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APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

**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: 203-094
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Product: Cobicistat 150 mg
Indication: CYP3A Inhibitor / Pharmacokinetic Enhancer
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1 Executive Summary

1.1 Introduction

Cobicistat (COBI, 150-mg) as a component of STRIBILD® (elvitegravir, cobicistat, emtricitabine, tenofovir disoproxil fumarate), is an approved drug which has been reviewed in NDA 203-100 for the treatment of human immunodeficiency virus-1 (HIV-1). COBI inhibits human CYP3A4 with high affinity and is 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.

1.2 Brief Discussion of Nonclinical Findings (from both this NDA as well as cross-referenced 203-100)

The most concerning safety pharmacological effects with COBI were limited to the cardiovascular system. In *in vitro* studies, COBI inhibited hERG potassium current (IC₅₀ 1.8 µM) and the hCav1.2 L-type calcium channel (IC₅₀ 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 (TDF) renal exposure in humans. The Sponsor plans to evaluate the effects of COBI on tenofovir.

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. Genotoxicity evaluation of several COBI product impurities also did not reveal any genotoxicity potential of the impurities.

In the 2-year carcinogenicity study in mice with COBI, no drug-related increase in tumor incidence was observed at exposures 7 to 16 times (males and females, respectively) the human systemic exposure at the therapeutic daily dose. In the 2-year carcinogenicity study in rats, increases in follicular cell adenomas and/or carcinomas in the thyroid gland were observed at doses of 25 and 50 mg/kg/day in males, and at 30 mg/kg/day in females. The follicular cell findings are considered to be rat-specific, secondary to hepatic microsomal enzyme induction and thyroid hormone imbalance, and are not relevant for humans. At the highest doses tested in the rat carcinogenicity study, systemic exposures were approximately 2 times the human systemic exposure at the therapeutic daily dose. In rats, COBI induces hepatic CYP3A activity due to a species-specific activation of PXR, which does not occur in humans. The observed toxicity profile on the thyroid is rodent-specific and it is unlikely that COBI presents a risk to the human thyroid. These effects, associated with liver enzyme induction, bear no relevance for man as a similar association between liver enzyme induction and carcinogenesis does not exist in humans.

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 on 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 150 mg tablets.

1.3 Recommendations

1.3.1 Approvability

There are no nonclinical pharmacology and toxicology issues which would preclude the approval of COBI 150 mg tablets

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

Pregnancy

(b) (4)

Pregnancy Category B

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

Animal Data

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 at the embryo-fetal No Observed Adverse Effects Levels (NOAELs) in rats and rabbits were respectively 1.4 and 3.3 times higher than the exposure in humans at the recommended daily dose of 150 mg.

Carcinogenesis, Mutagenesis, and Impairment of Fertility

Carcinogenesis

In a long-term carcinogenicity study in mice, no drug-related increases in tumor incidence were observed at doses up to 50 and 100 mg/kg/day (males and females, respectively). Cobicistat exposures at these doses were approximately 7 (male) and 16 (females) times, respectively, the human systemic exposure at the therapeutic daily dose. In a long-term carcinogenicity study of cobicistat in rats, an increased incidence of follicular cell adenomas and/or carcinomas in the thyroid gland was observed at doses of 25 and 50 mg/kg/day in males, and at 30 mg/kg/day in females. The follicular cell findings are considered to be rat-specific, secondary to hepatic microsomal enzyme induction and thyroid hormone imbalance, and are not relevant for humans. At the highest doses tested in the rat carcinogenicity study, systemic exposures were approximately 2 times the human systemic exposure at the therapeutic daily dose.

Mutagenesis

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

Impairment of Fertility

Cobicistat did not affect fertility in male or female rats at daily exposures (AUC) approximately 3-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 similar human exposures at the recommended 150 mg daily dose.

2 Drug Information

2.1 Drug

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

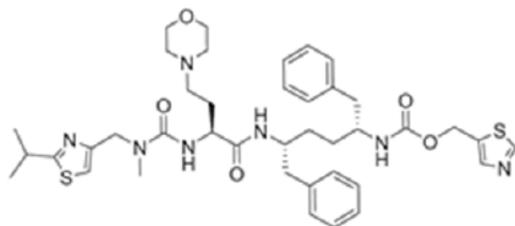
Generic Name - Cobicistat

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 - C₄₀H₅₃N₇O₅S₂ MW 776.0

Structure or Biochemical Description



Pharmacologic Class – CYP3A inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA 203-100 (Stribild®)

IND 101,283 (cobicistat, GS-9350)

2.3 Drug Formulation

The cobicistat (COBI) tablet is an immediate-release tablet containing 150 mg of cobicistat.

Table 1 - Qualitative and Quantitative Composition of Cobicistat Tablets

Components	% w/w	Unit Formula (mg/unit)	Quality Standard	Function
Tablet Core				
Cobicistat on Silicon Dioxide		(b) (4)	In-House	Active
Microcrystalline Cellulose			NF, Ph. Eur., JP	(b) (4)
Croscarmellose Sodium			NF, Ph. Eur., JP	(b) (4)
Magnesium Stearate			NF, Ph. Eur., JP	(b) (4)
Total				
Film Coat				
(b) (4)			In-House	(b) (4)
(b) (4)			USP, Ph. Eur.	(b) (4)



2.4 Comments on Novel Excipients

None. See tables below for excipients in the tablet as well as in the (b) (4) film coating for the tablets.

Table 2 - Excipients Used in Manufacturing of Cobicistat Tablets

Inactive Ingredient	Reference to Standards	Function
Croscarmellose Sodium	NF, Ph. Eur., JP	(b) (4)
Magnesium Stearate	NF, Ph. Eur., JP	(b) (4)
Microcrystalline Cellulose	NF, Ph. Eur., JP	(b) (4)

Table 3 - Quantitative Composition of (b) (4) (Tablet Film Coating)

Ingredient	Quantity % w/w	Reference to Standard
Polyvinyl Alcohol	(b) (4)	USP, Ph. Eur., JP
Titanium Dioxide (b) (4)	(b) (4)	USP, Ph. Eur., JP
Polyethylene Glycol (b) (4)	(b) (4)	NF, Ph. Eur., JP
Talc (b) (4)	(b) (4)	USP, Ph. Eur., JP
Sunset Yellow FCF (FD&C Yellow # 6) Aluminum Lake (b) (4)	(b) (4)	In-House
Iron Oxide Yellow (b) (4)	(b) (4)	NF, JP

2.5 Comments on Impurities/Degradants of Concern

None.

2.6 Proposed Clinical Population and Dosing Regimen

Population: COBI has no antiviral activity. COBI is administered with anti-HIV protease inhibitors in order to increase their exposure by inhibiting CYP3A metabolism. COBI is currently approved in the 4-drug fixed-dose combination tablet (Stribild®, STR) which is comprised of the integrase strand-transfer inhibitor (INSTI) elvitegravir (EVG), COBI, emtricitabine (FTC), and tenofovir (TDF). The NDA for STR (NDA 203-100).

The Sponsor is pursuing an indication for Cobicistat as a pharmacokinetic enhancer of the HIV-1 protease inhibitors atazanavir and darunavir in adults.

Dosing regimen: The proposed regimen for the COBI tablet is once daily oral administration at 150 mg.

2.7 Regulatory Background

COBI as a component of STRIBILD® is an approved drug which has been reviewed in NDA 203-100.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology

- Abbreviated Validation of a Method for the Determination of GS-9350 in Rabbit Plasma by HPLC with MS/MS Detection
- Quantitative Determination of Ritonavir in Rat Plasma by LC/MS/MS

Toxicology

Carcinogenicity

- 104-Week Oral Gavage Carcinogenicity Study with GS-9350 in Mice
- 104-Week Oral Gavage Carcinogenicity Study with GS-9350 in Rats

Genotoxicity

- (b) (4) Bacterial Reverse Mutation Test in *Salmonella typhimurium* and *Escherichia coli*
- (b) (4) In Mammalian Cell Mutation Test
- *In Silico* Evaluation of Potential Genotoxicity and Carcinogenicity for (b) (4)

3.2 Studies Not Reviewed

All studies were reviewed.

3.3 Previous Reviews Referenced

See final P/T review of NDA 203-100.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Study title: Abbreviated Validation of a Method for the Determination of GS-9350 in Rabbit Plasma by HPLC with MS/MS Detection

Study no.: BA-216-2004

Study report location: EDR

Conducting laboratory and location: (b) (4)

Date of study initiation: April 14, 2008 (protocol approval)

GLP compliance: Not stated. Compliance statement only refers to compliance with (b) (4) SOPs.

QA statement: Yes.

Drug, lot #, and % purity: GS-9350, Lot No. 08A00027 ((b) (4)), 97.4% pure

(b) (4), Lot No. 2 (b) (4)
 (u) (4), the internal standard
 (ISTD), 98.3% pure

Key Study Findings

A quantitative procedure for the determination of GS-9350 in rabbit plasma, over the concentration range of 5.00 to 1000 ng/mL, was successfully validated for use.

Study title: Quantitative Determination of Ritonavir in Rat Plasma by LC/MS/MS

Study no.: BA-183-2012
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 20 July 2006
 GLP compliance: Not stated. Compliance statement only refers to compliance with (b) (4) SOPs.
 QA statement: Yes.
 Drug, lot #, and % purity: See table below.

Figure 1- Analytical Reference Standards

<i>Compound</i>	<i>Lot Number</i>	<i>Correction Factor *</i>	<i>Expiration Date</i>	<i>Source</i>	<i>Storage Conditions</i>
(b) (4)					

Key Study Findings

The method described in this report has been validated for the determination of ritonavir in rat plasma.

7 Genetic Toxicology

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

Study title: (b) (4) **Bacterial Reverse Mutation Test in *Salmonella typhimurium* and *Escherichia coli***

Study no.: TX-216-2052
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 15 Sept 2011
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: (b) (4), Lot 4923-085-13, 99.7% pure
 (b) (4), Lot 4923-065-32, 99.5% pure
 pure
 (b) (4), Lot 4923-085-36, 99.9% pure
 (b) (4), Lot 4923-064-37, 99.6% pure

Key Study Findings

The COBI process impurities (b) (4) were negative in the Ames assay with or without S9 mix.

Methods

Strains: *S. typhimurium* TA1535 *hisG46 rfa ΔuvrB*
S. typhimurium TA1537 *hisC3076 rfa ΔuvrB*
S. typhimurium TA98 *hisD3052 rfa ΔuvrB*
 pKM101
S. typhimurium TA100 *hisG46 rfa ΔuvrB*
 pKM101
E. coli WP2 *trp uvrA*
 Concentrations in definitive study: up to 5000 µg/plate
 Basis of concentration selection: Standard test limit dose
 Negative control: DMSO, Sterile Water
 Positive control:
 Formulation/Vehicle: DMSO, Sterile Water
 Incubation & sampling time: Without S9 -- NaAz, 9AC, 2NF, NQO
 With S9 – 2AA, BaP

Study Validity

The study was valid.

Results

The purpose of this study was to evaluate the genotoxicity (b) (4) using the bacterial mutation test.

Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100, and Escherichia coli strain WP2 uvrA were treated with individual test articles at a range of concentrations up to 5000 µg/plate (the standard limit dose for this assay) in the presence and absence of a supplemented rat liver fraction (S9 mix) using the plate incorporation and pre-incubation versions of the bacterial mutation test.

Bacteria were incubated with standard positive control agents, and the response of the various bacterial strains to these agents confirmed the sensitivity of the test system and the activity of the S9 mix.

No substantial increases in the revertant colony counts were obtained with any strain following exposure (b) (4) in either the plate incorporation or pre-incubation assay in the absence or presence of S9 mix. It is concluded that none of the test articles, (b) (4) showed any evidence of genotoxic activity in this *in vitro* mutagenicity assay when tested in accordance with regulatory guidelines.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: (b) (4) **In Mammalian Cell Mutation Test**

Study no.:	TX-216-2053
Study report location:	FDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10 Aug 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4), Lot 4923-085-13, 99.7% pure (b) (4), Lot 4923-065-32, 99.5% pure pure (b) (4), Lot 4923-085-36, 99.9% pure (b) (4), Lot 4923-064-37, 99.6% pure

Key Study Findings

(b) (4) (COBI process impurities) did not show any evidence of genotoxicity in this *in vitro* test when tested in accordance with regulatory guidelines.

Methods

Cell line: Mouse lymphoma L5178Y TK+/- (clone

3.7.2C) from ATCC

Concentrations in definitive study: Up to 957 µg/mL (b) (4)
Up to 2318 µg/mL
Up to 419 µg/mL (b) (4)

Basis of concentration selection: Preliminary toxicity test (with cell survival) range up to 1703 (b) (4), 4126 (b) (4), and 4185 (b) (4) µg/mL at the final concentration.

Negative control: DMSO and sterile water

Positive control: With S9 – BaP (Benzo(a)pyrene)
Without S9 – NQO (4-nitroquinoline oxide)

Formulation/Vehicle: R0p consisted of RPMI 1640 medium supplemented with 0.1% v/v Pluronic F68, 1 mM sodium pyruvate and 50 µg gentamycin per mL. R2p and R10p consisted of R0p supplemented with 2% and 10% v/v heat-inactivated donor herd horse serum respectively. R2p was used for washing steps and intermediate dilutions. Selective medium consisted of R10p containing 5 µg trifluorothymidine (TFT) per mL.

Incubation & sampling time: See flowchart below

Figure 2 - Mouse Lymphoma Assay Procedures



Study Validity

The study was valid.

Results

The purpose of this study was to evaluate the genotoxicity of (b) (4) using a mammalian L5178 TK+/- mouse lymphoma cell mutation test.

A preliminary toxicity test was used to determine dose levels for the main test. In the main test, mouse lymphoma L5178Y TK+/- cells were incubated with the vehicle, each test article or positive control for 3 hours (with and without S9 metabolic activation) or 24 hours (without S9 only).

No substantial increases in mutation frequency were observed after treatment of cells with (b) (4) at dose levels up to the appropriate limit of toxicity.

7.4 Other Genetic Toxicity Studies

Study title: In Silico Evaluation of Potential Genotoxicity and Carcinogenicity (b) (4)

Study no: TX-216-2054
Study report location: EDR
Conducting laboratory and location: Gilead Sciences, California
Date of study initiation: January 24, 2012
GLP compliance: N/A
QA statement: No

Key Study Findings

(b) (4) is a potential impurity in (b) (4) a cobicistat starting material.

(b) (4) was evaluated *in silico* for potential toxicity using DEREK Nexus (Lhasa Ltd.) software. The computational assessment of toxicity was based on potential for mutagenicity, chromosome damage, genotoxicity and carcinogenicity.

The DEREK predictions for (b) (4) indicate nothing to report. Therefore, *in silico* results indicate no prediction for mutagenicity, chromosome damage, genotoxicity or carcinogenicity for (b) (4).

8 Carcinogenicity

Study title: 104-Week Oral Gavage Carcinogenicity Study with GS-9350 in Mice

Study no.: TX-216-2030
 Study report location: Electronic Document Room
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 30 June 2009
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: See tables below for drug lots and vehicle lots. Vehicle lots are being included due to the vehicle-associated toxicity noted in the male mice.
 CAC concurrence: Yes

Table 4 - Mouse Carcinogenicity Study: Test Article Information

Test Article ^a	Lot No.	Storage	Purity/Strength (%)	Retest Date	Reserve (Archive) Sample
GS-9350 in EtOH [Days 1 through 175 (males) and Days 1 through 173 (females)]	9350-AC-2E	In a refrigerator, set to maintain 2 to 8°C, and protected from light	98.6/52.9 ^b	(b) (4)	Collected ^c
GS-9350 Foam [beginning Day 176 (males) and 174 (females)]	3793-143-21	In a refrigerator, set to maintain 2 to 8°C, and protected from light with desiccant under nitrogen	99.1/97.1 ^d	(b) (4)	Collected ^e

Table 5 - Mouse Carcinogenicity Study: Vehicle Dosing Information

Study Days (intervals of use)	Study Weeks ^a	Description	Abbreviation
Days 1 - 94 (males) Days 1 - 92 (females)	Weeks 1 through 13	95% propylene glycol (PG, v/v)/5% EtOH (v/v), pH 2.3 ± 0.1 with hydrochloric acid	95/5 PG/EtOH, pH 2.3
Days 95 - 175 (males) Days 93 - 173 (females):	Weeks 14 through 25	95% PG (v/v)/5% EtOH (v/v)	95/5 PG/EtOH
Day 176 - termination (males) Day 174 - termination (females):	Week 26 through termination	10% PG in 90% 40 mM sodium acetate trihydrate buffer in reverse osmosis water (v/v), pH = 4.0 ± 0.1	10% PG

^a Ending study week is based on the last completed male study week.

Table 6 - Mouse Carcinogenicity Study: Vehicle Information

Vehicle Control Article Components ^a and Control Article	Manufacturer	Lot No.	Storage	Purity	Expiration Date ^b	Reserve (Archive) Sample
Propylene glycol	(b) (4)	H37599	Room temperature (15 to 30°C)	99.8%	09 Nov 2012	Collected from each lot ^{c,d}
		E38585		99.6%	14 Jan 2012	
		G33611		99.8%	01 Apr 2012	
		H01626		99.9%	23 Jul 2012	
		H10630		99.9%	31 Aug 2012	
		H24621		100.0%	31 Aug 2012	
		H34606		99.8%	21 Sep 2012	
		H37599		99.8%	09 Nov 2012	
		H39638		100.0%	29 Dec 2012	
		H39638		100.0%	15 Jan 2013	
		H42584		100.0%	10 Aug 2013	
		J04618		100.0%	10 Aug 2013	
		H37599		99.8%	22 Dec 2012	
J46626	99.9%	18 Apr 2014				
Ethanol		06862EH	Room temperature (15 to 30°C)	Meets USP specifications	14 Sep 2010	Collected from each lot ^{c,d}
		16979PH			30 Jan 2011	
		15596CK			28 Jul 2012	
Sodium acetate trihydrate		077K0156	Room temperature (15 to 30°C)	99.9%	14 Apr 2011	Collected from each lot ^c
		080M0118V			18 Nov 2013	
		G11473			24 Jul 2011	
Reverse osmosis water		NA	Ambient conditions	NA	NA	None required

Key Study Findings

The study was amended from the original protocol due to likely vehicle-associated effects. From Day 1 through Week 13, the vehicle was 95% propylene glycol (PG)/5% ethanol (EtOH) (v/v) (pH 2.3 ± 0.1). Due to increased mortality in vehicle control animals compared to water control animals, the vehicle was modified slightly to 95% PG/5% EtOH (v/v) (not pH-adjusted) and was used from Weeks 14 through 25. At Week 26, due to continued higher mortality in the vehicle control mice, the vehicle was changed to 10% PG in 90% 40 mM acetate buffer (v/v) (pH 4.0 ± 0.1) which was used for the remainder of the study.

There were no notable differences in GS-9350 exposures associated with the change in vehicles from vehicle 1 to vehicle 2. However, Vehicle 3 had an increase in exposure (AUC and C_{max}) compared to vehicle 1 and 2.

Both the Sponsor's analysis and the FDA analysis detected no significant positive trend and/or GS-9350-related increase in neoplasms was observed compared to the vehicle control. There was also no significant difference in neoplastic lesion incidence between the vehicle and water controls was observed. No GS-9350-related ophthalmic findings or development of hematologic neoplasia was observed.

Adequacy of Carcinogenicity Study

Although there were significant modifications to the vehicle (due to survival issues), an adequate number of mice survived an appropriate duration (i.e., Weeks 97 or 100, males and females respectively, of the dosing phase). The study achieved acceptable exposures to the test article for a valid evaluation of the carcinogenic potential of GS-9350.s

Appropriateness of Test Models

The test models were appropriate.

Evaluation of Tumor Findings

No significant increase in tumors was noted.

Methods

Doses: 0 (vehicle), 5, 15, and 50 mg/kg/day (males)
 0 (vehicle), 10, 30, and 100 mg/kg/day
 (females)

Frequency of dosing: Daily
 Dose volume: 5 mL/kg

Route of administration: Oral gavage

Formulation/Vehicle: 3 vehicles (see narrative below)

1. 95% PG/5%EtOH pH 2.3 (week 0-13)
2. 95% PG/5%EtOH no pH adjustment (week 14-25)
3. 10% PG in 90% 40 mM acetate buffer pH 4.0 (week 25 till sacrifice)

Basis of dose selection: MTD and AUC ratio

Species/Strain: Crl:CD1(ICR) mice were received (b) (4)

Number/Sex/Group: 60/sex/group for main study. 11 or 49/sex/group for TK. See table below.

Age: 6-7 weeks old

Animal housing: Individually housed

Paradigm for dietary restriction: Rodent diet (Harlan #2016C) ad libitum

Dual control employed: Yes. Water control (groups 1 and 6)

Interim sacrifice: None

Satellite groups: TK animals were separate from main study animals

Deviation from study protocol: Several protocol deviations were noted. The most significant deviation was the change in vehicles while on study (see explanatory narrative below). The Sponsor performed comparison PK studies and the change in vehicles did not decrease exposure. Exposures actually increased with the last vehicle change, which was acceptable.

Vehicle change explanation from the Sponsor's Application: "From Day 1 through Week 13, the vehicle was 95% propylene glycol (PG)/5% ethanol (EtOH) (v/v) (pH 2.3 ± 0.1). Due to increased mortality in vehicle control animals compared to water control animals, the vehicle was modified slightly to 95% PG/5% EtOH (v/v) (not pH-adjusted) and was used from Weeks 14 through 25. At Week 26, due to continued higher mortality in the vehicle control mice, the vehicle was changed to 10% PG in 90% 40 mM acetate buffer (v/v) (pH 4.0 ± 0.1) which was used for the remainder of the study. Toxicokinetic data indicate that there were no notable differences in GS-9350 exposures associated with the change in vehicles."

Table 7 - Carcinogenicity Study Design - Mice

Group	No. of Animals		Dose Level (mg/kg/day)		Dose Concentration (mg/mL)	
	Male	Female	Male	Female	Male	Female
Carcinogenicity Animals						
1 (Control) ^a	60	60	0	0	0	0
2 (Vehicle Control) ^b	60	60	0	0	0	0
3 (Low)	60	60	5	10	1	2
4 (Mid)	60	60	15	30	3	6
5 (High)	60	60	50	100	10	20
Toxicokinetic Animals						
6 (Control) ^a	11	11	0	0	0	0
7 (Vehicle Control) ^b	11	11	0	0	0	0
8 (Low)	49	49	5	10	1	2
9 (Mid)	49	49	15	30	3	6
10 (High)	49	49	50	100	10	20
Sentinel Animals^c						
11	15	15	c	c	c	c

a Groups 1 and 6 received the control article (reverse osmosis water), only.

b Groups 2 and 7 received the vehicle control article only.

c Group 11 was not dosed.

Observations and Results

Mortality

Checked 2x daily. There were increased mortalities (males and females) in the vehicle control, compared to water control. See Figures below for both male and female survival curves.

Due to the increases in deaths, the Sponsor changed vehicles at multiple time points (indicated by the arrows on the figure below).

An adequate number of mice survived an appropriate duration (i.e., at least to Week 88 of the dosing phase) to result in acceptable exposure to the test article for a valid evaluation of the carcinogenic potential of GS-9350.

Figure 3 - Adjusted Percent Survival (Male mice)

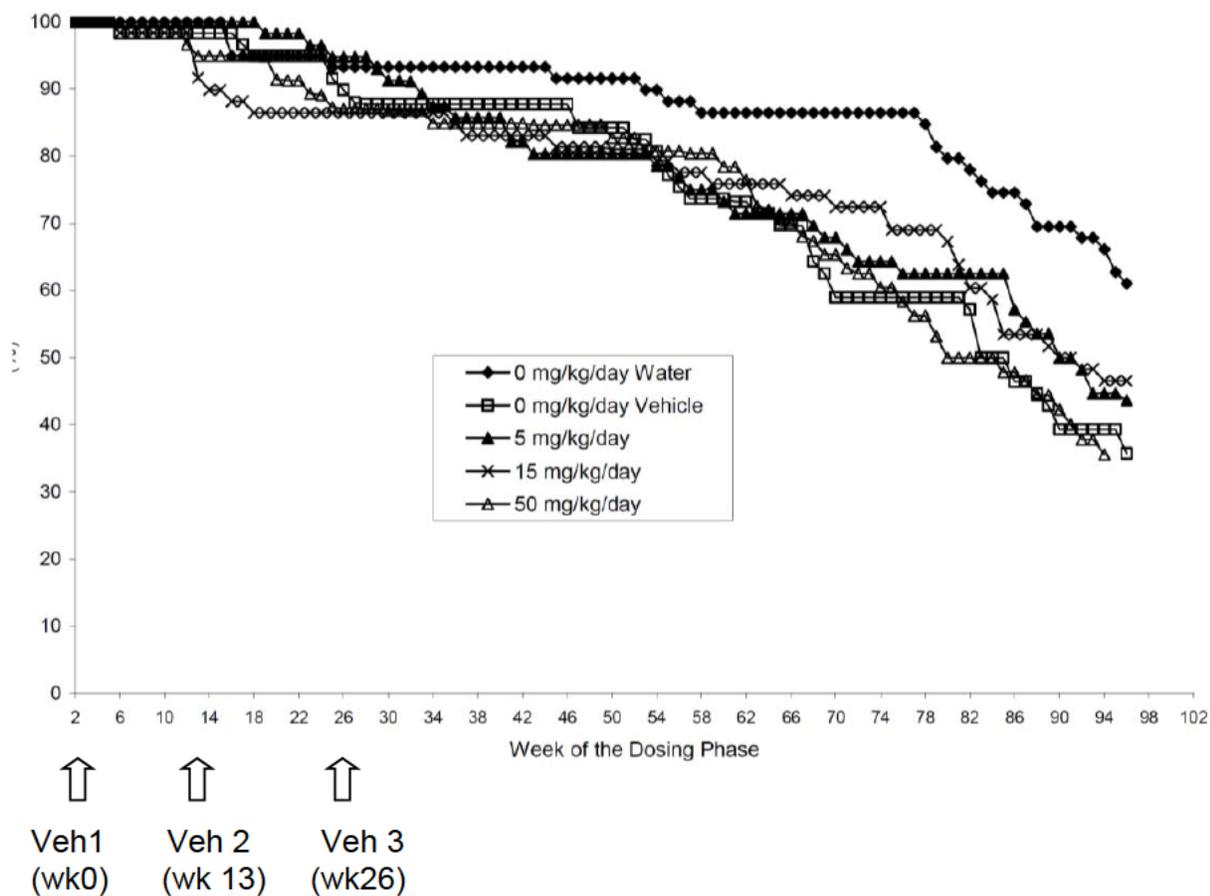
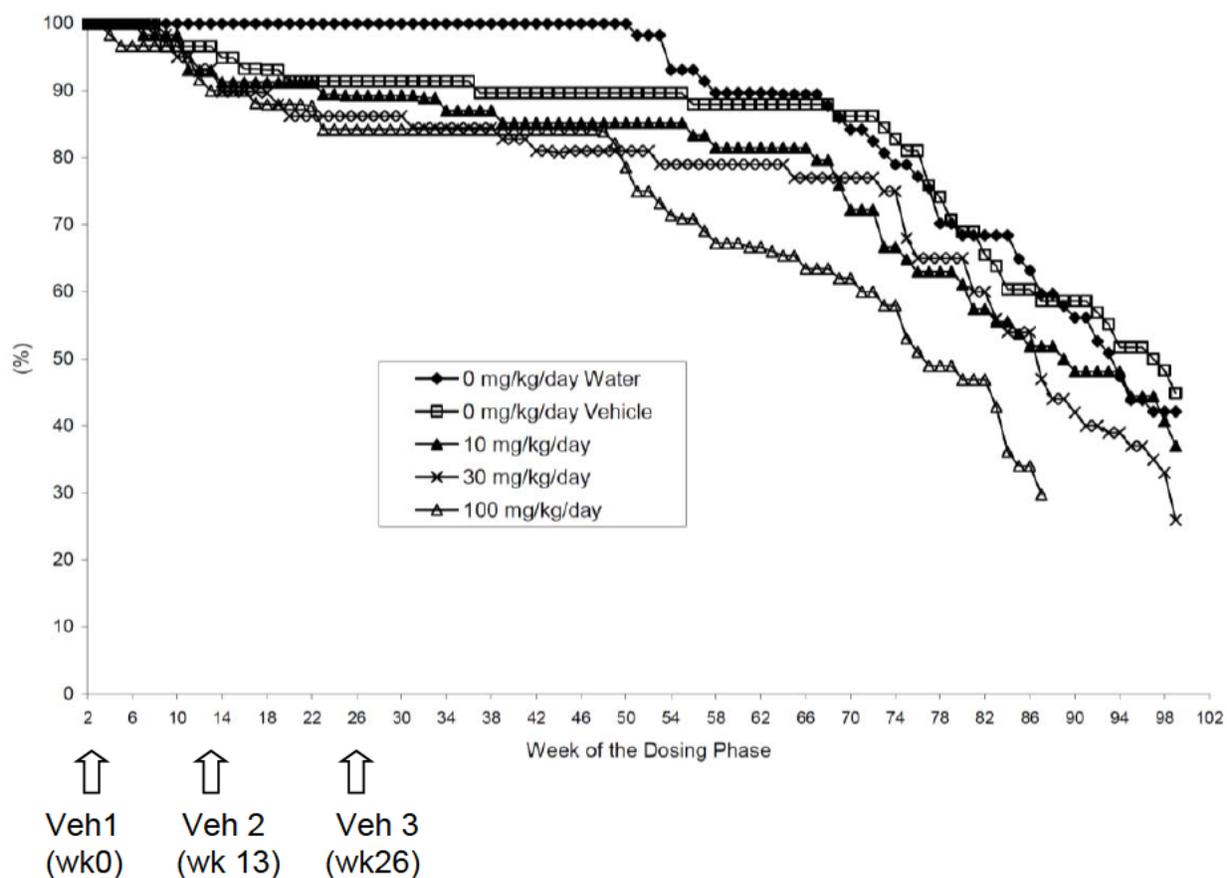


Figure 4 - Adjusted Percent Survival (Female mice)



Decreases in unadjusted survival rate occurred for the high-dose group approximately by Week 26 in both sexes and by Week 52 in the GS-9350 groups compared with the vehicle and/or water controls. See table below.

Table 8 - Unadjusted Survival Rates (%) for Carcinogenicity Animals

Sex	Males					Females					
	Dose Level (mg/kg/day)	0 (control)	0 (vehicle control)	5	15	50	0 (control)	0 (vehicle control)	10	30	100
Week 14		98	97	97	88	93	98	93	87	88	90
Week 26		92	88	90	85	78	97	88	83	83	80
Week 52		90	78	75	78	72	95	87	77	77	70
Week 78		83	55	58	67	45	67	72	57	62	40
Week 91		68	37	47	48	30	53	57	43	38	NA ^d
Termination		60 ^a	33 ^a	40 ^a	45 ^a	22 ^b	38 ^c	38 ^c	32 ^c	25 ^c	23 ^d

NA = Not applicable.

Percent unadjusted survival at the end of each study week = [(survival/60) x 100].

- a Terminal sacrifice on Day 673 (Week 97).
- b Terminal sacrifice on Day 665 (Week 95).
- c Terminal sacrifice on Day 699 (Week 100).
- d Terminal sacrifice on Day 611 (Week 88).

Adjusted survival rates for carcinogenicity animals through the scheduled sacrifices (during Weeks 95 and 97 for males and Weeks 88 and 100 for females) are presented in the table below.

Table 9 - Adjusted Survival Rates (%) for Carcinogenicity Animals

Sex	Males					Females					
	Dose Level (mg/kg/day)	0 (control)	0 (vehicle control)	5	15	50	0 (control)	0 (vehicle control)	10	30	100
Week 14		100	98	100	90	95	100	95	91	90	90
Week 26		93	90	95	86	87	100	91	89	86	84
Week 52		92	82	80	81	83	98	90	85	82	75
Week 78		85	59	63	69	56	70	74	63	67	49
Week 91		69	39	50	50	40	56	59	48	42	NA ^d
Termination		61 ^a	36 ^a	44 ^a	47 ^a	29 ^b	40 ^c	40 ^c	35 ^c	28 ^c	30 ^d

NA = Not applicable.

Percent adjusted survival at the end of each study week = [(survival/(60-no. of accidental deaths)) x 100]

- a Terminal sacrifice on Day 673 (Week 97).
- b Terminal sacrifice on Day 665 (Week 95).
- c Terminal sacrifice on Day 699 (Week 100).
- d Terminal sacrifice on Day 611 (Week 88).

Clinical Signs

Checked 2x daily. Clinical signs with higher occurrence in both GS-9350 and vehicle control animals compared with water control mice included decreased feces, hypoactivity, and audible/labored/irregular respiration. In GS-9350-dosed animals, a slightly higher occurrence of hunched posture and thin appearance was observed in 50 mg/kg/day males and 100 mg/kg/day females, respectively. The remaining clinical signs had a similar occurrence for all treated and control animals. Sore/scabs were observed on the ears, neck, and other areas of control and treated animals. Some of the skin lesions were diagnosed as ulcerative dermatitis by a veterinarian. In severe cases, animals were sacrificed due to skin lesions. These types of skin lesions are common and were not considered test article-related.

Body Weights

Recorded twice during the predose phase, weekly during Weeks 1 through 26, and once every 4 weeks thereafter.

In males, no significant differences in mean body weight were observed between the GS-9350-treated groups and vehicle control (see Table below). There was a transient decrease in the high dose in the week 26-54 interval. However, overall, there were no differences at final sacrifice.

In females, significantly ($p \leq 0.05$) higher mean body weights were noted intermittently during the first 13 weeks of the study in the 100 mg/kg/day group compared with vehicle

control, but mean values were generally similar to the water control for the same 13 week period. Beginning Week 11 through the end of the dosing period, mean body weights for the vehicle control females were generally significantly lower than the mean water control values. There were no significant GS-9350-related effects on mean body weight in females compared with vehicle controls from Week 14 through the end of the dosing period. Based on the magnitude of the change, the body weight effects were not considered adverse.

Table 10 - Mean Body Weight of Mice (Percent Difference from Vehicle Control)

Sex	Males				Females				
	Dose Level (mg/kg/day)	0 (water)	5	15	50	0 (water)	10	30	100
Week 13		↑3.6	↑0.8	0.0	↑0.3	↑6.5*	↑1.7	↑1.7	↑5.1*
Week 26		↑5.5	↓0.7	0.0	↓0.24	↑11.7*	↑1.3	↑2.3	↑2.9
Week 54		↑4.7	↑1.1	↑0.7	↓4.3	↑12.0*	0.0	↓0.3	↑0.3
Week 78		↑2.9	↓0.9	↓1.77	↓8.2	↑13.7*	↓1.4	↓1.1	↓6.6
Week 86		↑5.2	↓3.6	↑0.45	↓4.3	↑15.2*	↓3.4	↓0.6	↓7.9
Final Assessment ^a		↑4.6	↓2.5	↓0.2	↓5.9	↑10.9*	↓7.3	↓6.0	b

↓ = Decrease; ↑ = Increase

* = Significant at $p \leq 0.05$. Significance is based on comparison with the mean vehicle control values, not percent changes.

a The final assessment was made during Week 94 for males and Week 98 for females

b Group 5 females underwent an early scheduled sacrifice during Week 86 due to reduced survival.

Table 11 - Mean Body Weight Change of Mice (Percent Difference from Vehicle Control)

Sex	Males				Females				
	Dose Level (mg/kg/day)	0 (water)	5	15	50	0 (water)	10	30	100
Weeks 1-26		↑37.5*	↑2.3	↑4.5	↓2.3	↑49.2*	↑6.2	↑6.2	↑9.2
Weeks 26-54		↓14.8	↑22.2	↑7.4	↓107.4*	↑15.8	↓31.6	↓57.9	↓52.6
Weeks 54-78		↓150.0	↓187.5	↓212.5	↓375.0	↓12.0	↓8.0	↓12.0	↓76.0
Weeks 1- Termination ^a		↑22.0	↓8.3	↑2.8	↓17.4	↑36.4*	↓14.4	↓10.2	↓22.0

↓ = Decrease; ↑ = Increase.

* = Significant at $p \leq 0.05$. Significance is based on comparison with the mean vehicle control values, not percent changes.

a Termination (final assessment) was made during Week 94 for males and Week 86 for females.

Feed Consumption

Measured weekly for Weeks 1 through 25 and once every 4 weeks thereafter for the week prior to body weight collection.

The complicating factor for food consumption was a potential vehicle effect compared to water controls. All animals administered vehicle or GS-9350 formulations (in vehicle) had consistently lower mean food consumption values compared to water control.

In general, mean food consumption for treated males was increased compared to vehicle controls and was slightly reduced compared to the water controls during the first 13 weeks. After Week 13, GS-9350-treated males exhibited a trend of reduced mean food consumption compared with vehicle controls, but the decreases were slight, did not demonstrate dose dependency.

Mean food consumption values for GS-9350-treated females were generally similar to those of the vehicle controls and slightly reduced versus water controls during the first 13 weeks. Beginning with Week 37, treated females generally had reduced mean food consumption compared with vehicle controls, reaching statistical significance ($p \leq 0.05$) during Weeks 37, 45 through 73, 85, and 89 for females administered 30 mg/kg/day. Females administered 100 mg/kg/day had consistently reduced mean food consumption beginning Week 11 through the remainder of the study, except at three intervals. Changes in mean food consumption did not demonstrate dose dependency.

Table 12 - Mean Food Consumption of Mice (Percent Difference from Vehicle Control)

	Sex				Sex			
	Males				Females			
Dose Level (mg/kg/day)	0 (water)	5	15	50	0 (water)	10	30	100
Week 1	↑ 0.3	0.0	↑ 0.3	↓ 0.3	↑ 2.3	↑ 5.2	↑ 5.2	↑ 2.3
Week 12	↑ 6.0	↑ 0.6	↑ 2.4	↑ 1.2	↑ 6.5	↓ 0.7	↑ 3.3	0.0
Week 25	↑ 5.2	↓ 0.3	↓ 1.7	↓ 2.0	↑ 9.5*	↑ 0.9	↓ 6.1	↓ 2.4
Week 53	↓ 5.2	↓ 4.1	↓ 8.2*	↓ 13.7*	↑ 2.9	↓ 4.9	↓ 9.6*	↓ 3.2
Week 77	↑ 6.2	↓ 3.1	↓ 6.2	↓ 5.4	↑ 4.9	↓ 5.1	↓ 7.7	↑ 0.3
Week 85	↑ 5.3	↓ 10.9*	↓ 4.2	↓ 1.1	↑ 3.9	↓ 2.2	↓ 12.4*	↓ 3.6
Final Assessment ^a	↑ 1.2	↓ 0.6	↓ 1.7	↓ 2.0	↑ 10.2	↓ 5.4	↓ 10.2	NA ^b

↓ = Decrease; ↑ = Increase

* = Significant at $p \leq 0.05$. Significance is based on comparison with the mean vehicle control values, not percent changes.

a The final assessment was made during Week 93 for males and Week 97 for females

b Group 5 females underwent an early scheduled sacrifice during Week 86 due to reduced survival.

Clinical Pathology

Blood samples were collected for hematology from nonfasted animals via cardiac puncture. Samples were collected at each early scheduled sacrifice as follows: Group 5 females (Day 611/Week 88), Group 5 males (Day 665/Week 95), Group 1 through 4 males (Day 673/Week 97), and Group 1 through 4 females (Day 699/Week 100).

No evidence of GS-9350-related hematologic neoplasia was observed in males administered 5, 15, or 50 mg/kg/day for up to 96 weeks of dosing or in females administered 10, 30, or 100 mg/kg/day for up to 100 weeks of dosing.

There was 1 male (15 mg/kg) and 1 female (vehicle control) that had lymphocyte counts >10,000 cells/ μ L. The finding in the male was likely not related to drug since it was not found in any other animal or at any higher doses. The female (control) showed a spontaneous myelogenous leukemia.

Gross Pathology

Replaced Animals: Unscheduled Sacrifices and Deaths

Fifty animals in the carcinogenicity and toxicokinetic groups died or were euthanized in a moribund condition (and subsequently replaced) in the first 28 days of study. The causes of death, based on macroscopic and limited microscopic examinations were potential gavage accident (28/50), airway erosion (11/50), undetermined (8/50), handling trauma (2/50), and hemorrhage (1/50). See table below.

Table 13 - Cause of Death for the Replaced Mice - Days 1 through 28

Dose Level (mg/kg/day)	GS-9350										
	Sex	Males ^a					Females ^a				
		0 (control)	0 (vehicle control)	5	15	50 ^b	0 (control)	0 (vehicle control)	10	30	100
Undetermined	0	1	0	0	3	0	1	0	1	2	
Potential Gavage Accident	1	2	1	2	3	0	4	8	3	4	
Airway Erosion	0	1	0	1	3	0	3	1	2	0	
Hemorrhage	0	1	0	0	0	0	0	0	0	0	
Handling Trauma	0	0	0	0	0	0	0	1	0	1	
Total	1	5	1	3	9	0	8	10	6	7	

a Carcinogenicity and toxicokinetic animals are combined.

b Includes one animal sacrificed but not replaced on Day 30 of the dosing phase with limited tissue collection.

The increase in vehicle deaths compared to the single water control death (due to gavage error) suggests an increase in deaths due to the vehicle. The increased number of deaths in the males appears to suggest both a drug and vehicle effect. In females, there was no increase in deaths in the female animals with dose.

Carcinogenicity Animals: Unscheduled Sacrifices and Deaths

None of the macroscopic observations were suggestive of a systemic GS-9350-related effect. Distended gastrointestinal tract was observed in a few animals. See table below.

Table 14 - Incidence of Distended Gastrointestinal Tract Observations among Unscheduled Sacrifices in Mice

Dose Level (mg/kg/day)	GS-9350										
	Sex	Males					Females				
		0 (control)	0 (vehicle control)	5	15	50	0 (control)	0 (vehicle control)	10	30	100
Gastrointestinal tract distention	1	4	4	3	6	2	1	5	5	6	

The incidence in males was suggestive of a vehicle control article-related effect. In females, a higher incidence was noted in GS-9350-treated groups suggesting a test article effect, but the overall incidence remained low and there was no dose-dependency so the increased incidence was not attributed to GS-9350.

The Sponsor proposed that the distended GI tract was secondary to upper airway occlusion. *“Because mice are obligate nose breathers, occlusions in the upper airways sometimes act as one-way valves, allowing air to be inhaled but poorly exhaled. As a result, air enters the gastrointestinal tract and causes distention.”*

Increases in gavage and dosing procedure-related injuries as well as direct irritant effects in the lungs and upper airways were noted among carcinogenicity animals given the vehicle control or test article compared with water control. See table below.

Table 15 - Select Microscopic Observations in Unscheduled Sacrifices and Early Deaths in Mice

Dose Level (mg/kg/day)	Sex	GS-9350									
		Males					Females				
		0 (control)	0 (vehicle control)	5	15	50 ^a	0 (control)	0 (vehicle control)	10	30	100
Number of animals examined		24	40	36	33	46 ^a	37	37	41	45	46
Deaths Attributed to Dosing and											
Direct Irritation ^b		1	8	9	4	21	3	4	12	15	19
Nasal Turbinates											
Infiltrate, Neutrophils, Airway		4	20	21	20	43	7	17	27	28	36
Liver											
Pigment, Kupffer cells		0	0	0	0	10	4	3	0	3	18
Hypertrophy, Hepatocellular		1	6	2	6	15	2	2	1	0	4

The nasal infiltrates was most pronounced at the highest doses (50 mg/kg in males and 100 mg/kg in females). Liver effects increased with dose in both sexes.

Histopathology

At sacrifice.

Peer Review – Yes. Noted were the gavage accidents (more than ½ the deaths) and vehicle toxicity resulting in airway erosion and chronic injury in the other deaths.

Neoplastic - No significant positive trend and/or test article-related increase in neoplasms was present.

Non Neoplastic - Two nonadverse, drug-related effects were noted in the liver of females given 100 mg/kg/day and males given 50 mg/kg/day. Hepatocellular hypertrophy, considered likely secondary to hepatic enzyme induction, was increased in the high dose males and females compared to both control groups. The incidence of pigmented Kupffer cells was increased in high dose males, and marginally increased in high dose females when compared to either the vehicle or water controls.

Table 16 - Incidence of Select Nasal Turbinate and Liver Observations in Scheduled Sacrifices in Mice

Sex	GS-9350									
	Males					Females				
	0 (control)	0 (vehicle control)	5	15	50	0 (control)	0 (vehicle control)	10	30	100
Number of animals examined	36	20	24	27	13	23	23	19	15	14
Nasal Turbinates										
Infiltrate, Neutrophils, Airway	6	5	8	10	7	1	11	10	13	10
Liver										
Pigment, Kupffer cells	3	1	2	0	11	10	7	2	4	13
Hypertrophy, Hepatocellular	3	2	5	2	8	0	0	0	0	5

Toxicokinetics

Measured on day 1 and week 29 (predose and approximately 1, 2, 4, 8, 12, and 24 hours postdose).

Exposure to GS-9350 generally increased with the dose from 5 to 50 mg/kg for males and from 10 to 100 mg/kg for females. Mean concentrations of GS-9350 were generally higher after multiple dosing.

After oral gavage administration, GS-9350 was readily absorbed, with T_{max} values ranging from 1.00 to 2.00 hours on Day 1 and from 1.00 to 4.00 hours during Week 29.

Values for C_{max} and AUC_{0-t} were generally higher during Week 29 compared with Day 1. These results indicated potential accumulation of GS-9350 after multiple dosing in mice.

Increases in C_{max} and AUC_{0-t} for males and females were not consistently dose-proportional.

Table 17 - Toxicokinetic Parameters for GS-9350 in Mouse Plasma - Day 1 and Week 29

Dose Group	Dose Level (mg/kg/day)	Sex	AUC_{0-t} (ng.hr/mL)		C_{max} (µg/mL)	
			Day 1	Week 29	Day 1	Week 29
8	5	Male	600	4049	194	2310
	10	Female	4262	13874	1997	7877
9	15	Male	14261	12399	3343	4418
	30	Female	35999	72118	7067	13333
10	50	Male	53451	74954	9353	10130
	100	Female	100007	173893	16767	21567

There were no notable differences in GS-9350 exposures associated with the change in vehicles from vehicle 1 to vehicle 2. However, Vehicle 3 had an increase in exposure (AUC and C_{max}) compared to vehicle 1 and 2 (see tables below).

Table 18 - Mean COBI AUC Values in Mice Following Multiple Daily Oral Gavage Doses in Vehicles 1, 2 and 3

Dose Level (mg/kg/day)	Sex	AUC _{0-t} (µg•h/mL)			
		Vehicle 1 ¹	Vehicle 1 ²	Vehicle 2 ³	Vehicle 3 ⁴
5	Male	0.5	0.6	0.9	4.05
10	Female	3.8	2.6	4.1	13.9
15	Male	7.6	7.6	11.0	12.4
30	Female	32.4	43.9	36.0	72.1
50	Male	32.5	51.0	54.8	75.0
100	Female	75.7	151.0	169.7	174

1 Vehicle 1 = 95% PG, 5% EtOH, pH 2.3 (Male values at Week 13 [TX-216-2026]; females values at Day 14 [TX-216-2025])

2 Vehicle 1 = 95% PG, 5% EtOH, pH 2.3 (Day 14; TX-216-2032)

3 Vehicle 2 = 95% PG, 5% EtOH (Day 14, TX-216-2032)

4 Vehicle 3 = 10% PG (Week 29, TX-216-2030)

Table 19 - Mean COBI C_{max} Values in Mice Following Multiple Daily Oral Gavage Doses in Vehicles 1, 2 and 3

Dose Level (mg/kg/day)	Sex	C _{max} (µg/mL)			
		Vehicle 1 ¹	Vehicle 1 ²	Vehicle 2 ³	Vehicle 3 ⁴
5	Male	0.4	0.2	0.2	2.31
10	Female	1.0	0.9	1.9	7.88
15	Male	3.6	1.6	3.8	4.42
30	Female	7.2	6.6	8.4	13.3
50	Male	8.9	8.8	9.4	10.1
100	Female	13.9	14.6	19.8	21.6

1 Vehicle 1 = 95% PG, 5% EtOH, pH 2.3 (male values at Week 13 [TX-216-2026]; females values at Day 14 [TX-216-2025])

2 Vehicle 1 = 95% PG, 5% EtOH, pH 2.3 (Day 14; TX-216-2032)

3 Vehicle 2 = 95% PG, 5% EtOH (Day 14, TX-216-2032)

4 Vehicle 3 = 10% PG (Week 29, TX-216-2030)

Dosing Solution Analysis

Stability of samples in 95/5 PG/EtOH (pH 2.3), 95/5 PG/EtOH (no pH adjustment), or 10% PG) was confirmed for at least 15 days at room temperature.

Homogeneity was not necessary for any of the solutions.

Study title: 104-Week Oral Gavage Carcinogenicity Study with GS-9350 in Rats

Study no.: TX-216-2031
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 16 July 2009
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: See lot information in the table below.
 CAC concurrence: Yes

Table 20 - Test Article Information for Study TX-216-2031

Test Article ^a	Lot No.	Storage	Purity/Strength (%)	Retest Date ^{(b) (4)}	Reserve (Archive) Sample ^b
GS-9350	9350-AC-2E Crop 2	In a refrigerator, set to maintain 2 to 8°C, and protected from light	98.6/52.9 ^c	(b) (4)	Collected
	9350-AC-2E Crop 1		99.2/48.5 ^d		

Key Study Findings

Nonneoplastic and neoplastic microscopic findings attributed to GS-9350 were limited to the thyroid and liver. Findings included: thyroid follicular cell adenoma M/F; thyroid follicular carcinoma, M only, thyroid follicular cell hypertrophy, M/F; centrilobular hepatocyte hypertrophy, M/F; karyocytomegaly, M/F. There were also findings in the adrenal medulla (benign pheochromocytoma, males only), which were considered benign.

The Sponsor's evaluation found statistical significance for follicular cell adenomas and carcinomas (combined) in males and females. The FDA analysis also detected statistical significance for follicular cell adenomas and carcinomas (combined) in males and females.

These findings are considered secondary to the hepatic microsomal enzyme induction (due to the mechanism of action of GS-9350 as a CYP3A4 inhibitor) as well as thyroid hormone imbalance previously observed in repeat dose studies in rats with GS-9350.

Adequacy of Carcinogenicity Study

The study achieved acceptable exposures to the test article for a valid evaluation of the carcinogenic potential of GS-9350.

Appropriateness of Test Models

The test models were appropriate.

Evaluation of Tumor Findings

The evaluation of the tumor findings was acceptable.

Methods

Doses: See table below.
 Frequency of dosing: Daily
 Dose volume: 2 ml/kg
 Route of administration: Oral gavage
 Formulation/Vehicle: 95% propylene glycol (PG; v/v) and 5% EtOH (v/v), pH 2.3 (\pm 0.1)
 Basis of dose selection: MTD and AUC
 Species/Strain: Sprague Dawley rats (b) (4)
 Number/Sex/Group: See table below.
 Age: 6-7 weeks old at initiation of dosing
 Animal housing: Individual housing
 Paradigm for dietary restriction: Rodent chow ad libitum unless fasted for study-specific procedures
 Dual control employed: Yes
 Interim sacrifice: No
 Satellite groups: Yes (see table below)
 Deviation from study protocol: No significant deviations were noted that would affect data interpretation or data quality/integrity.

Table 21 - Study Design for Study TX-216-2031

Group	No. of Animals		Dose Level (mg/kg/day)		Dose Concentration (mg/mL)	
	Male	Female	Male	Female	Male	Female
Carcinogenicity Animals						
1 (Control) ^a	65	65	0	0	0	0
2 (Vehicle Control) ^b	65	65	0	0	0	0
3 (Low)	65	65	10	5	5	2.5
4 (Mid)	65	65	25	15	12.5	7.5
5 (High)	65	65	50	30	25	15
Toxicokinetic Animals						
6 (Control) ^a	6	6	0	0	0	0
7 (Vehicle Control) ^b	6	6	0	0	0	0
8 (Low)	12	12	10	5	5	2.5
9 (Mid)	12	12	25	15	12.5	7.5
10 (High)	12	12	50	30	25	15
Sentinel Animals^c						
Group 11	10	10	c	c	c	c

a Groups 1 and 6 received the control article only.

b Groups 2 and 7 received vehicle control article, only.

c Group 11 was not dosed.

Observations and Results

Mortality

Checked 2x daily. The number of surviving animals in the vehicle control groups reached 20 animals earlier than expected; therefore, males were dosed for a minimum of 97 weeks and females were dosed for a minimum of 102 weeks.

Administration of GS-9350 did not have a negative impact on survival, and sufficient numbers of animals survived to adequately evaluate carcinogenicity. Relative to the vehicle control group, administration of GS-9350 was associated with significantly lower mortality for males administered 25 or 50 mg/kg/day. Mortality relative to the water control group was significantly increased for vehicle control males. In females, no statistically significant effects on mortality occurred.

Neoplasia was the most frequent cause of death in unscheduled deaths of males (pituitary) and females (pituitary and mammary), with a similar incidence across water and vehicle control and GS-9350-treated groups.

Figure 5 – Adjusted Percent Survival (Male rats)

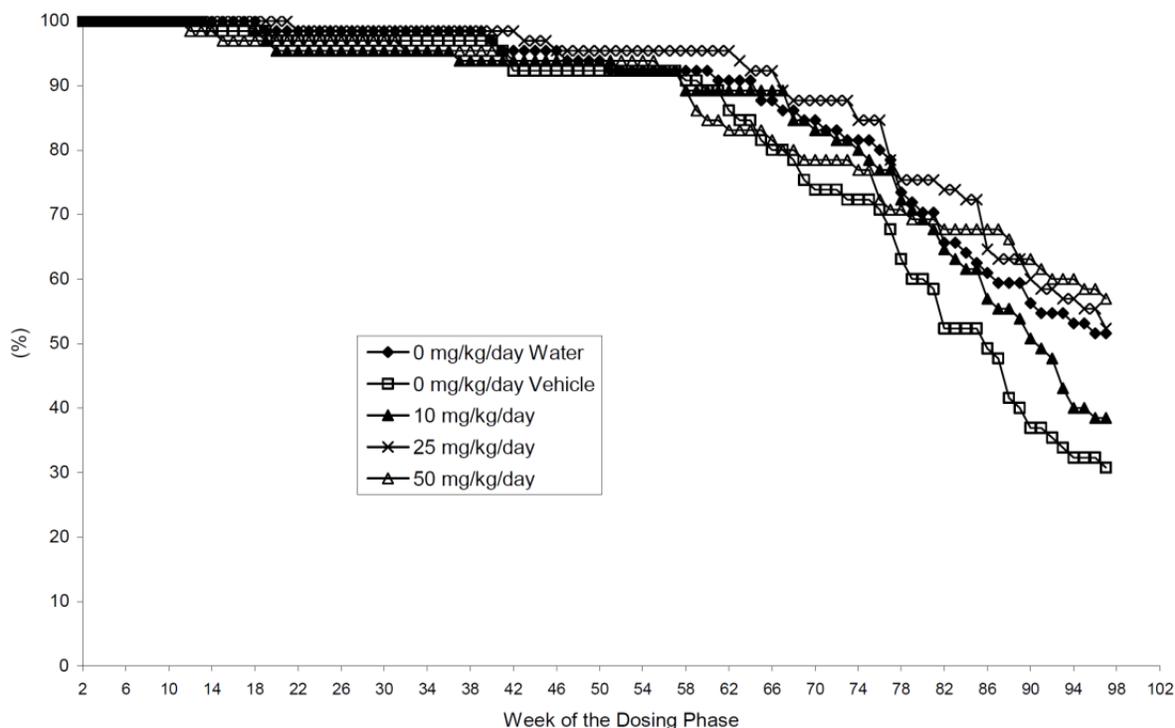
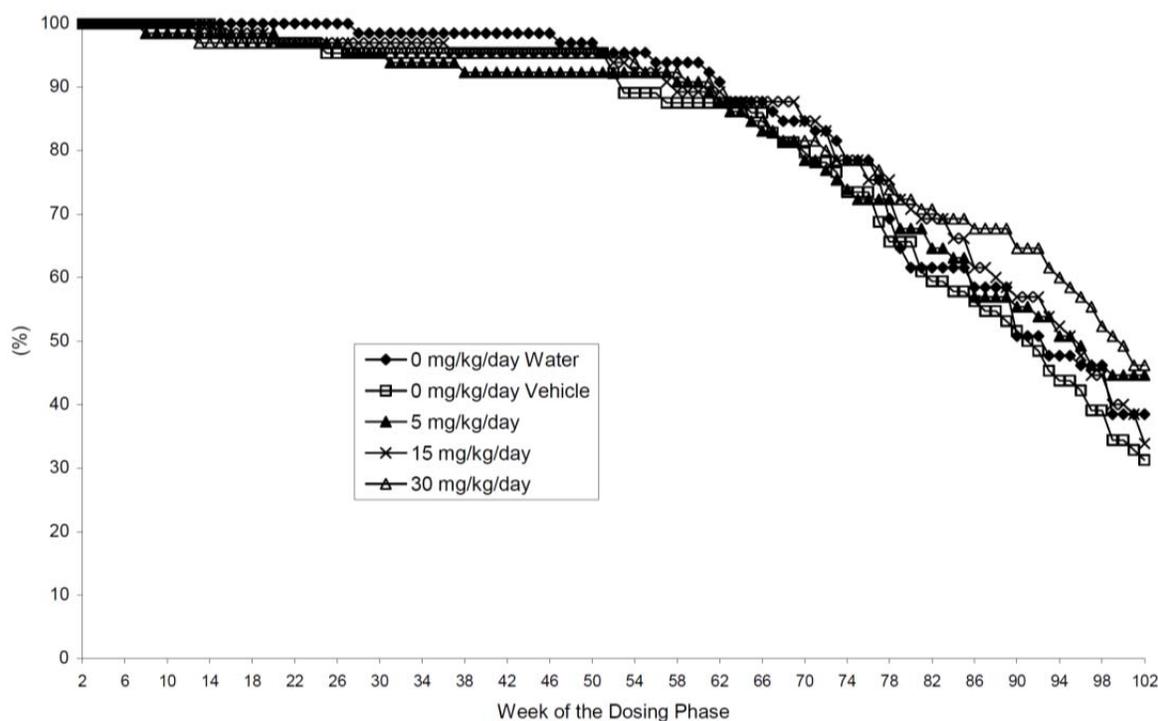


Figure 6 – Adjusted Percent Survival (Female rats)

Clinical Signs

Checked 2x daily. Clinical signs were noted infrequently, without a relationship to dose, and were those commonly observed in aged rats; therefore, they were considered unrelated to GS-9350. In addition to masses, most carcinogenicity animals were noted with at least one of the following clinical signs: hunched (females only), thin, or swollen appearance; limited use of limbs/paws (males only); few feces; clear oral discharge; alopecia; rough or red haircoat; and sore/scab, red, or scaly skin.

Body Weights

Once predose, weekly through week 26, then once every 4 weeks till sacrifice.

Males:

There were no notable differences in mean body weight or mean body weight gain between water and vehicle control groups, although mean body weights were often increased in the vehicle control group. At the final assessment (Week 98), mean body weight relative to vehicle controls was decreased 12 or 9% for males administered 25 or 50 mg/kg/day, respectively. However, relative to water controls, mean body weight were slightly decreased in males administered 25 or 50 mg/kg/day at Week 98.

Females:

In females, there were no differences in body weight or mean body weight gain between water and vehicle control groups. There were transient increases (Week 1 to 26 intervals at 15 and 30 mg/kg/day) and decreases (Weeks 78 to 102 at 15 mg/kg/day) in body weight gain. However, at the final assessment (Week 102), mean body weight and mean body weight gain relative to both water and vehicle controls were not notably different for females administered GS-9350 at any dose level.

Figure 7 – Mean Body Weights (Male rats)

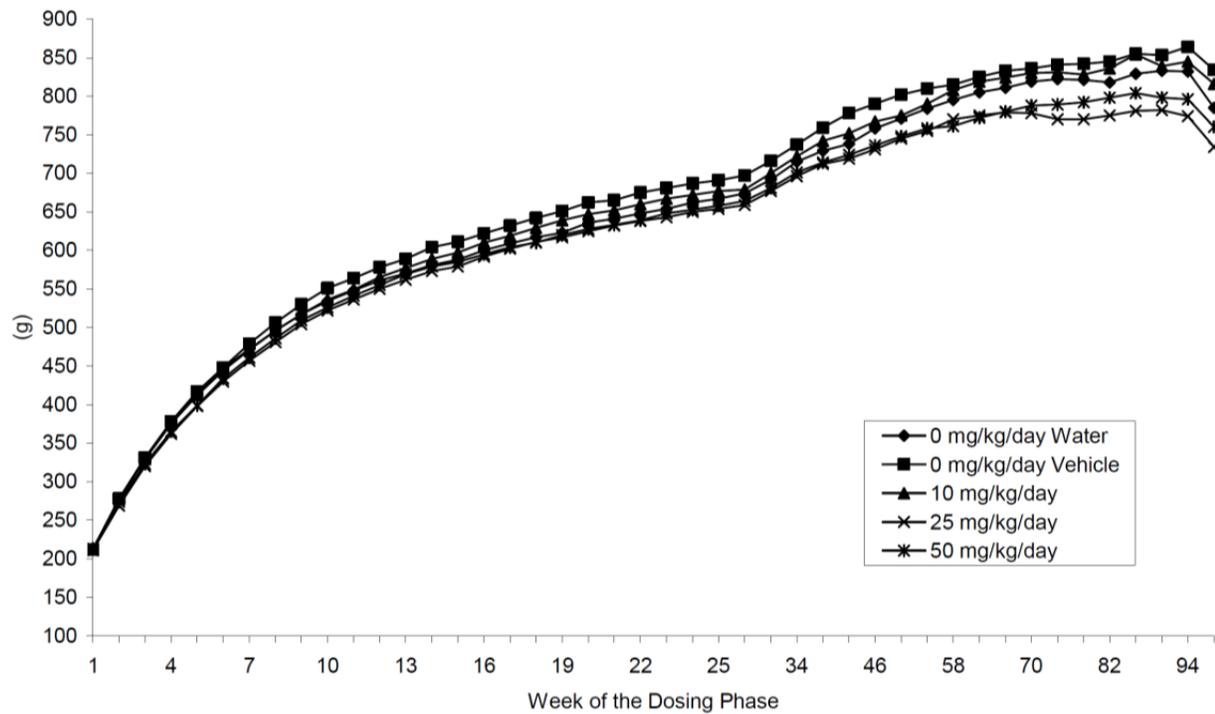
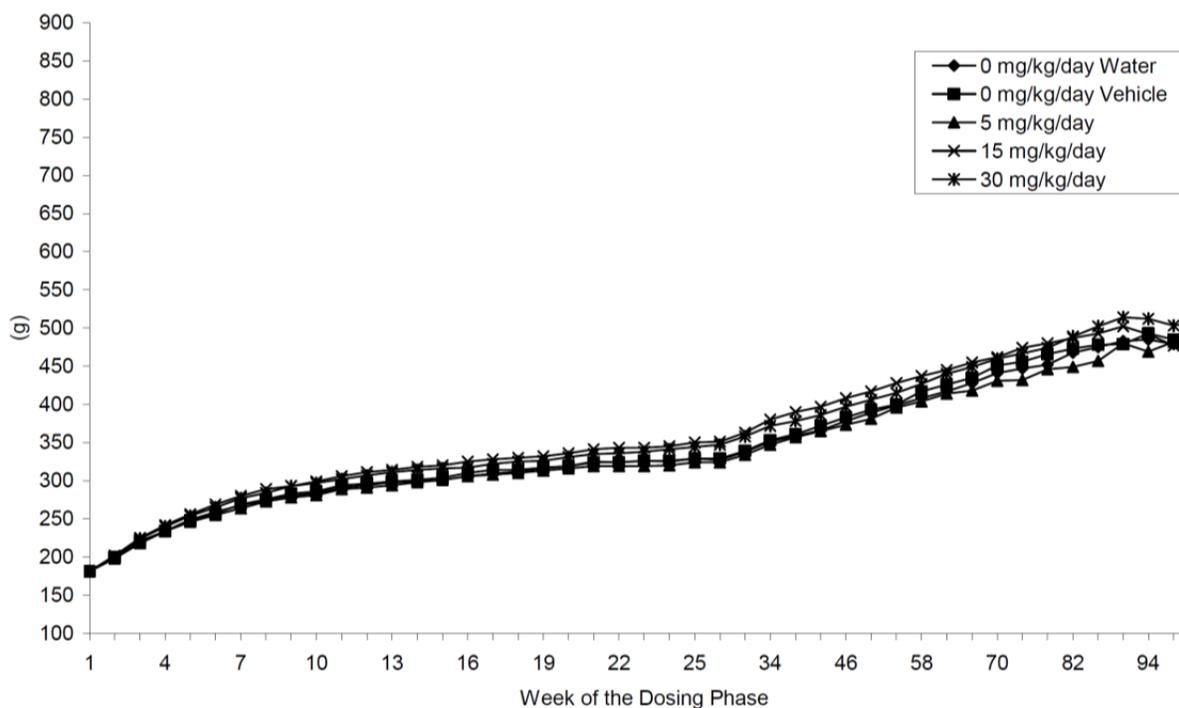


Figure 8 – Mean Body Weights (Female rats)



Feed Consumption

Weekly through week 25, then once every 4 weeks till sacrifice. Mean food consumption relative to vehicle controls was minimally decreased (<12%) at most of the assessment intervals for males at all dose levels through 77 weeks of dosing.

Food consumption for females administered GS-9350 was essentially comparable with water and vehicle control group values throughout the dosing phase. The effect on food consumption in males was considered related to GS-9350, but given that it did not result in adverse effects on body weight or other signs of toxicity, decreased food consumption was not considered adverse.

Figure 9 – Mean Food Consumption (Male rats)

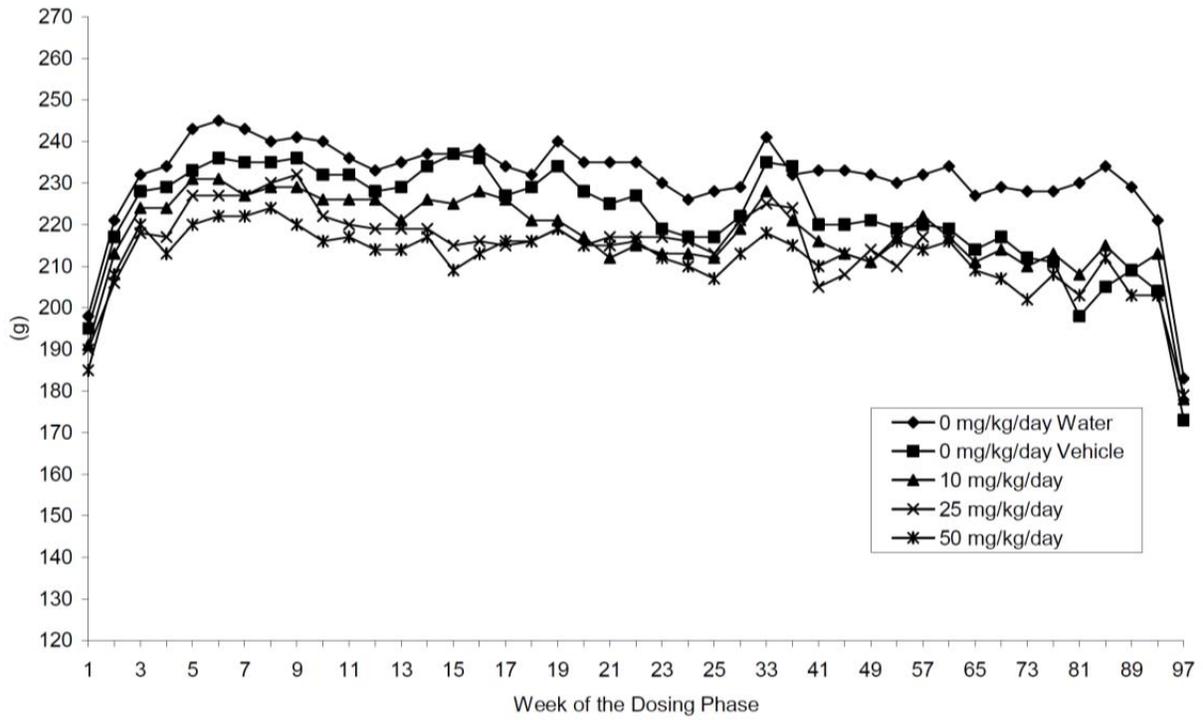
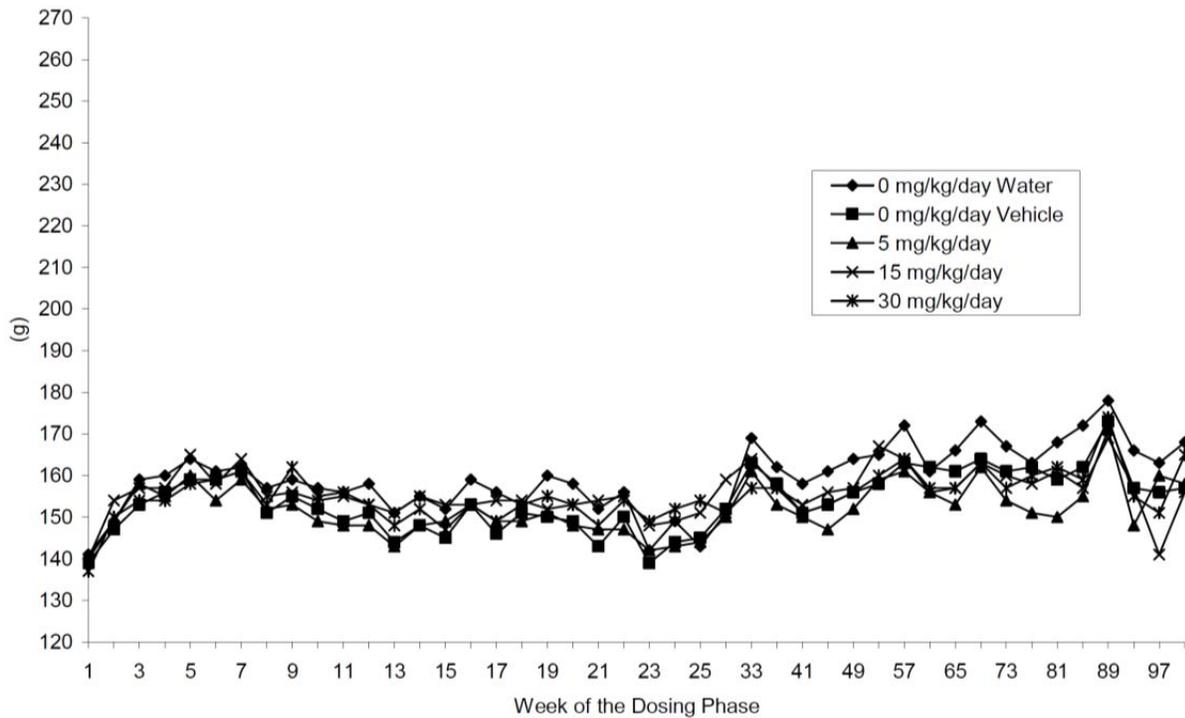


Figure 10 – Mean Food Consumption (Female rats)



Clinical Pathology

Collected from the first 10 surviving animals/sex/group during Months 12 (Days 358 and 357 for males and females, respectively) and 18 (Days 527 and 526 for males and females, respectively) of the dosing phase and from all surviving animals at their respective scheduled sacrifice.

GS-9350 exhibited no evidence of hematopoietic neoplasia and there were no notable effects in hematology test results.

Gross Pathology

At sacrifice. No major effects noted.

Macroscopic observations, including those commonly observed in the pituitary (mass), brain (depressed area due to pituitary mass), skin (confirmed mass), and mammary gland (confirmed mass), represented incidental, background findings that typically occur in long-term studies in rats and were not directly related to administration of the test article.

Histopathology

At sacrifice.

Peer Review – Yes.

Nonneoplastic and neoplastic microscopic findings attributed to GS-9350 were limited to the thyroid and liver. There were also findings in the adrenal medulla, which were considered benign.

These findings are considered secondary to the hepatic microsomal enzyme induction (due to the mechanism of action of GS-9350 as a CYP3A4 inhibitor) as well as thyroid hormone imbalance previously observed in repeat dose studies in rats with GS-9350.

Neoplastic

Thyroid

A significant positive trend for thyroid follicular cell adenoma (common tumors) was present in males and females, with a significant increase in pairwise comparison noted only in males given 50 mg/kg/day. Thyroid follicular carcinoma incidences demonstrated a significant positive trend for common tumors in males but not females; however, the effect was not significant by pairwise comparison for any of the male treatment groups. Thyroid follicular cell adenoma incidences in females showed a significant positive trend for common tumors; however, the effect was not significant by pairwise comparison for any of the treatment groups for common tumors. A significant positive trend was noted for combined thyroid follicular cell adenoma and carcinoma (common tumors), with a significant pairwise comparison increase in males and females administered ≥ 25 mg/kg/day and 30 mg/kg/day, respectively.

Table 22 - Incidence of GS-9350-Related Neoplastic Findings in the Thyroid in the Rat

	Sex	GS-9350										
		Males					Females					
		Dose Level (mg/kg/day)		0	0	10	25	50	0	0	5	15
		(water)	(vehicle)				(water)	(vehicle)				
Thyroid	Number Examined	65	65	65	65	65	65	65	65	65	65	65
	B-Adenoma, Follicular Cell	3	0	1	5	15	0	0	0	0	2	6
	M-Carcinoma, Follicular Cell	0	0	0	1	5	0	0	0	0	1	2
	B-Adenoma and M-Carcinoma, Follicular Cell	3	0	1	6	20	0	0	0	0	3	8

Adrenal Medulla

The incidence of benign pheochromocytoma in males was dose dependently increased compared with the vehicle control group; the positive trend test was not significant for this common tumor.

Table 23 - Incidence of Neoplastic Findings in the Adrenal Medulla in the Rat

	Sex	GS-9350										
		Males					Females					
		Dose Level (mg/kg/day)		0	0	10	25	50	0	0	5	15
		(water)	(vehicle)				(water)	(vehicle)				
Adrenal, Medulla	Number Examined	65	65	65	65	65	65	65	65	65	65	65
	Hyperplasia	2	0	1	3	0	0	3	0	0	0	1
	Pheochromocytoma, Benign	2	1	3	6	7	1	0	1	1	1	1
	Pheochromocytoma, Malignant	0	1	0	2	0	0	0	1	1	0	0
	Pheochromocytoma, Benign and/or Malignant	2	2	3	8	7	1	0	2	2	1	1

Non Neoplastic**Thyroid**

Thyroid follicular cell hypertrophy, a nonneoplastic GS-9350-related microscopic finding, occurred at an increased incidence in males administered ≥ 25 mg/kg/day and females administered ≥ 15 mg/kg/day.

Table 24 - Incidence of GS-9350-Related Nonneoplastic Microscopic Findings in the Thyroid in the Rat

Dose Level (mg/kg/day)	Sex	GS-9350									
		Males					Females				
		0 (water)	0 (vehicle)	10	25	50	0 (water)	0 (vehicle)	5	15	30
Thyroid	Number Examined	65	65	65	65	65	65	65	65	65	65
	Hypertrophy, Follicular Cell	5	1	3	17	25	1	1	3	9	16
	Hyperplasia, Follicular Cell	3	2	3	3	5	0	0	1	4	4

Liver

GS-9350-related nonneoplastic microscopic findings considered secondary to enzyme induction in the liver included centrilobular hepatocyte hypertrophy in males administered ≥ 10 mg/kg/day and females administered 30 mg/kg/day and hepatocyte karyocytomegaly in males administered ≥ 25 mg/kg/day. Although the hepatocyte karyocytomegaly in females was not convincingly increased over control animals, an increasing trend was noted and consistent with the overall hepatic changes.

Table 25 - Incidence of GS-9350-Related Nonneoplastic Microscopic Findings in the Liver in the Rat

Dose Level (mg/kg/day)	Sex	GS-9350									
		Males					Females				
		0 (water)	0 (vehicle)	10	25	50	0 (water)	0 (vehicle)	5	15	30
Liver	Number Examined	65	65	65	65	65	65	65	65	65	65
	Karyocytomegaly, Hepatocyte	1	0	1	8	13	4	3	3	7	8
	Hypertrophy, Hepatocyte, Centrilobular	6	5	16	25	27	1	0	0	0	12

Toxicokinetics

Recorded once during the predose phase, weekly for Weeks 1 through 26, and once every 4 weeks thereafter.

Exposure to GS-9350 increased with the increase in dose level from 10 to 50 mg/kg/day for males and from 5 to 30 mg/kg/day for females. Increases in C_{max} and AUC_{0-t} for males and females were generally greater-than-dose proportional. Accumulation of GS-9350 was observed after multiple dosing, notably in males administered 10 or 25 mg/kg/day and females administered 5 mg/kg/day.

Table 26 - Toxicokinetic Parameters for GS-9350 in Rat Plasma - Day 1 and Week 26

Dose Group	Dose Level (mg/kg/day)	Sex	AUC _{0-t} (ng.hr/mL)		C _{max} (ng/mL)	
			Day 1	Week 26	Day 1	Week 26
8	10	Male	89.5	1305	37.4	430
	5	Female	38.2	807	17.0	725
9	25	Male	2568	9077	544	1753
	15	Female	3882	6376	1051	1745
10	50	Male	20832	22649	2999	2424
	30	Female	12041	19853	2183	3233

Dosing Solution Analysis

Mean values for concentration verification analyses from Weeks 1, 4, 13, 26, 52, and 78 for all test article formulations ranged between 93.0 and 105.2% of target concentration, thereby meeting acceptance criteria. Mean values for formulations from Week 103 targeted at 2.5, 7.5, and 15 mg/mL were 88.6, 87.9, and 86.5% of target concentration, respectively. The recoveries for the Week 103 dose preparations had no impact on the study results because these formulations were only slightly lower than targeted concentrations and they were administered only to females on the final 2 days of dosing.

No test article was detected in water and vehicle control article formulations for Weeks 1, 4, 13, 52, 78, and 103. Vehicle control article formulated for administration to Groups 2 and 7 during Weeks 26 and 28 through 29 had positive detection of test article. The Sponsor proposed that the lot of the 1N sodium hydroxide used for the preparation of these batches of vehicle control article was a possible cause. The 1N sodium hydroxide was changed in these particular weeks compared to earlier dosing formulations. However, it could not be confirmed if the response was GS-9350 or matrix background.

The low levels of positive detection likely had no impact on the study results. All Week 26 bioanalytical samples for the vehicle control group were lower than the limit of GS-9350 quantitation in plasma (<5.00 ng/mL).

11 Integrated Summary and Safety Evaluation

Cobicistat (COBI) is an approved drug as a component of STRIBILD (Elvitegravir 150 mg/Cobicistat 150 mg/Emtricitabine 200 mg/Tenofovir DF 300 mg Tablet) for the treatment of HIV-1 infection.

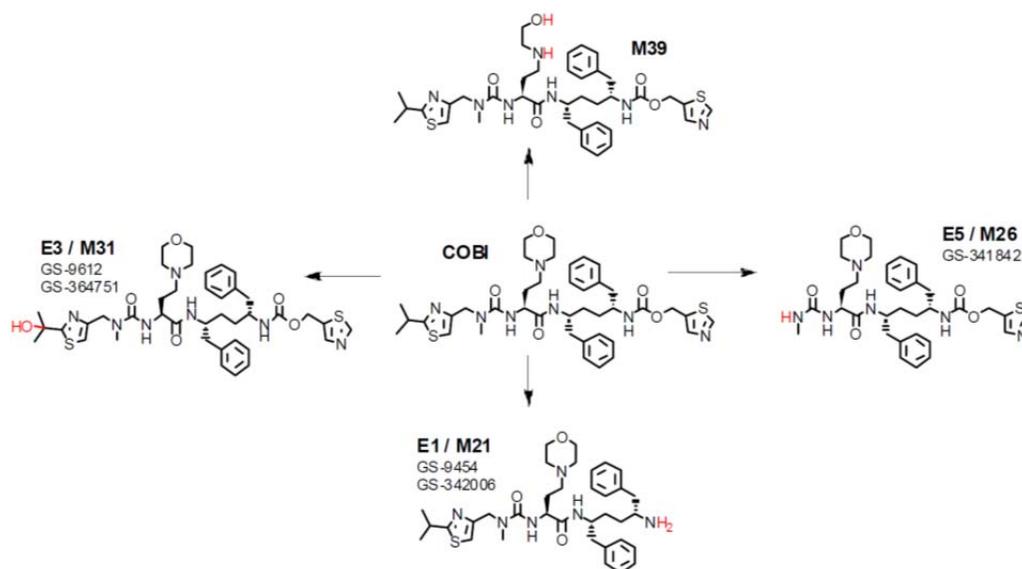
All nonclinical studies required to support chronic use have been performed and submitted as a part of the nonclinical assessment for COBI.

No significant effects were noted that would preclude approval of COBI as a CYP3A inhibitor 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 ($IC_{50} = 1.8 \mu\text{M}$). Furthermore, COBI inhibited the hCav1.2 L-type calcium channel at a moderately low concentration ($IC_{50} = 6 \mu\text{M}$). During further characterization *in vitro* utilizing rabbit hearts, COBI caused negative inotropic effects and shortening of the APD at $\geq 1 \mu\text{M}$ 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 $\geq 1.5 \mu\text{M}$. 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 11 - 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 Division requested additional studies in PMR requests 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 (atazanavir). 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 27 – 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.

In the 2-year carcinogenicity study in mice with COBI, no drug-related increase in tumor incidence was observed at exposures 7 to 16 times (males and females, respectively)

the human systemic exposure at the therapeutic daily dose. In the 2-year carcinogenicity study in rats, increases in follicular cell adenomas and/or carcinomas in the thyroid gland were observed at doses of 25 and 50 mg/kg/day in males, and at 30 mg/kg/day in females. The follicular cell findings are considered to be rat-specific, secondary to hepatic microsomal enzyme induction and thyroid hormone imbalance, and are not relevant for humans. At the highest doses tested in the rat carcinogenicity study, systemic exposures were approximately 2 times the human systemic exposure at the therapeutic daily dose. In rats, COBI induces hepatic CYP3A activity due to a species-specific activation of PXR, which does not occur in humans. The observed toxicity profile on the thyroid is rodent-specific and it is unlikely that COBI presents a risk to the human thyroid. These effects, associated with liver enzyme induction, bear no relevance for man as a similar association between liver enzyme induction and carcinogenesis does not exist in humans.

Table 28 - Estimated Safety Margins for COBI 150 mg Based on Exposure (AUC) at the High Dose in the Carcinogenicity Studies

Label Section Number	Study Type	Study Number	Dose (mg/kg/day)	AUC _{0-t} (µg•h/mL)	Safety Margin ^a
13.1 (Carcinogenesis, Mutagenesis, Impairment of Fertility)	Carcinogenesis				
	Mouse	TX-216-2030	50 (males)	75.0	7
			100 (females)	174	16
Rat	TX-216-2031	50 (males)	22.6	2.1 ^b	
		30 (females)	19.9	1.8	

a Human AUC_{tau} 10.9 µg•h/mL (m2.7.2, Section 3.4.2)

b Male and females values combined to provide approximate exposure margin of 2 in draft label.

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 F₁ 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 29 - 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 irritation, 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 due to the

presence of [REDACTED]^{(b) (4)}, respectively. In addition, [REDACTED]^{(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 203-100 review) for that information.

The overall nonclinical program of COBI was considered adequate to support the safety of COBI 150 mg tablets.

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/s/

LAINÉ P MYERS
03/21/2013

HANAN N GHANTOUS
03/21/2013

I concur with Dr. Myers conclusion "There are no nonclinical pharmacology and toxicology issues which would preclude the approval of COBI 150 mg tablets."

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 203094 Applicant: Gilead Sciences Stamp Date: 6/28/2012

Drug Name: Cobicistat NDA/BLA Type: NDA

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?	X		2 method validations 2 carci studies (one rat, one mouse) 1 Ames assay 1 Mouse lymphoma assay 1 In silico evaluation
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
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)?	X		The prior NDA (#203100) for cobicistat did not include the carci studies. The carci studies for cobicistat are included as part of this NDA to fulfill that requirement.
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 <u>submitted</u> a rationale to justify the alternative route?	Yes		Oral route
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	Yes		Included in the final study reports.
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	Yes		Included in the prior NDA (#203100)

**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		Genotoxicity assays (in vivo, in vitro, and in silico) performed to address impurities from the prior NDA submission (#203100)
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
Reviewing Pharmacologist

July 11, 2012
Date

Hanan Ghantous, PhD, DABT
Team Leader/Supervisor

July 11, 2012
Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

LAINÉ P MYERS
08/01/2012

HANAN N GHANTOUS
08/01/2012