyroid changes, effects were primarily observed at the high-dose with or without impurities. Toxicokinetic analysis verified exposure to GS-9350.

Overall, administration of GS-9350 spiked with impurities was consistent with effects noted in the concurrent GS-9350 alone group. Based on the absence of adverse effects at any dose level, the NOAEL was 100 mg/kg/day of GS-9350 spiked with impurities or GS-9350 alone.

Title: 4-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Dogs with a 4-Week Recovery Phase (Study no. TX-216-2005)

Summary – *Mortality, clinical signs, body weights, food consumption, ophthalmoscopy, ECGs, blood pressure, hematology, coagulation, clinical chemistry, urinalysis, gross pathology, organ weights, histopathology, microsomal enzymes, and toxicokinetics were evaluated in Beagle dogs after oral administration of 5, 15, and 45/30 mg/kg/day GS-9350 for 4 weeks followed by a 4-week recovery phase. The vehicle/control article was 5% ethanol (v/v) and 95% propylene glycol (v/v). Effects included salivation and emesis as well as decreased body weight. Toxicity was observed in the liver as demonstrated by increased ALT, ALP, bilirubin, and organ weight as well as hepatocellular vacuolation. Effects appeared to be reversible and, with the exception of clinical signs and increased liver weight parameters, were limited to the high-dose group. Toxicokinetic analysis verified exposure to GS-9350.*

Based on the presence of clinical signs and minimal increase in liver weight parameters, the NOAEL was 15 mg/kg/day of GS-9350. See Dr. Myer's review for detailed review.

Combination Studies

Title: A 14-Day Study of Non-Degraded and Degraded TDF/FTC by Oral Gavage in Rats (Study no. TX-164-2005)

Summary – *Mortality, clinical signs, body weights, food consumption, hematology, coagulation, clinical chemistry, urinalysis, gross pathology, organ weights, histopathology, and toxicokinetics were evaluated in male Sprague-Dawley rats after oral administration of 30/20, 100/67, and 300/200 mg/kg/day of degraded tenofovir disproxil fumarate/emtricitabine for 14 days. For comparison, groups were also given 30/20, 100/67, and 300/200 mg/kg/day of undegraded tenofovir disproxil fumarate/emtricitabine. The vehicle/control article was 0.5% (w/v) carboxymethylcellulose, 0.9% (w/v) benzyl alcohol, 0.5% (w/v) polysorbate 20 in sterile water for injection. Minor changes in clinical pathology parameters included decreased hematocrit, hemoglobin, MCV, MCH, and RDW as well as increased ALT. Histopathological effects were*

limited to the duodenum (cryptal epithelium, hyperplasia; single cell necrosis). Effects were generally limited to the high-dose groups received degraded or undegraded test articles. Toxicokinetic analysis verified exposure to tenofovir and emtricitabine.

Overall, the effects of degraded tenofovir disproxil fumarate/emtricitabine were consistent with effects noted in the concurrent undegraded tenofovir disproxil fumarate/emtricitabine group. Based on the absence of adverse effects at any dose level, the NOAEL was 300/200 mg/kg/day of degraded or undegraded tenofovir disproxil fumarate/emtricitabine.

Title: 28-Day Oral Gavage Qualification Toxicity and Toxicokinetic Study with GS-9350/GS-9137 Tablets in Rat (Study no. TX-236-2002)

Summary – *Mortality, clinical signs, body weights, food consumption, hematology, coagulation, clinical chemistry, gross pathology, organ weights, histopathology, and toxicokinetics were evaluated in female Sprague-Dawley rats after oral administration of 30/30 and 50/50 mg/kg/day of degraded GS-9350/GS-9137 for 28 days. For comparison, groups were also given 30/30 and 50/50 mg/kg/day of undegraded GS-9350/GS-9137. The vehicle/control article was 0.5% (w/v) carboxymethylcellulose, 10% (w/v) propylene glycol, in 40 mm sodium acetate buffer (pH adjusted to 4). Minor changes in clinical pathology parameters included increased platelets as well as increased globulin, total protein, A:G ratio, and cholesterol. An increase in liver weight parameters was also observed. Effects were generally limited to the high-dose groups received degraded or undegraded test articles. Toxicokinetic analysis verified exposure to GS-9350 and GS-9137.*

Overall, the effects of degraded GS-9350/GS-9137 were consistent with effects noted in the concurrent undegraded GS-9350/GS-9137 group. Based on the absence of adverse effects at any dose level, the NOAEL was 50/50 mg/kg/day of degraded or undegraded GS-9350/GS-9137.

Appendix C3 – *In Silico* Evaluations

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Appendix C4 – Material Safety Data Sheet (b) (4)

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Appendix C5 – ToxNet Entry (b) (4)

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/s/

LAINE P MYERS
06/29/2012

HANAN N GHANTOUS
06/29/2012

Comments on N203100 : elvitegravir, cobicistat, emtricitabine, tenofovir DF
From: A. Jacobs, AD

Date: June 21, 2012

1. I concur that there are no pharm/tox approval issues
2. I concur that there are no adverse effects noted relative to pregnancy

I have discussed other comments with the reviewers and they will address them as appropriate

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/s/

ABIGAIL C JACOBS
06/25/2012

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 203-100 **Applicant: Gilead Sciences** **Stamp Date: October 27, 2011**

Drug Name: EVG/COBI/FTC/TDF **NDA/BLA Type: New**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	yes		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	yes		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	yes		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	yes		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	yes		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	yes		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	yes		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	yes		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	yes		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	yes		
11	Has the applicant addressed any abuse potential issues in the submission?			N/A
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

If the NDA/BLA is not fileable from the pharmacology/toxicology 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.

None.

L. Peyton Myers, PhD
Pritam S. Verma, PhD — December 16, 2011
Reviewing Pharmacologists — Date

Hanan Ghantous, PhD, DABT — December 16, 2011
Team Leader/Supervisor — Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

LAINE P MYERS
12/16/2011

HANAN N GHANTOUS
12/19/2011