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RESEARCH**

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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

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Applicant: Gilead
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1 Executive Summary

1.1 Introduction

Eplclusa™, a once daily fixed-dose combination (FDC) tablet containing velpatasvir and sofosbuvir, is intended for the treatment of chronic hepatitis C virus (HCV) genotypes 1-6 infection in adult patients. Velpatasvir (VEL, GS-5816) is a specific inhibitor of nonstructural protein 5A (NS5A) of HCV that has displayed potent inhibition of HCV replication *in vitro*. Sofosbuvir (SOF, GS-7977) is a nucleotide prodrug of 2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate that is converted intracellularly to the active uridine triphosphate (GS-461203) within tissues. GS-461203 is a specific inhibitor of nonstructural protein 5B (NS5B) of HCV that has displayed potent inhibition of HCV replicon ribonucleic acid (RNA) replication *in vitro*. SOF (Sovaldi, in a combination antiviral treatment regimen with ribavirin) was approved for marketing in the U.S. in December 2013 (refer to NDA-204,671), and as a component of a FDC with ledipasvir (Harvoni) in October 2014 (refer to NDA-205834).

The nonclinical safety profile of VEL has been evaluated in: safety pharmacology studies in rats and dogs; repeat-dose toxicology studies in mice, rats and dogs for up to 1, 6 and 9 months duration, respectively; up to 2-week repeat-dose toxicology studies to qualify impurities; phototoxicity studies in mice and rats; fertility and pre- and post-natal developmental studies in rats; embryo-fetal developmental studies in mice, rats, and rabbits; and genetic toxicology studies (Ames, *in vitro* chromosomal aberration and *in vivo* rat micronucleus assays). In addition, numerous *in vitro* and *in vivo* nonclinical pharmacokinetic studies evaluating the absorption, distribution, metabolism and excretion of VEL have been conducted in mice, rats, dogs, and monkeys, while rat and mouse carcinogenicity studies with VEL are currently in progress. Nonclinical safety studies for SOF to support the FDC were reviewed previously. Refer to the Pharmacology/Toxicology reviews for NDA-204,671 and NDA-205834 for a detailed summary of SOF nonclinical data (as well as Table 44 in this review for updated exposure margins).

1.2 Brief Discussion of Nonclinical Findings

No clear target organs of toxicity were identified in repeat-dose toxicology studies in mice, rats, and dogs administered VEL doses up to 1500, 200, and 100 mg/kg/day for 1, 6 and 9 months, respectively. VEL exposures at these doses were 68, 4, and 9 times the exposure in humans at the recommended human dose for the FDC. VEL was not genotoxic and had no effects on reproduction or development in mice, rats, and rabbits. Carcinogenicity studies, a 6 month transgenic rasH2 mouse study and a 2 year rat study, are currently on-going.

The oral FDC of VEL/SOF doubled the clinical exposure of SOF (and its main circulating metabolite, GS-331007) which appears to be the result of increased intestinal absorption of SOF due to VEL inhibition of intestinal efflux transporters. However, there was only a marginal change in safety multiples for SOF, as SOF alone was not associated with significant toxicity. Therefore, in respect to the FDC of VEL/SOF, no specific overlapping toxicity of potential significant clinical concern was identified in animals.

VEL had moderate oral bioavailability (~25-30% in rats, monkeys and dogs) with T_{max} values of ~6 hours at the highest feasible exposure in each model. While multiple formulations of VEL were examined, higher exposures were not reached due to low intrinsic solubility of GS-5816 and saturation of absorption. Nonetheless, adequate circulating VEL exposure levels were achieved using the optimized vehicle in the toxicology studies. VEL was highly bound to plasma protein (>99%) in all species examined and had wide tissue distribution in rodents, including the gall bladder (and bile), liver, adrenal gland, pancreas, kidney, small intestine, and the eye (harderian gland and uveal tract). VEL was rapidly eliminated from most tissues and mainly excreted in the bile within 24 hours, except from the eye which maintained VEL exposure at 168 hours postdose (the final observation). Additional assessments in pigmented rats revealed binding with melanin-containing tissues (including the eye), but VEL was reversibly bound to melanin and did not reach meaningful concentrations in the eye (<1% of absorbed dose). Follow-up studies in rats and rabbits suggest that VEL was neither phototoxic nor an ocular irritant. Although several minor metabolites were identified, unchanged parent drug was the predominant circulating component (in mice, rats, dogs, and human subjects) as well as the primary drug component in feces, with the major route of VEL elimination as biliary excretion (<0.3% excreted in urine).

1.3 Recommendations

1.3.1 Approvability

Yes. The sponsor provided sufficient nonclinical safety information on velpatasvir in support of approval for marketing in the U.S. Sofosbuvir (Sovaldi, in a combination antiviral treatment regimen with ribavirin) was approved for marketing in the U.S. in December 2013.

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

The sponsor's draft product label will be reviewed in a subsequent labeling review.

2 Drug Information

2.1 Drug

2.1.1 Sofosbuvir

CAS Registry Number: 1190307-88-0

Generic Name: Sofosbuvir (SOF)

Code Name: GS-7977

Chemical Name: (S)-Isopropyl 2-(((S)-(((2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-(phenoxy)phosphorylamino)propanoate

Molecular Formula/Molecular Weight: C₂₂H₂₉FN₃O₉P/ 529.45 g/mol

Structure or Biochemical Description:

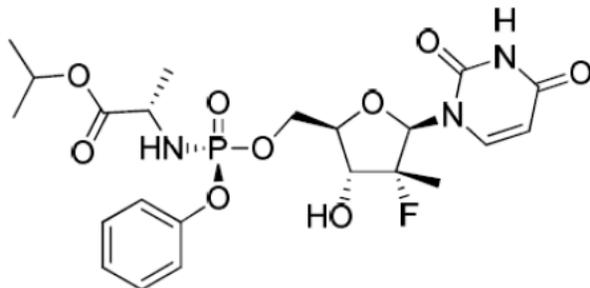


Figure 1: Sofosbuvir Structure

Pharmacologic Class: NS5B inhibitor

2.1.2 Velpatasvir

CAS Registry Number: 1377049-84-7

Generic Name: Velpatasvir (VEL)

Code Name: GS-5816 (GS-589916)

Chemical Name: (b) (4)

Molecular Formula/Molecular Weight: C₄₉H₅₄N₈O₈/883.00

Structure or Biochemical Description:



Figure 2: Velpatasvir Structure

Pharmacologic Class: NS5A inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA 204,671 (Solvadi); NDA 205,834 (Harvoni); IND 106,739 (Sofosbuvir); IND 115,670 (Velpatasvir); IND 118,605 (SOF/VEL)

2.3 Drug Formulation

SOF/VEL fixed-dose combination tablets contain 400 mg of sofosbuvir and 100 mg of velpatasvir. The tablet formulation utilizes (b) (4).

The tablets are pink, diamond-shaped, film-coated tablets, debossed with “GSI” on one side and “7916” on the other side. The quantitative composition of the tablets is listed in the sponsor’s table below.

Table 1: Quantitative Composition of Sofosbuvir/Velpatasvir Tablets

Component	Composition (% w/w)	Unit Formula (mg/tablet)	Quality Standard	Function
Intragranular				
Sofosbuvir ^a	(b) (4)	400.0	In-house	Active Ingredient
Velpatasvir ^{b,c}	(b) (4)	100.0	In-house	Active Ingredient
Copovidone ^{b,c,d}	(b) (4)	(b) (4)	NF, Ph. Eur.	(b) (4)
(b) (4)	(b) (4)	(b) (4)	USP, Ph. Eur.	(b) (4)
Microcrystalline Cellulose ^{a,c}	(b) (4)	(b) (4)	NF, Ph. Eur.	(b) (4)
Croscarmellose Sodium	(b) (4)	(b) (4)	NF, Ph. Eur.	(b) (4)
Magnesium Stearate	(b) (4)	(b) (4)	NF, Ph. Eur.	(b) (4)
(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)	NF, Ph. Eur.	(b) (4)
Total Tablet Core Weight	100.0	1000	—	—
Film-Coat				
(b) (4)	(b) (4)	(b) (4)	In-house	Film-coat
(b) (4)	(b) (4)	(b) (4)	USP, Ph. Eur.	(b) (4)
a	(b) (4)			
b	(b) (4)			
c	(b) (4)			
d	(b) (4)			
e	(b) (4)			
f	(b) (4)			
g	(b) (4)			
h	(b) (4)			

2.4 Comments on Novel Excipients

No novel excipients are used to manufacture SOF/VEL tablets.

2.5 Comments on Impurities/Degradants of Concern

The proposed specifications for impurities in the SOF/VEL drug substance were deemed acceptable based on results from general toxicology studies and/or assessments of potential mutagenicity using (quantitative) structure-activity relationship (Q)SAR predictions. For further information, please see the impurity review by Dr. Mark Powley in 11 Appendix/Attachments.

2.6 Proposed Clinical Population and Dosing Regimen

Epclusa™ (SOF/VEL) is an oral fixed-dose combination tablet (400/100 mg) taken once daily for the treatment of chronic hepatitis C virus infection in adults. The proposed recommended treatment regime for all HCV genotypes is for 12 weeks in patients without cirrhosis (or compensated cirrhosis) and for 12 weeks plus ribavirin in patients with decompensated cirrhosis.

2.7 Regulatory Background

This application is being submitted in support of a NDA for a FDC containing the active ingredients sofosbuvir (SOF) and velpatasvir (VEL). SOF has been fully reviewed under NDA 204671. VEL is a new chemical entity and this NDA contains full nonclinical data sets (except for carcinogenicity studies that currently are on-going). This NDA is supported by right of reference to the following Gilead applications: NDA 204,671 (Solvadi), NDA 205,834 (Harvoni), IND 106,739 (Sofosbuvir), IND 115,670 (Velpatasvir), and IND 118,605 (SOF/VEL).

3 Studies Submitted

3.1 Studies Reviewed for VEL

Study Title	Study #
Secondary Pharmacology	
Cellular Cytotoxicity Evaluation of VEL in Multiple Cell Lines	PC-281-2014
In Vitro Receptor Binding of GS-589916	PC-281-2001
Safety Pharmacology	
Cardiovascular Safety Pharmacology Evaluation of GS-5816 Administered by Oral Gavage to Male Telemetry-Instrumented Conscious Dogs	PC-281-2003
Central Nervous System Safety Pharmacology Evaluation of GS- 5816 following Oral Administration to Male Rats	PC-281-2004
Respiratory Safety Pharmacology Evaluation Using Head-Out Plethysmography of GS-5816 following Oral Administration to Male Rats	PC-281-2005
Effect of GS-5816 on Cloned hERG Potassium Channels Expressed in	PC-281-2006

Human Embryonic Kidney Cells	
ADME/Pharmacokinetics	
Absorption	
Pharmacokinetics of GS-5816 in Sprague-Dawley Rats	AD-281-2002
Pharmacokinetics of GS-5816 in Beagle dogs	AD-281-2003
Pharmacokinetics of GS-5816 in Cynomolgus Monkeys	AD-281-2004
Pharmacokinetics of GS-5816 Following Single Ascending Oral Doses in Beagle Dogs	AD-281-2013
Pharmacokinetics of GS-5816 Following Single Ascending Oral Doses in Sprague-Dawley Rats	AD-281-2014
Pharmacokinetics of GS-5816 Following Single Oral Doses in Various Formulations in Sprague-Dawley Rats	AD-281-2020
Pharmacokinetics of GS-5816 Following Single Ascending Oral Doses to CD-1 Mice	AD-281-2028
Pharmacokinetics of GS-5816 Following Single Ascending Oral Doses in Female New Zealand White Rabbits	AD-281-2032
Pharmacokinetics of GS-5816 Following Single Ascending Oral Doses to 001178-W (wild type) Mice	AD-281-2034
Pharmacokinetics of GS-5816 Following a Single Oral Dose in Various Formulations to Rabbits	AD-281-2035
Pharmacokinetics of GS-5816 Following Single Ascending Oral Doses in Solution to Rabbits	AD-281-2036
Distribution	
In Vitro Protein Binding Determination of GS-5816 by Equilibrium Dialysis	AD-281-2001
Pharmacokinetics, Absorption, Distribution, and Excretion of ¹⁴ C-GS-5816 following Oral Administration to Rats	AD-281-2018
Pharmacokinetics, Absorption, Distribution, and Excretion of ¹⁴ C-GS-5816 Following Oral Administration to Mice	AD-281-2021
In Vitro Human Plasma Protein Binding Determination of GS-5816 by Equilibrium Dialysis	AD-281-2029
Placental Transfer and Lacteal Excretion of ¹⁴ C-GS-5816 Following Administration of a Single Oral Dose to Pregnant and Lactating Rats	AD-281-2031
In Vitro CD-1 Mouse Plasma Protein Binding Determination of GS-5816 by Equilibrium Dialysis	AD-281-2037
Pharmacokinetics of GS-5816 in Female New Zealand White Rabbits	AD-281-2038
Metabolism	
In Vitro Metabolic Stability of GS-5816 in Hepatic Subcellular Fractions from Human, Dog, Rat and Monkey and in Cryopreserved Human Hepatocytes	AD-281-2006
Cytochrome P450 Metabolic Reaction Phenotyping of GS-5816	AD-281-2007
In Vitro Metabolic Stability of GS-5816 in Hepatic Subcellular Fraction from CD-1 Mice	AD-281-2039
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Rats After Oral Administration of ¹⁴ C-	AD-281-2019

GS-5816	
Profiling and Identification of Metabolites in Selected Plasma, Urine, and Feces Samples from Mice After Oral Administration of ¹⁴ C-GS-5816	AD-281-2022
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Dogs After Oral Administration of ¹⁴ C-GS-5816	AD-281-2024
Excretion	
Pharmacokinetics of GS-5816 in Bile-Duct Cannulated Rats	AD-281-2005
Pharmacokinetics, Absorption, Distribution, and Excretion of ¹⁴ C-GS-5816 Following Oral Administration to Mice	AD-281-2021
Pharmacokinetics, Absorption, and Excretion of ¹⁴ C-GS-5816 Following Oral Administration to Intact and Bile Duct-Cannulated Dogs	AD-281-2023
Pharmacokinetic Drug Interactions	
In Vitro Assessment of Human Liver Cytochrome P450 Inhibition Potential of GS-5816	AD-281-2008
In Vitro Assessment of Induction Potential of GS-5816 in Humans	AD-281-2009
General Toxicology	
Single Dose Toxicity-None Submitted	
Repeat Dose Toxicity	
4-Week Oral Gavage Dose Range-Finding Toxicity and Toxicokinetic Study with GS-5816 in Model 001178-W (Wild-Type), CByB6F1-Tg(HRAS) ^{2Jic} Mice	TX-281-2028
5-Day Oral Gavage Toxicity and Toxicokinetic Study with GS-5816 in Male Rats	TX-281-2001
2-Week Oral Gavage Toxicity and Toxicokinetic Study with GS- 5816 in Rats with a 1-Week Recovery and a Micronucleus Assessment	TX-281-2003
2-Week Oral Gavage Toxicity and Toxicokinetic Study with GS- 5816 in Dogs with a 1-Week Recovery	TX-281-2004
26-Week Oral Gavage Toxicity and Toxicokinetic Study with GS- 5816 in Rats with a 13-Week Interim Necropsy and a 4-Week Recovery Phase	TX-281-2007
39-Week Oral Gavage Toxicity and Toxicokinetic Study with GS- 5816 in Dogs with a 13-Week Interim Necropsy and a 4-Week Recovery	TX-281-2008
Genetic Toxicology	
Bacterial Reverse Mutation Assay with a Confirmatory Assay with GS-5816	TX-281-2005
Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes with GS-5816	TX-281-2006
2-Week Oral Gavage Toxicity and Toxicokinetic Study with GS- 5816 in Rats with a 1-Week Recovery and a Micronucleus Assessment	TX-281-2003
Reproductive and Developmental Toxicology	
Oral Gavage Dose Range-finding Developmental Toxicity and Toxicokinetics Study with GS-5816 in Rats	TX-281-2009

Oral Gavage Dose Range-finding Developmental Toxicity and Toxicokinetics Study with GS-5816 in Rabbits	TX-281-2010
Oral Gavage Study of Fertility and Early Embryonic Development to Implantation with GS-5816 in Rats	TX-281-2012
Oral Gavage Study for Effects on Embryo-Fetal Development with GS-5816 in Rats	TX-281-2013
Oral Gavage Study for Effects on Embryo-fetal Development and Toxicokinetic with GS-5816 in Rabbits	TX-281-2014
Oral Gavage Study for Effects on Embryo-fetal Development and Toxicokinetic with GS-5816 in CD-1 Mice	TX-281-2032
An Oral (Gavage) Study of the Effects of GS-5816 on Pre- and Postnatal Development, Including Maternal Function in Rats	TX-281-2027
Local Tolerance	
GS-5816: The Bovine Corneal Opacity and Permeability Assay (BCOP)	TX-281-2039
GS-5816: Skin Irritation to the Rabbit	TX-281-2040
Antigenicity	
GS-5816: Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (Individual animal approach)	TX-281-2041
Impurities	
Bacterial Reverse Mutation Assay Plate Incorporation Method with (b) (4)	TX-281-2033
2-Week Oral Gavage Qualification Toxicity and Toxicokinetic Study with GS-5816 in Rats	TX-281-2042
Other	
Neutral Red Uptake Phototoxicity Assay of GS-5816 in Balb/c 3T3 Mouse Fibroblasts	TX-281-2015
A Multiple Dose Phototoxicity Study to Determine the Effects of Oral Administration of GS-5816 on Skin in Pigmented Rats	TX-281-2016

3.2 Studies Not Reviewed

Study Title	Study #
Secondary Pharmacodynamics	
Lead Profiling Screen Study of GS-589916	PC-281-2001
Pharmacodynamic Drug Interactions	
In Vitro Assessment of GS-5816 Inhibition of Human OATP1B1, OATP1B3, Pgp and BCRP	AD-281-2010
In Vitro Assessment of GS-5816 as a Substrate for Human OATP1B1 and OATP1B3	AD-281-2011
In Vitro Assessment of GS-5816 Inhibition of Human MRP2, BSEP, and NTCP	AD-281-2012
In Vitro Assessment of GS-5816 Inhibition of Human OATP1B1, OATP1B3, Pgp and BCRP	AD-281-2010

In Vitro Assessment of Human UGT1A1 Inhibition Potential of GS-5816	AD-281-2016
Evaluation of Induction Potential of GS-5816 in Cultured Human Hepatocytes	AD-281-2025
Studies to Determine if GS-5816 is an Inhibitor of OCT1, OCT2, MATE1, OAT1, and OAT3 or Substrate for OCT1	AD-281-2026
In Vitro Inhibition Studies of GS-5816 with Human OATP1A2 and OATP2B1 Transporters	AD-281-2040
Effect of P-glycoprotein and BCRP Expression on GS-5816 Accumulation In Vitro	AD-281-2041

3.3 Previous Reviews Referenced

GS-5816 nonclinical safety studies, including safety pharmacology, ADME, repeat-dose toxicology, genetic toxicology, and reproductive toxicology studies have been reviewed by Dr. Pritam Verma, and are summarized (as appropriate) in sections of this review, with complete reviews of pivotal studies included within the review text. SOF was reviewed under NDA 204,671.

4 Pharmacology

4.1 Primary Pharmacology

VEL

VEL inhibits HCV replication by interfering with the viral NS5A protein, showing antiviral activity against HCV genotypes 1 to 6 with mean EC₅₀ values ranging from 0.002 to 0.13 nM.

SOF/VEL

The combination of SOF and VEL exhibit additive antiviral activity and lack cross-resistance making SOF/VEL FDC a favorable therapy for treatment of HCV in the clinic. Additional complete details of the pharmacodynamics of VEL and SOF/VEL can be found in the clinical virology review.

4.2 Secondary Pharmacology

VEL

In Vitro Receptor Binding of GS-589916 (PC-281-2001)

VEL was evaluated against a panel of mammalian enzymes, ion channels, and receptors for potential off-target activity, and no significant responses were observed at 10 μM VEL.

Cellular Cytotoxicity Evaluation of GS-5816 in Multiple Cell Lines (PC-281-2014)

The cytotoxicity of GS-5816 was evaluated in two hepatic cell lines (Huh7 and HepG2), a prostate carcinoma cell line (PC-3), a lymphoma cell line (MT-4), and normal lung fibroblasts (MRC-5) following five days of VEL exposure. GS-5816 had no observed cellular cytotoxicity (> 44,444 nM) in four of the five tested cell lines. CC₅₀

value in PC-3 cells was 4,028 nM, which is similar to that observed for other NS5A inhibitors. Overall, VEL has low cellular cytotoxicity.

SOF/VEL

Due to the low potential for off-target activity and cytotoxicity by SOF (NDA 204671) and VEL and no significant changes in cell viability when combined up to concentrations of 320/ 0.064 nM (SOF/VEL), no additional secondary pharmacology studies were deemed necessary.

4.3 Safety Pharmacology

VEL

Effects of GS-5816 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (PC-281-2006)

To examine the *in vitro* effect of GS-5816 on potassium ion channels, GS-5816 at concentrations of 3 and 6.5 μ M were evaluated on the hERG (human ether-à-go-go-related gene) channel current. Test concentrations met the appropriate acceptance criteria. GS-5816 inhibited hERG current by $0.7 \pm 0.3\%$ at 3 μ M ($n = 3$) and $0.9 \pm 1.0\%$ at 6.5 μ M ($n = 3$) versus $0.7 \pm 0.7\%$ ($n = 4$) in control. Testing was conducted at these concentrations because GS-5816 was insoluble at higher concentrations. hERG inhibition was not statistically significant compared to vehicle control. Due to the solubility limitations, the IC_{50} for the inhibitory effect of GS-5816 on hERG potassium current was estimated to be greater than 6.5 μ M. Under identical conditions, the positive control (60 nM terfenadine) inhibited hERG potassium current by $80.7 \pm 1.8\%$ ($n = 2$), confirming the sensitivity of the test system.

Cardiovascular Safety Pharmacology Evaluation of GS-5816 Administered by Oral Gavage to Male Telemetry-Instrumented Conscious Dogs (PC-281-2003)

To evaluate the potential cardiovascular effects of GS-5816, male Beagle dogs ($n=4$) were administered a single dose via oral gavage using a Latin square design on days 1, 8, 15, and 22. Each dog received vehicle or GS-5816 at doses of 5, 20 and 100 mg/kg. No GS-5816-related mortality, morbidity, or effects on body weight/food consumption were noted. Assessments of cardiovascular function were based on hemodynamic and electrocardiographic (ECG) parameters where telemetry data was continuously recorded for at least 90 minutes prior to dosing and at least 25 hours post-dose. Plasma levels at 4.5 hours postdose confirmed all dogs were exposed to test article. No GS-5816 related effects were noted on qualitative or quantitative ECG or hemodynamic parameters. Based on these results, the NOEL for cardiovascular parameters was 100 mg/kg.

Central Nervous System Safety Pharmacology Evaluation of GS-5816 following Oral Administration to Male Rats (PC-281-2004)

To evaluate the neurological effects of GS-5816, thirty-two male Hsd:Sprague Dawley (SD) rats were randomized to four groups (eight rats/group) and administered a single dose by oral gavage of vehicle or GS-5816 at dose levels of 20, 60, or 200 mg/kg.

Neurological assessments were collected predose and 1, 3, 5, 8, and 24 hours postdose in a modified Irwin battery, including home cage, hand-held, open-field, and elicited response observations. No effects related to GS-5816 were observed for clinical signs or body temperature. No neurological effects related to GS-5816 were evident during the modified Irwin observational battery. Based on these results, the NOEL for neurological function in rats was 200 mg/kg.

Respiratory Safety Pharmacology Evaluation of GS-5816 Using Head-Out Plethysmography following Oral Gavage Administration to Male Rats (PC-281-2005)

To evaluate the respiratory effects of GS-5816, thirty-two male SD rats were randomized to four groups (eight rats/group) and administered a single dose by oral gavage of vehicle or GS-5816 at dose levels of 20, 60, and 200 mg/kg. Plethysmography data, including tidal volume, respiration rate, and minute volume, were collected continuously for at least 2.5 hours 2 days prior to dosing, for at least 6.5 hours from 2 to 8 hours postdose, and for at least 2.5 hours beginning approximately 22.5 hours postdose. Plethysmography parameters were analyzed as 30-minute averages at baseline, from 2 through 8 hours postdose, and at 24 hours postdose. All rats survived until the scheduled sacrifice and no mortality, morbidity, or signs of toxicity were observed. No GS-5816-related changes in respiration rate, tidal volume, or minute volume were observed. Based on these results, the NOEL for respiratory function in rats was 200 mg/kg.

SOF/VEL

No combination safety pharmacology studies were performed as neither agent had biologically meaningful vital organ effects alone. Thus, the combination is unlikely to have significant effects on the respiratory, CNS, or cardiovascular systems.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

Mice:

Pharmacokinetics of GS-5816 Following Single Ascending Oral Doses to CD-1 Mice (AD-281-2028)

To evaluate the plasma exposure of GS-5816, CD-1 mice were administered single ascending oral doses of GS-5816 in a suspension formulation at 30, 100, 300 and 1000 mg/kg. The concentration of GS-5816 in plasma was determined by an LC/MS/MS method. The exposure to GS-5816 in CD-1 mouse plasma increased with dose from 30 to 1000 mg/kg (sponsor's table below). The increase in C_{max} and AUC₀₋₂₄ was less than dose proportional between the 30 to 1000 mg/kg doses.

Table 2: Mean Pharmacokinetic Parameters of GS-5816 after a Single Oral Gavage Dose to CD-1 Male Mice

Dose (mg/kg)	C _{max} (µg/mL)	AUC ₀₋₂₄ (µg·h/mL)
30	4.01	37.1
100	7.43	81.7
300	10.5	176
1000	17.4	249

Pharmacokinetics of GS-5816 Following Single Ascending Oral Doses to 001178-W (wild type) Mice (AD-281-2034)

To determine the systemic exposure of GS-5816 administered as a single dose via oral gavage to 001178-W (wild type) mice, 9 animals/group/sex were dosed 100, 300, and 1000 mg/kg of GS-5816. Blood samples were collected from three animals/group/time point at approximately 1, 2, 4, 8, 12, and 24 hours postdose. Plasma samples were assayed via LC/MS/MS method. Exposure to GS-5816 in 001178-W wild type mouse plasma increased with the increase in dose level from 100 to 1000 mg/kg (sponsor's table below). The increases in C_{max} and AUC₀₋₂₄ were less than dose proportional between the 100 to 1000 mg/kg doses. Gender-based differences were less than 2-fold in GS-5816 C_{max} and AUC₀₋₂₄ values.

Table 3: Mean Pharmacokinetic Parameters of GS-5816 after a Single Oral Gavage Dose to 001178-W (wild type) Mice

Dose (mg/kg)	Sex	C _{max} (µg/mL)	AUC ₀₋₂₄ (µg·h/mL)
100	Male	8.57	107
100	Female	11.5	112
300	Male	9.96	156
300	Female	11.1	180
1000	Male	14.0	223
1000	Female	17.2	266

Rats:

Pharmacokinetics of GS-5816 Following Single Ascending Oral Doses in Sprague-Dawley Rats (AD-281-2014)

GS-5816 was administered orally to male Sprague-Dawley (SD) rats at 10, 30, 75, 150, 300, and 600 mg/kg (3/group). Results are shown in sponsor's table below. The systemic plasma exposure of GS-5816 in SD rats increased with doses between 10 to 300 mg/kg. The increase in plasma exposure was approximately proportional with dose between 10 to 150 mg/kg, but was less than proportional with dose between 150 and 300 mg/kg. The plasma exposure of GS-5816 decreased slightly from 300 and 600 mg/kg, revealing a point of exposure saturation in rats around the 300 mg/kg dose.

Table 4: Pharmacokinetic Parameters of GS-5816 Following Single Ascending Oral Doses in SD Rats

Dosage (mg/kg)	AUC _(0-24hr)		C _{max}		T _{max} (hr)
	(μM•hr)	(μg•hr/mL)	(μM)	(μg/mL)	
10	1.65 ± 0.13	1.46 ± 0.11	0.22 ± 0.05	0.19 ± 0.04	2.67 ± 1.15
30	6.43 ± 1.65	5.68 ± 1.46	0.58 ± 0.12	0.51 ± 0.11	4.00 ± 0.00
75	10.8 ± 1.1	9.54 ± 0.96	1.13 ± 0.35	1.00 ± 0.31	4.00 ± 0.00
150	19.3 ± 2.5	17.0 ± 2.24	1.48 ± 0.07	1.31 ± 0.06	6.00 ± 0.00
300	27.3 ± 9.3	24.1 ± 8.2	1.84 ± 0.64	1.62 ± 0.57	10.0 ± 3.46
600	21.2 ± 2.1	18.7 ± 1.8	1.41 ± 0.26	1.25 ± 0.23	10.7 ± 2.31

Pharmacokinetics of GS-5816 Following Single Oral Doses in Various Formulations in Sprague-Dawley Rats (AD-281-2020)

In order to determine the optimal formulation for development, GS-5816 was administered orally at 10 and 30 mg/kg to Sprague-Dawley (SD) rats in 3 different formulations (3 males/group). Results are shown in sponsor's table below.

Table 5: Pharmacokinetic Parameters of GS-5816 in SD Rats Following Single Oral Doses of GS-5816 in Differing Formulations

	GS-5816 Formulation					
	Formulation 1 ^a		Formulation 2 ^b		Formulation 3 ^c	
Dosage (mg/kg)	10	30	10	30	10	30
C _{max} (nM)	223 ± 52	581 ± 125	106 ± 13	410 ± 51	218 ± 57	549 ± 112
T _{max} (hr)	2.7 ± 1.2	4.0 ± 3.5	7.3 ± 4.2	8.7 ± 3.1	4.7 ± 1.2	4.7 ± 1.2
AUC _(0-last) (nM•hr)	1648 ± 126	6431 ± 1654	1155 ± 413	4943 ± 1720	1649 ± 351	4676 ± 979
AUC _(0-∞) (nM•hr)	1722 ± 145	6475 ± 1665	1811 ± 1289	4978 ± 1737	1706 ± 322	4706 ± 995

- a Formulation 1 -
- b Formulation 2 -
- c Formulation 3 -

(b) (4)

Pharmacokinetics of IV/Oral GS-5816 in Sprague Dawley Rats (AD-281-2002)

GS-5816 was administered intravenously (IV) by a 30-minute infusion at 1 mg/kg and orally at 2 mg/kg to Sprague Dawley (SD) male rats (3/group). Results are shown in sponsor's table below. The systemic plasma clearance for IV GS-5816 in SD rats was low (0.94 L/hr/kg), and the volume of distribution (1.61 L/kg) was greater than total body water volume. The absolute oral bioavailability was 27.7% on average.

Table 6: Pharmacokinetic Parameters of GS-5816 Following Intravenous and Oral Administration of GS-5816 to Male Rats

Parameter	Route of Administration	
	IV Infusion	Oral
Dosage (mg/kg)	1.0	2.0
T _{max} (hr)	0.48 ± 0.0	1.0 ± 0.0
C _{max} (nM)	1210 ± 433	116 ± 53
t _{1/2} (hr)	2.36 ± 0.26	2.33 ± 0.38
AUC _(0-last) (nM•hr)	1310 ± 239	670 ± 435
AUC _(0-∞) (nM•hr)	1320 ± 240	709 ± 478
MRT (hr)	1.72 ± 0.18	—
CL (L/h/kg)	0.94 ± 0.19	—
V _{ss} (L/kg)	1.61 ± 0.31	—
F (%)	—	27.7 ± 18.7

Dogs:

Pharmacokinetics of GS-5816 Following Single Ascending Oral Doses in Beagle Dogs (AD-281-2013)

GS-5816 was administered orally to male beagle dogs at 3, 10, 30, 100 and 200 mg/kg (3/group). The plasma exposure of GS-5816 increased with dose between 3 and 100 mg/kg. The increase in GS-5816 plasma exposure was approximately proportional with doses between 3 and 30 mg/kg, but was less than proportional with doses between 30 to 100 mg/kg. The plasma exposure of GS-5816 decreased slightly between 100 to 200 mg/kg, revealing a point of exposure saturation in dogs around the 100 mg/kg dose.

Table 7: Pharmacokinetic Parameters of GS-5816 Following Single Ascending Oral Doses of GS-5816 to Beagle Dogs

Dosage (mg/kg)	AUC _(0-24hr)		C _{max}		T _{max} (hr)
	(µM•hr)	(µg•hr/mL)	(µM)	(µg/mL)	
3	1.64 ± 0.91	1.45 ± 0.80	0.289 ± 0.175	0.255 ± 0.155	3.33 ± 1.15
10	2.49 ± 0.41	2.20 ± 0.36	0.446 ± 0.165	0.394 ± 0.146	2.00 ± 0.0
30	11.7 ± 1.4	10.3 ± 1.24	1.10 ± 0.18	0.974 ± 0.162	10.0 ± 12.2
100	16.8 ± 1.6	14.8 ± 1.4	1.40 ± 0.147	1.24 ± 0.13	6.67 ± 4.62
200	9.79 ± 1.68	8.65 ± 1.49	1.30 ± 0.57	1.14 ± 0.51	10.7 ± 11.5

Pharmacokinetics of IV/Oral GS-5816 in Beagle Dogs (AD-281-2003)

GS-5816 was administered intravenously by a 30-minute infusion at 0.25 mg/kg and orally at 0.5 mg/kg to male beagle dogs (3/group). Results are shown in sponsor's table below. The systemic plasma clearance GS-5816 in beagle dogs was low (0.25 L/hr/kg),

and the volume of distribution (1.46 L/kg) was greater than total body water volume. The absolute oral bioavailability was 25.0% on average.

Table 8: Pharmacokinetic Parameters of GS-5816 Following IV and Oral Doses of GS-5816 to Male Beagle Dogs

Parameter	Route of Administration	
	IV Infusion	Oral
Dosage (mg/kg)	0.25	0.5
T _{max} (hr)	0.48 ± 0.0	1.3 ± 0.6
C _{max} (nM)	448 ± 157	71.3 ± 27.2
t _{1/2} (hr)	5.51 ± 0.46	9.1 ± 5.1
AUC _(0-last) (nM•hr)	931 ± 217	490 ± 252
AUC _(0-∞) (nM•hr)	1010 ± 187	585 ± 343
MRT (hr)	5.70 ± 0.70	-
CL (L/h/kg)	0.25 ± 0.04	-
V _{ss} (L/kg)	1.46 ± 0.43	-
F (%)	-	25.0 ± 12.9

Monkeys:

Pharmacokinetics of IV/Oral GS-5816 in Cynomolgus Monkeys (AD-281-2004)

GS-5816 was administered intravenously by a 30-minute infusion at 0.5 mg/kg and orally at 1 mg/kg to male cynomolgus monkeys. Results are shown in sponsor's table below. The systemic plasma clearance for GS-5816 in cynomolgus monkeys was low (0.30 L/hr/kg), and the volume of distribution (1.58 L/kg) was greater than total body water volume. The absolute oral bioavailability was 29.7% on average.

Table 9: Pharmacokinetic Parameters of GS-5816 Following IV and Oral Doses of GS-5816 to Male Cynomolgus Monkeys

Parameter	Route of Administration	
	IV Infusion	Oral
Dosage (mg/kg)	0.5	1.0
T _{max} (hr)	0.40 ± 0.13	3.33 ± 1.15
C _{max} (nM)	897 ± 275	157 ± 25
t _{1/2} (hr)	4.18 ± 3.65	5.49 ± 0.20
AUC _(0-last) (nM•hr)	1960 ± 766	1220 ± 122
AUC _(0-∞) (nM•hr)	2080 ± 705	1280 ± 123
MRT (hr)	5.16 ± 1.21	-
CL (L/h/kg)	0.30 ± 0.09	-
V _{ss} (L/kg)	1.58 ± 0.62	-
F (%)	-	29.7 ± 2.8

Rabbits:**Pharmacokinetics of GS-5816 Following Single Ascending Oral Doses in Female New Zealand White Rabbits (AD-281-2032)**

GS-5816 was administered orally to female New Zealand white (NZW) rabbits (3 female/group) at 100, 300, 600, and 1000 mg/kg. The plasma exposure of GS-5816 increased with dose in the range of 100 to 600 mg/kg (sponsor's table below). The increase in exposure was less than proportional with dose from 100 to 600 mg/kg. There was no increase in exposure from 600 to 1000 mg/kg, suggesting saturation of absorption was reached at the 600 mg/kg dose.

Table 10: Pharmacokinetic Parameters of GS-5816 Following Single Ascending Oral Doses of GS-5816 to Female NZW Rabbits

Dosage (mg/kg)	PK Parameters			
	AUC _{0-24h} (ng•h/mL)	C _{max} (ng/mL)	T _{max} (h)	C _{24h} (ng/mL)
100 ^a	710 ± 216	133 ± 34	2.7 ± 1.2	7.6 ± 3.7
300 ^a	980 ± 305	115 ± 40	4.0 ± 2.0	23.0 ± 17.3
600 ^a	2870 ± NC	225 ± NC	8.0 ± NC	99 ± NC
1000 ^a	2080 ± 929	134 ± 50	6.7 ± 5.0	90.2 ± 41.1

a Vehicle consisted 0.5% hydroxyl propyl methylcellulose (HPMC), 0.1% Tween 20, 0.9% benzyl alcohol, and 98.5% water

Pharmacokinetics of GS-5816 Following a Single Oral Dose in Various Formulations to Rabbits (AD-281-2035)

GS-5816 in 3 different formulations (600 mg/kg) was administered orally to female NZW Rabbits (4 females/group). The plasma exposure of GS-5816 in rabbits is shown in the sponsor's table below.

Table 11: Pharmacokinetic Parameters of GS-5816 in Female NZW Rabbits Following a Single Oral Dose of GS-5816 (600 mg/kg) in Various Formulations

Formulation	PK Parameters			
	AUC _{0-24h} (ng•h/mL)	C _{max} (ng/mL)	T _{max} (h)	C _{24h} (ng/mL)
A ^a	987 ± 228	61.3 ± 12.1	10.0 ± 9.4	51.5 ± 15.6
B ^b	2150 ± 809	148 ± 40	5.5 ± 1.0	74.4 ± 26.4
C ^c	3280 ± 903	246 ± 75	4.0 ± 1.6	128 ± 48

a Formulation A consisted of

b Formulation B consisted of

c Formulation C consisted of

(b) (4)

Pharmacokinetics of GS-5816 Following Single Ascending Oral Doses in Solution to Rabbits (AD-281-2036)

GS-5816 was administered orally to female NZW rabbits at 100, 300, 600 mg/kg (3/group). The systemic exposure of GS-5816 in rabbit plasma increased with dose in

the range of 100 to 300 mg/kg, but did not increase further with dose from 300 to 600 mg/kg, suggesting saturation of absorption was reached at the 300 mg/kg dose.

Table 12: Pharmacokinetic Parameters of GS-5816 Following Single Ascending Oral Doses of GS-5816 to Female NZW Rabbits

Dosage (mg/kg)	PK Parameters			
	AUC _{0-24h} (ng•h/mL)	C _{max} (ng/mL)	T _{max} (h)	C _{24h} (ng/mL)
100 ^a	904 ± 595	135 ± 59.4	2.3 ± 1.5	8.42 ± NC
300 ^a	2640 ± 3360	240 ± 265	4.0 ± 2.0	40.5 ± 47.5
600 ^a	1980 ± 703	239 ± 176	4.7 ± 1.2	57.5 ± 21.6

a Dosing vehicle consisted of 25% Solutol HS-15 and 75% propylene glycol.

Distribution

In Vitro Assessments:

In Vitro CD-1 Mouse Plasma Protein Binding Determination of GS-5816 by Equilibrium Dialysis (AD-281-2037)

Equilibrium dialysis was conducted at 37°C using CD-1 mouse plasma spiked with GS-5816 at final concentrations of 2 µM. The dialysis was performed in duplicate for 3 hr. CD-1 mouse plasma protein binding of GS-5816 was high; less than 0.1% of GS-5816 was free under the tested conditions (sponsor's table below).

Table 13: Protein Binding of GS-5816 in CD-1 Mouse Plasma

Matrix	Conc. (µM) ^a	Free Fraction (%) ^b	Bound (%) ^b	Study
CD-1 mouse Plasma	2.00	<0.1	>99.9	Gilead# 140416-454

a: Initial concentration in protein-containing dialysis cell

b: Mean ± Standard Deviation (n = 2)

In Vitro Human Plasma Protein Binding Determination of GS-5816 by Equilibrium Dialysis (AD-281-2029)

Equilibrium dialysis was conducted at 37°C using human plasma spiked with GS-5816 at final concentrations of 0.1 µM, 0.25 µM, 0.5 µM, 1 µM, and 2 µM. The dialysis was performed in duplicate for 3 hr. The human plasma protein binding of GS-5816 was high; less than 0.5% of GS-5816 was free under all tested conditions (sponsor's table below).

Table 14: Protein Binding of GS-5816 in Human Plasma at Different GS-5816 Concentrations

Matrix	Conc. (μM) ^a	Free Fraction (%) ^b	Bound (%) ^b	Study
Human Plasma	2.00	0.47 \pm 0.00	99.53 \pm 0.00	Gilead# 131114-428
Human Plasma	1.00	0.38 \pm 0.01	99.62 \pm 0.01	
Human Plasma	0.50	0.29 \pm 0.07	99.71 \pm 0.07	
Human Plasma	0.25	0.29 \pm 0.08	99.71 \pm 0.08	
Human Plasma	0.10	0.49 \pm 0.09	99.51 \pm 0.09	

a: Initial concentration in protein-containing dialysis cell

b: Mean \pm Standard Deviation (n = 2)

In Vitro Protein Binding Determination of GS-5816 by Equilibrium Dialysis (AD-281-2001)

Protein binding of GS-5816 in Sprague-Dawley rat, beagle dog, cynomolgus monkey, rhesus monkey, and human plasma were assessed by equilibrium dialysis. Equilibrium dialysis was conducted at 37°C at a final concentration of 2 μM . The relative protein binding of GS-5816 to cell culture medium (CCM) and human plasma was determined by direct competitive dialysis. The extent of plasma protein binding of GS-5816 was high; less than 0.5% free fraction in all species tested (sponsor's table below).

Table 15: Protein Binding of GS-5816 in Plasma from Different Species

Matrix	Conc. (μM) ^a	Free Fraction (%) ^b	Study
Human Plasma	2	0.30 \pm 0.02	(b) (4) = 60D-1140
Beagle Dog Plasma	2	0.19 \pm 0.02	
Sprague-Dawley Rat Plasma	2	0.22 \pm 0.03	
Cynomolgus Monkey Plasma	2	0.41 \pm 0.07	
Rhesus Monkey Plasma	2	0.28 \pm 0.01	

a: Initial concentration in protein-containing dialysis cell

b: Mean \pm Standard Deviation (n = 3)

Using competitive equilibrium dialysis between CCM containing 10% fetal bovine serum and 100% human plasma, the ratio of GS-5816 bound to CCM vs. human plasma was 51.9 % (sponsor's table below).

Table 16: Relative Protein Binding of GS-5816 in CCM vs. Human Plasma

Matrices	Conc. (μM) ^a	Ratio ^b	Mean Ratio	Study
CCM versus Human Plasma	2	49.06 \pm 2.66	51.89 \pm 5.47	Gilead# 110912-307
		54.71 \pm 7.12		Gilead# 110928-312

a: Initial concentration in each dialysis cell

b: Corrected final concentration in plasma / corrected final concentration in CCM; Mean \pm Standard Deviation, (n = 2)

Mice:

Pharmacokinetics, Absorption, Distribution, and Excretion of ¹⁴C-GS-5816 Following Oral Administration to Mice (AD-281-2021)

The absorption, distribution, and excretion of radioactivity were determined after administration of a single oral dose (target 20 mg/kg) of ¹⁴C-GS-5816 to male CD-1 (n=43) and rasH2 transgenic (n=15) mice. In CD-1 mice, the highest mean concentrations of radioactivity in blood and plasma 2 hours postdose were 3420 and 6840 ng ¹⁴C-GS-5816/g, respectively. The highest mean concentrations of radioactivity in blood and plasma from male rasH2 transgenic mice 3 hours postdose were 2030 and 4130 ng equivalents ¹⁴C-GS-5816/g, respectively (sponsor's table below).

Table 17: Pharmacokinetic Parameters for ¹⁴C-GS-5816 in Blood and Plasma From CD-1 and rasH2 Transgenic Mice After a Single Administration

Matrix	T _{max} (hours)	C _{max} (ng eq/g)	t _{1/2} (hours)	AUC ₀₋₂₄ (ng eq·hours/g)	AUC ₀₋₁₆₈ (ng eq·hours/g)
<u>Group 2 (CD-1)</u>					
Blood	2.00	3420	3.30	24982	25183
Plasma	2.00	6840	3.23	50141	50502
<u>Group 4 (rasH2 transgenic)</u>					
Blood	3.00	2030	N.C.	11614	N.C.
Plasma	3.00	4130	N.C.	24960	N.C.

eq Equivalents ¹⁴C-GS-5816.

N.C. Not calculated due to insufficient data in the elimination phase.

¹⁴C-GS-5816-derived radioactivity was widely distributed and reached maximum concentration by 3 hours postdose. The tissues showing the highest concentrations of radioactivity included gall bladder, liver, kidney cortex, Harderian gland, kidneys, and kidney medulla.

¹⁴C-GS-5816-derived radioactivity was rapidly excreted, primarily in feces, within the first 24 hours after dosing. By 168 hours postdose, CD-1 mice had excreted 95.9 and 0.262% of the administered ¹⁴C-GS-5816 in feces and urine, respectively. The average overall recovery of radioactivity after oral dosing to male CD-1 mice was 96.5%.

Rats:**Pharmacokinetics, Absorption, Distribution, and Excretion of ¹⁴C-GS-5816 following Oral Administration to Rats (AD-281-2018)**

The absorption, distribution, and excretion of a single oral dose (target 30 mg/kg) of ¹⁴C-GS-5816 to male bile duct-intact, bile duct-cannulated (SD), and intact Long Evans (LE) rats was evaluated for 72 hrs for blood/plasma analysis and out to 168 hrs for sample/tissue collection. SD/LE rats reached maximum concentrations in the blood by 4 hours postdose. Radioactivity in blood and plasma declined 12 hours to BLQ and remained 72 hours postdose. The elimination half-life of radioactivity in blood and plasma was approximately 2.9 hours. ¹⁴C-GS-5816-derived radioactivity was widely distributed to most tissues by 4 hours postdose in both rats. The tissues showing the highest maximum concentrations of radioactivity included liver, adrenal glands, kidney medulla, kidney(s), kidney cortex, small intestine, and pancreas. No radioactivity was detected in the brain, suggesting ¹⁴C-GS-5816-derived radioactivity didn't cross the blood: brain barrier. Radioactivity was eliminated from all tissues by 48 hours, with the exception of Harderian gland, eye uveal tract, and preputial gland. At 168 hours postdose, radioactivity was observed in eye uveal tract only.

Distribution trends in the pigmented uveal tract of the eye and pigmented skin suggested that ¹⁴C-GS-5816-related radioactivity was associated with the melanin-containing tissues in the pigmented rat, but was not irreversibly bound to melanin. The estimated absorbed dose in uveal tract of the eye was 25.2 mRad, representing 0.503% of the allowable 5000 mrem exposure limit.

Most of the radioactivity was excreted after oral administration by 24 hours postdose (approximately 91 and 92% in intact and bile duct-cannulated rats, respectively). Most of the absorbed dose was excreted in the bile. Based on the radioactivity excreted in urine and bile after oral administration, a minimum of approximately 14% of the orally administered dose was absorbed.

Placental Transfer and Lactal Excretion of ¹⁴C-GS-5816 Following Administration of a Single Oral Dose to Pregnant and Lactating Rats (AD-281-2031)

The placental transfer and lactal excretion were determined after administration of a single oral dose (target 30 mg/kg) of ¹⁴C-GS-5816 to lactating and pregnant rats. After dosing, radioactivity in tissues from pregnant rats was quantitated by whole-body autoradiography and concentrations of radioactivity in blood, plasma, and milk were determined at selected times through 24 hours postdose.

For animals dosed on Gestation Day 13 (18), ¹⁴C-GS-5816-derived radioactivity was distributed to most of the maternal tissues by 1 hour postdose, and reached maximum concentration by 2 (4) hours postdose. The maternal tissues showing the highest maximum concentrations of radioactivity included liver, adrenal gland, small intestine, kidney cortex, cecum, kidney(s), and kidney medulla (and pancreas).

After oral administration to lactating rats, the peak mean concentrations of radioactivity in blood and plasma were 457 and 742 ng ¹⁴C-GS-5816/g, respectively, observed at 2 hours postdose. The peak mean concentration of radioactivity in milk was observed at 4 hours postdose (see sponsor's table).

Table 18: Pharmacokinetic Parameters for Radioactivity in Blood, Plasma, and Milk From Lactating Rats After Single Oral Dose of ¹⁴C-GS-5816 (30 mg/kg)

Matrix	T _{max} (hours)	C _{max} (ng eq/g)	t _{1/2} (hours)	AUC ₀₋₄ (ng eq-hours/g)	AUC _{0-∞} (ng eq-hours/g)
Blood	2	457	2.01	1959	NC
Plasma	2	742	1.85	3198	NC
Milk	4	988	NC	5553	NC

eq Equivalents ¹⁴C-GS-5816.
 NC Not calculated due to insufficient data.

Rabbits:

Pharmacokinetics of GS-5816 in Female NZW Rabbits (AD-281-2038)

GS-5816 was administered intravenously via a 30-minute infusion at 5 mg/kg to 4 female NZW rabbits. The concentrations of GS-5816 in rabbit plasma were determined with an LC/MS/MS method. The systemic plasma clearance (CL) of GS-5816 was 0.44 L/h/kg and the volume of distribution (V_{ss}) was 1.55 L/kg following a 30-min IV infusion (sponsor's table below).

Table 19: Pharmacokinetic Parameters of GS-5816 Following IV Administration to Female NZW Rabbits

Parameter	Route of Administration
	IV Infusion
Dosage (mg/kg)	5.0
T _{max} (h)	0.48 ± 0.00
C _{max} (ng/mL)	8940 ± 614
AUC _{0-24h} (ng·h/mL)	11600 ± 2380
AUC _{inf} (ng·h/mL)	11800 ± 2560
t _{1/2} (h)	5.05 ± 1.14
MRT (h)	3.64 ± 0.78
CL (L/h/kg)	0.44 ± 0.09
V _{ss} (L/kg)	1.55 ± 0.22

Metabolism

In Vitro Assessments:

In Vitro Metabolic Stability of GS-5816 in Hepatic Microsomal Fraction from CD-1 Mouse (AD-281-2039)

The metabolic stability of GS-5816 was assessed *in vitro* in pooled hepatic microsomal fractions from CD-1 mouse. GS-5816 showed a moderately low, indicating low hepatic clearance in mouse (see sponsor's table).

Table 20: In Vitro Rate of Metabolism of GS-5816 in Mice Hepatic Microsomes

Species	$t_{1/2}$ (min)	Predicted Hepatic CL (L/hr/kg)	Predicted Hepatic Extraction (%)
Mouse	272	0.98	18.2

Cytochrome P450 Metabolic Reaction Phenotyping of GS-5816 (AD-281-2007)

To determine if specific cytochrome P450 (CYP450) enzymes have the potential to metabolize GS-5816, GS-5816 was incubated with seven individual cDNA expressed human CYP450 enzyme preparations co-expressed with human NADPH CYP450 reductase. Compounds known to be metabolized by each CYP450 enzyme were used as controls. GS-5816 was not a substrate for recombinant CYP1A2, CYP2C9, CYP2C19, or CYP2D6. Metabolism of GS-5816 with CYP2B6, CYP2C8 and CYP3A4 was detectable but comparatively slow, so inhibitors or inducers of these seven major CYP enzymes should not affect the pharmacokinetics of GS-5816 in a clinically significant manner.

In Vitro Metabolic Stability of GS-5816 in Hepatic Subcellular Fractions from Human, Dog, Rat and Monkey and in Cryopreserved Human Hepatocytes (AD-281-2006)

The metabolic stability of GS-5816 was assessed *in vitro* in pooled hepatic microsomal fractions from SD rats, beagle dogs, cynomolgus monkeys and humans via the disappearance rate of GS-5816. The stability was also assessed in cryopreserved human hepatocytes. The predicted hepatic clearance was low in cryopreserved human hepatocytes and hepatic microsomes (all species; sponsor's tables below).

Table 21: In Vitro Rate of Metabolism of GS-5816 in Cryopreserved Human Hepatocytes

Species	$t_{1/2}$ (h)	Predicted Hepatic Cl (L/hr/kg)	Predicted Hepatic Extraction (%)
Human	> 39.5	< 0.07	< 5.1

Table 22: In Vitro Rate of Metabolism of GS-5816 in Hepatic Microsomes

Species	t _{1/2} (min)	Predicted Hepatic Cl (L/hr/kg)	Predicted Hepatic Extraction (%)
Rat	192	0.743	17.7
Dog	163	0.369	20.5
Monkey	> 395	< 0.17	< 10.6
Human	> 395	< 0.17	< 12.7

Profiling and Identification of Metabolites in Selected Plasma, Urine, and Feces Samples from Mice After Oral Administration of ¹⁴C-GS-5816 (AD-281-2022)**Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Rats After Oral Administration of ¹⁴C-GS-5816 (AD-281-2019)****Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Dogs After Oral Administration of ¹⁴C-GS-5816 (AD-281-2024)**

Unchanged VEL was the most abundant circulating component, accounting for > 71% of the total plasma exposure of VEL-derived radioactivity in mice, rats, dogs, and human subjects following oral administration (AD-281-2022, AD-281-2019, and AD-281-2024; and GS-US-281-1055). There were no major circulating metabolites in human plasma. Unchanged VEL was also the most abundant component in feces across species (mice, rats, dogs, and human subjects), accounting for > 63% of the administered dose, reflecting unabsorbed drug from the gastrointestinal tract or eliminated in bile. Unchanged parent drug accounted for 11.7% and 55.5% of the radioactivity recovered in bile from bile duct-cannulated rats and dogs, respectively. Several metabolites were identified, primarily formed via oxidation and/or O-demethylation. No unique metabolites were observed in human samples. The proposed biotransformation pathways are summarized in the sponsor's table below.

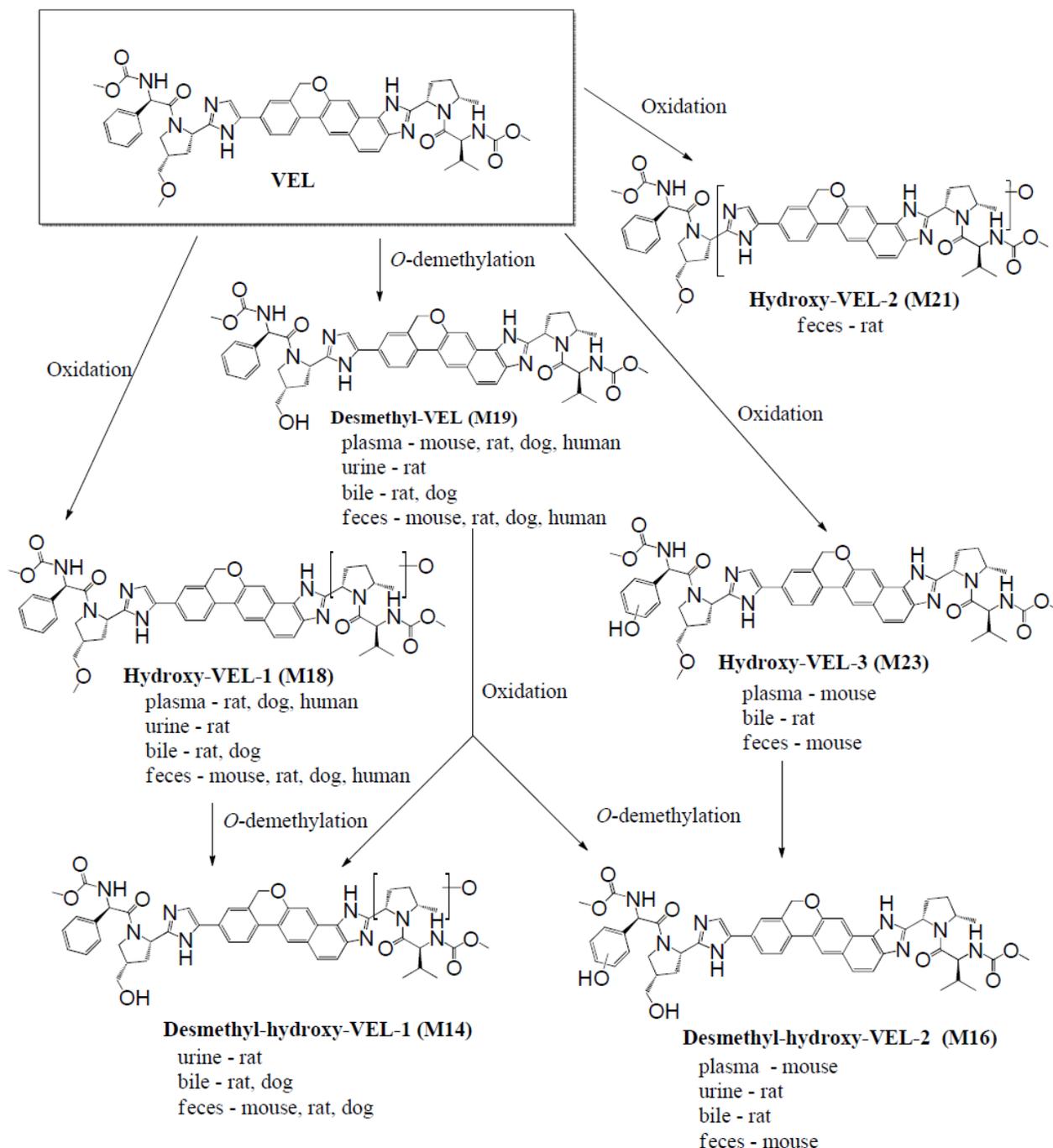


Figure 3: Proposed Biotransformation Pathway of Velpatasvir Based on Metabolite Identification in Plasma, Urine, Bile, and Feces Following Oral Administration of ¹⁴C- Velpatasvir

Excretion

Pharmacokinetics of GS-5816 in Bile-Duct Cannulated Rats (AD-281-2005)

GS-5816 was administered intravenously by a 30-minute infusion (2 mg/kg) to bile-duct

cannulated male SD rats (n=3). The concentrations of GS-5816 in rat plasma, bile, and urine were determined by LC/MS/MS method. Approximately 16% of total dose was recovered in bile as GS-5816. Only trace amounts of GS-5816 was detected in urine. The systemic clearance of GS-5816 (0.93 L/hr) and the volume of distribution (1.7 L/kg) in bile-duct cannulated rats were consistent with the previous pharmacokinetic study (AD-281-2002) in regular SD rats.

Pharmacokinetics, Absorption, and Excretion of ¹⁴C-GS-5816 Following Oral Administration to Intact and Bile Duct-Cannulated Dogs (AD-281-2023)

The absorption, distribution, and excretion of ¹⁴C-GS-5816 were determined after administration of a single 10-mg/kg oral dose to bile duct-intact and bile duct cannulated (BDC) male beagle dogs (3/group). The maximum blood and plasma concentrations were 717 and 1110 ng ¹⁴C-GS-5816/g at 2.67 and 3.00 hours postdose, respectively. The observed concentrations of radioactivity in blood and plasma then declined to 217 and 344 ng ¹⁴C-GS-5816/g, respectively, by 8 hours postdose and were below the limit of quantitation (BLQ) thereafter. The mean elimination half-life of total radioactivity in blood and plasma from intact male dogs was 2.80 hours for both matrices. In intact dogs, elimination of radioactivity was rapid, with approximately 92% of the dose recovered in feces and urine 48 hours postdose. Means of 93.6 and 0.201% of the administered radioactivity were excreted in feces and urine, respectively, at 168 hours postdose. The overall mean recovery of radioactivity after oral dosing to bile duct-intact dogs was 94.9%.

In BDC dogs, means of 71.2, 18.7, and 0.245% of the administered radioactivity were excreted in feces, bile, and urine, respectively, at 168 hours postdose. Based on the radioactivity excreted in urine and bile, a minimum of approximately 19% of the orally administered dose was absorbed. The elimination of a large amount of radioactivity in bile from BDC dogs indicates that biliary excretion was a major route of elimination of ¹⁴C-GS-5816. The overall recovery of radioactivity after oral dosing to BDC dogs was 91.1%.

Pharmacokinetic Drug Interactions

In Vitro Assessment of Human Liver Cytochrome P450 Inhibition Potential of GS-5816 (AD-281-2008)

To assess the potential for GS-5816 to inhibit CYP450 enzymes, the rates of enzyme-specific metabolite formation from probe substrates by hepatic microsomal fractions were determined in the presence and absence of test compound and, where possible, IC₅₀ values were determined. At concentrations up to 25 μM GS-5816 had no inhibitory effect on the activities of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 or CYP3A.

Table 23: IC50 Values for Major Human CYP450 Enzymes for GS-5816 and Positive Control Inhibitors

Enzyme	Activity	Calculated IC ₅₀ (μM)	
		Control Inhibitor ^a	GS-5816
CYP1A2	Ethoxyresorufin O-deethylase	0.06	> 25
CYP2B6	Bupropion 4-hydroxylase	0.68	> 25
CYP2C8	Paclitaxel 6α-hydroxylase	0.55	> 25
CYP2C9	Tolbutamide 4-hydroxylase	0.60	> 25
CYP2C19	S Mephenytoin 4'-hydroxylase	7.08	> 25
CYP2D6	Dextromethorphan O-demethylase	0.04	> 25
CYP3A	Midazolam 1'-hydroxylase	0.04	> 25
	Testosterone 6β-hydroxylase	0.09	> 25

^a Control Inhibitors: CYP1A2, α-Naphthoflavone (0–3 μM); CYP2B6 ticlopidine (0-10 μM; CYP2C8 Montelukast (0–3 μM); CYP2C9, Sulfaphenazole (0–10 μM); CYP2C19, Tranylcypromine (0–50 μM); CYP2D6, Quinidine (0–3 μM); CYP3A, Ketoconazole (0–3 μM).

In Vitro Assessment of Induction Potential of GS-5816 in Humans (AD-281-2009)

The potential for induction of human drug metabolizing enzymes and transporters through the activation of the aryl hydrocarbon receptor (AhR) or the pregnane X receptor (PXR) by GS-5816 was assessed in vitro. These data suggest GS-5816 would not activate PXR or AhR regulated genes at concentrations that could be achieved in humans following oral administration. Thus the liability of GS-5816 to cause drug-drug interactions through induction of human drug-metabolizing cytochrome P450 enzymes, particularly those of the CYP1A, CYP2B, CYP2C, CYP3A subfamilies, and other proteins regulated by these xenobiotic receptors is low.

6 General Toxicology

6.1 Single-Dose Toxicity

No formal single-dose toxicity studies with GS-5816 have been conducted. Doses up to 1000 mg/kg in mice, 600 mg/kg in rats, and 200 mg/kg in dogs were well-tolerated in single-dose PK studies (Studies AD-281-2028, AD-281-2014, and AD-281-2013, respectively).

6.2 Repeat-Dose Toxicity

6.2.1 5-day Rat

5-Day Oral Gavage Toxicity and Toxicokinetic Study with GS-5816 in Male Rats (TX-281-2001)

Oral administration of GS-5816 at dose levels of 0, 50, 150, and 450 mg/kg/day for 5 days was well-tolerated and no treatment-related findings were noted. Minimally to

mildly lower white blood cell, absolute neutrophil, absolute monocyte, and absolute large unstained cell counts at all dose levels were considered incidental. Control animals had slightly lower albumin and slightly higher globulin concentrations, suggesting the possibility of a mild inflammatory process that may have increased their leukocyte counts. At the terminal sacrifice on Day 6, a statistically significant increase (1.2-fold) in mean epididymis weights (unadjusted weight) was noted in animals administered 50 mg/kg/day and a statistically significant decrease (-11%) in mean liver weights was noted in animals administered 150 mg/kg/day. Other small differences between control and GS-5816-dosed animals, such as minimally higher platelet count, glucose, and sodium for animals administered 450 mg/kg/day, were considered incidental. None of the differences were considered adverse because of their small magnitudes, lack of dose dependency, and lack of microscopic correlates. Exposure (AUC_{0-24}) increased with the increase in GS-5816 dose level; however, exposures were similar at 150 and 450 mg/kg/day (see sponsor's table). Day 5 C_{max} and AUC_{0-24} values were comparable with those on Day 1, indicating no accumulation at these dose levels for GS-5816.

Table 24: Toxicokinetic Parameters for GS-5816 in Rat Plasma on Day 5

Dose Level (mg/kg/day)	AUC_{0-24} (ng•hr/mL)	C_{max} (ng/mL)	T_{max} (hr)	C_{24hr} (ng/mL)
50	5500 ± 1340	810 ± 98.0	3.33 ± 1.15	3.07 ± NC
150	12800 ± 3910	1300 ± 188	4.33 ± 3.51	100 ± 153
450	16100 ± 6550	1420 ± 472	6.67 ± 2.31	26.7 ± 21.9

BLOQ: Below the limit of quantitation (< 1.77 ng/mL)

NC: Not calculated.

6.2.2.1 2-week Rat

2-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-5816 in Rats with a 1-Week Recovery and a Micronucleus Assessment (TX-281-2003)

No GS-5816 related effects were noted following oral administration at 0, 20, 60, and 200 mg/kg/day for 2 weeks to male and female rats. Bone marrow and supplementary liver samples (hepatic microsomal fractions via differential centrifugation) were extracted from designated micronucleus animals prior to exsanguination. GS-5816 did not induce increases in micronucleated polychromatic erythrocytes (PCEs) or decreases in PCE:normochromatic erythrocytes (NCEs) ratios at any dose examined, and resulted in no notable change (≥ 2 -fold increase or $\geq 50\%$ decrease) in microsomal protein yield, cytochrome P450 content, and in CYP1A, CYP2B, CYP2C, CYP2E, CYP3A, CYP4A and UDPGT activities compared to vehicle control animals. Exposure to GS-5816 increased with increasing dose levels from 20 to 200 mg/kg/day (sponsor's table below). The increases in C_{max} and AUC_{0-t} were less than dose proportional between the 20 and 200 mg/kg/day dose levels. Gender-based differences in C_{max} and AUC_{0-t} values of GS-5816 were generally less than 2-fold. No accumulation of GS-5816 was observed after multiple days of dosing in rats.

Table 25: Toxicokinetic Parameters for GS-5816 in Rat Plasma - Day 1 and Day 14

Dose Level (mg/kg/day)	Sex	Day 1		Day 14	
		C _{max} (ng/mL)	AUC _{0-t} (ng·hr/mL)	C _{max} (ng/mL)	AUC _{0-t} (ng·hr/mL)
20	M	597	5263	800	5569
	F	608	4522	578	3193
	MF	603	4894	645	4689
60	M	914	10371	1323	14529
	F	967	9770	1018	8364
	MF	934	10070	1134	11447
200	M	1167	17285	1607	21396
	F	1154	15852	1041	12865
	MF	1060	16568	1291	17130

Note: Combined data are based on the analysis of the combined concentration data of both sexes.

F = Female M = Male MF = Male and female combined data.

6.2.2.2 2-week Rat- Lot (Impurity) Qualification

14-Day Oral Gavage Qualification Toxicity and Toxicokinetic Study with GS-5816 in Male Sprague-Dawley Rats (TX-281-2042)

Comparison of different lots of GS-5816 (GS-5816 at 200 mg/kg/day and GS-5816-B at 60 and 200 mg/kg/day) yielded similar effects of decreased red cell mass characterized by minimally decreased red blood cell count, hemoglobin concentration, and hematocrit in animals. No clear GS-5816- or GS-5816-B-related effects were noted on coagulation, clinical chemistry, or urinalysis test results. There were no adverse effects after oral administration of GS-5816 or GS-5816-B up to 200 mg/kg/day for 14 days.

Exposure to GS-5816 increased with the increase in GS-5816-B dose level from 60 to 200 mg/kg/day (sponsor's table below). The increases in C_{max} and AUC₀₋₂₄ were less-than-dose proportional between the 60 and 200 mg/kg/day GS-5816-B dose levels. No accumulation of GS-5816 was observed after multiple doses of GS-5816 or GS-5816-B in animals. Exposure to GS-5816 was similar when GS-5816 or GS-5816-B was administered orally to animals at 200 mg/kg/day.

Table 26: Toxicokinetic Parameters for GS-5816 and GS-5816-B in Male Rat Plasma: Days 1 and 14

Interval	Dose Group	Dose Level (mg/kg/day)	C _{max} (ng/mL)	T _{max} (h)	AUC ₀₋₂₄ (ng·h/mL)	C _{last} (ng/mL)	T _{last} (h)
Day 1	2	200 (GS-5816)	1440	8.00	12600	4.23	24.0
	3	60 (GS-5816-B)	726	4.00	5320	4.37	24.0
	4	200 (GS-5816-B)	1230	4.00	12900	6.05	24.0
Day 14	2	200 (GS-5816)	952	4.00	7620	6.61	24.0
	3	60 (GS-5816-B)	831	4.00	5490	4.90	24.0
	4	200 (GS-5816-B)	1300	4.00	9330	9.72	24.0

Note: AUC₀₋₂₄ is equivalent to AUC_{0-t}.

6.2.3 2-week Dog

2-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-5816 in Dogs with a 1-Week Recovery (TX-281-2004)

No GS-5816-related effects were noted following oral administration of 0, 5, 20, and 100 mg/kg/day for 2 weeks to male and female Beagle dogs. Statistically significant decreases in absolute or relative heart weights in males and females administered 100 mg/kg/day and decreases in relative adrenal weights in males administered 20 and 100 mg/kg/day and females administered 100 mg/kg/day were not considered test article-related because of overlapping individual variation and a lack of microscopic correlates. Like rats, supplementary liver samples (hepatic microsomal fractions via differential centrifugation) were extracted from designated animals prior to exsanguination, and there were no notable changes (≥ 2 -fold increase or $\geq 50\%$ decrease) in microsomal protein yield, cytochrome P450 content, and in CYP1A, CYP2B, CYP2C, CYP2E, CYP3A, CYP4A and UDPGT activities compared to vehicle control animals. The mean concentration-time profiles for males, females, and combined sexes showed that exposure to GS-5816 increased with increasing dose level from 5 to 100 mg/kg/day (sponsor's table below). Gender-based differences in C_{max} and AUC_{0-t} were generally less than 2-fold. Increases in mean C_{max} and AUC_{0-t} values (sexes combined) were approximately dose-proportional between 5 and 20 mg/kg/day, and less than dose-proportional between 20 and 100 mg/kg/day. Values for mean C_{max} and AUC_{0-t} were generally similar on Days 1 and 14, indicating no accumulation of GS-5816 after multiple days of dosing in dogs.

Table 27: Toxicokinetic Parameters for GS-5816 in Dog Plasma - Day 1 and Day 14

Dose Level (mg/kg/day)	Sex		Day 1		Day 14	
			C _{max} (ng/mL)	AUC ₀₋₄ (ng-hr/mL)	C _{max} (ng/mL)	AUC ₀₋₄ (ng-hr/mL)
5	M	Mean	169	1069	228	1528
		SD	115	472	74	611
		N	3	3	3	3
	F	Mean	344	2094	401	2230
		SD	12	522	77	540
		N	3	3	3	3
	MF	Mean	257	1582	314	1879
		SD	120	717	117	643
		N	6	6	6	6
20	M	Mean	930	9615	950	10761
		SD	76	1424	121	3872
		N	3	3	3	3
	F	Mean	848	6137	996	9648
		SD	123	851	335	2681
		N	3	3	3	3
	MF	Mean	889	7876	973	10204
		SD	102	2175	227	3040
		N	6	6	6	6
100	M	Mean	1532	18920	2006	26167
		SD	496	6157	521	9340
		N	5	5	5	5
	F	Mean	1065	8725	1426	18853
		SD	480	3961	658	9559
		N	5	5	5	5
	MF	Mean	1299	13823	1716	22510
		SD	522	7259	637	9708
		N	10	10	10	10

F = Female; M = Male; MF = Combined sexes.

6.2.4 4-week Mouse

4-Week Oral Gavage Dose Range-Finding Toxicity and Toxicokinetic Study with GS-5816 in Model 001178-W (Wild Type) CByB6F1-Tg (HRAS)2Jic Mice

Study no.: TX-281-2028
 Study report location: electronic
 Conducting laboratory and location: (b) (4)
 Date of study initiation: August 28, 2014
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: GS-5816, 5816-AC-1P, 98.6%

Key Study Findings: GS-5816-related effects on clinical pathology test results were limited to mildly lower mean white blood cell count, absolute neutrophil and absolute lymphocyte count in males administered 1500 mg/kg/day. These changes were not considered adverse. The NOAEL for GS-5816 was 1500 mg/kg/day (C_{max} and AUC₀₋₂₄ values of 16,300 ng/mL and 220,000 ng-hr/mL, respectively).

Methods

Doses:	0, 100, 300 and 1500 mg/kg/day
Frequency of dosing:	daily
Route of administration:	oral
Dose volume:	10 ml/kg
Formulation/Vehicle:	(0.2% [w/v] hydroxypropyl methylcellulose [HPMC, Methocel K100 Premium LV], 0.2% [v/v] Tween 20, and 99.6% [v/v] deionized water) only
Species/Strain:	CByB6F1-Tg(HRAS)2Jic Model 1178-wild type mice
Number/Sex/Group:	10/sex/group
Age:	8 weeks old
Weight:	21.9 to 32.7 g for males and 18.2 to 25.7 g for females
Satellite groups:	6-36/sex/group
Unique study design:	none
Deviation from study protocol:	none

Observations and Results

Mortality: All animals were checked twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress. No GS-5816-related deaths occurred. All remaining animals survived to their scheduled sacrifice.

Clinical Signs: Cageside observations were conducted once daily during the predose and dosing phases. Detailed observations were conducted for each animal once during the predose phase, and they were conducted for each toxicity animal prior to dosing on Day 1 and weekly (based on Day 1) throughout the dosing phase. No GS-5816-related clinical observations were noted.

Body Weights: Body weights for all surviving animals were recorded once during the predose phase, before dosing on Day 1, and weekly thereafter during the dosing phase. No GS-5816-related effects on body weight or body weight gain were noted.

Feed Consumption: Quantitative food consumption for each cage of toxicity animals was recorded weekly (based on Day 1) during the dosing phase unless food was spilled. No GS-5816-related effects on food consumption were noted.

Clinical Pathology: Blood samples were collected from non-fasted toxicity animals via a cardiac puncture on the day of scheduled sacrifice (Day 30). Blood samples for hematology were collected from five animals/sex/group and samples collected for clinical chemistry were collected from up to five animals/sex/group.

Hematology: Mildly lower mean white blood cell count, absolute neutrophil and absolute lymphocyte counts were seen in males administered 1500 mg/kg/day. These differences were not statistically significant, had no microscopic correlate.

Clinical Chemistry: GS-5816 administration had no effects on clinical chemistry test results.

Organ Weights: In high dose females, mean liver/gallbladder weight was 122% of the control value with liver/gallbladder: body weight and liver/gallbladder: brain ratios also significantly higher than control ratios. Morphological correlates were not present in the liver and this weight difference was considered an incidental finding. Liver/gallbladder weights were not increased in the high dosed males. In high dose females, the mean uterine weight was 175% of the control value with uterus: body weight and uterus: brain ratios also significantly higher than control ratios. Morphological correlates were not present in the uterus and this weight difference was considered an incidental finding.

Gross Pathology: No GS-5816-related macroscopic observations were present.

Histopathology: Adequate Battery: yes Peer Review: yes

Histological Findings: No GS-5816-related microscopic observations were noted.

Toxicokinetics: Blood samples were collected from three toxicokinetic animals/sex/group/time point in Groups 2 through 4 on Day 1 and during Week 4 predose (Week 4 only) and at approximately 1, 2 (Day 1 only), 4, 8, 12, and 24 hours postdose. Blood samples were also collected from three toxicokinetic animals/sex in the vehicle control group on Day 1 and during Week 4 at approximately 4 hours postdose. Exposure to GS-5816 generally increased with the increase in dose level from 100 to 1500 mg/kg/day (sponsor's table below). The increases in C_{max} and AUC_{0-24} were less than dose proportional between the 100 to 300 and 300 to 1500 mg/kg/day dose levels. Gender-based differences were less than 2-fold in GS-5816 C_{max} and AUC_{0-24} values. No accumulation of GS-5816 was observed after multiple doses in mice.

Table 28: Mean Toxicokinetic Parameters of GS-5816 in the 4-Week Oral Gavage Study in 001178-W (wild type) Mice

Dose (mg/kg/day)	Sex	C_{max} (µg/mL)		AUC_{0-24} (µg·h/mL)	
		Day 1	Week 4	Day 1	Week 4
100	Male	8.24	8.56	69.2	83.3
300	Male	12.3	7.72	122	86.7
1500	Male	13.4	14.2	146	170
100	Female	10.8	8.02	79.5	64.9
300	Female	11.8	11.7	105	82.3
1500	Female	18.3	18.3	261	269

6.2.5 13-week Rat

26-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-5816 in Rats with a 13-Week Interim Necropsy and a 4-Week Recovery Phase

Study no.:	TX-281-2007
Study report location:	electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 2, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Gs-5816, 5816-XA-1, 95.9%

Key Study Findings: No GS-5816-related effects were noted following oral administration of GS-5816 for 13 weeks at doses up to 200 mg/kg/day in male and female rats. The NOAEL for GS-5816 at the interim sacrifice was 200 mg/kg/day (C_{max} 1190 ng/mL and AUC_{0-t} 15,500 ng·hr/mL, sexes combined).

Methods

Doses:	0, 20, 60 and 200 mg/kg/day
Frequency of dosing:	daily
Route of administration:	oral
Dose volume:	5 ml/kg
Formulation/Vehicle:	[45% (v/v) propylene glycol and 15% (v/v) Kolliphor® HS-15 prepared in water, pH 2.0]
Species/Strain:	Rats/Hsd:Sprague Dawley
Number/Sex/Group:	10/sex/group
Age:	6-7 weeks old
Weight:	186 to 223 g for males and 133 to 176 g for females
Satellite groups:	9/sex/group
Unique study design:	none
Deviation from study protocol:	none

Observations and Results

Mortality: All animals were checked twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress. No test article-related deaths occurred during the first 13 weeks of the study. A single mammary gland carcinoma in one female administered 200 mg/kg/day was considered an incidental event commonly seen in Sprague Dawley rats, and disuse of a hind limb in one female administered 60 mg/kg/day leading to humane sacrifice was presumptively due to accidental injury unrelated to GS-5816 administration.

Clinical Signs: Cageside observations were conducted once daily during the predose and dosing phases, except on days when detailed observations were conducted. No GS-5816-related clinical observations were noted.

Body Weights: Body weights were recorded for all animals on Day 3 of the predose phase, prior to dosing on Day 1, and weekly (based on Day 1 of the dosing phase) thereafter. No GS-5816-related effects were noted on mean body weight or mean body weight change. No GS-5816-related effects on body weight or body weight gain were noted.

Feed Consumption: Measured quantitatively for toxicity animals weekly during the dosing phase. No GS-5816-related effects on food consumption occurred. No GS-5816-related effects on food consumption were noted.

Ophthalmic Findings: Examinations were performed once during the predose phase and for toxicity animals only on Day 86 of the dosing phase. No visible lesions were noted during ophthalmic examinations.

Clinical Pathology: For hematology, coagulation, and clinical chemistry blood and urine was collected on the day of interim sacrifice from all surviving animals scheduled for the interim sacrifice only.

Hematology: No GS-5816-related changes were present in hematology test results.

Clinical Chemistry: No changes were noted.

Urinalysis: Parameters were unaffected.

Gross Pathology: No GS-5816-related macroscopic observations were present at the 13-week interim necropsy.

Organ Weights: No GS-5816-related organ weight changes were present at the 13-week interim necropsy.

Histopathology: Adequate Battery: yes Peer Review: yes

Histological Findings: No GS-5816-related microscopic observations were present at the 13-week interim necropsy.

Toxicokinetics: Blood samples were collected from toxicokinetic animals via a jugular vein on Day 1 and during Week 13 of the dosing phase predose. Exposure to GS-5816 increased with the increase in dose level from 20 to 200 mg/kg/day (sponsor's table below). The increases in C_{max} and AUC_{0-t} were generally less than proportional between all dose levels. Gender-based differences in plasma concentration of GS-5816 were less than 2-fold in C_{max} and AUC_{0-t} values. No accumulation of GS-5816 was observed after multiple dosing in rats.

Table 29: Toxicokinetic Parameters for GS-5816 in Rat Plasma - Day 1 and Week 13 (Day 90)

Interval	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)
Day 1	2	20	M	480	2.00	2960	12.0	23.1
			F	630	2.00	2950	12.0	27.9
			MF	555	2.00	3120	24.0	2.22 ^a
	3	60	M	917	4.00	6980	24.0	3.41
			F	1340	4.00	9170	24.0	4.27
			MF	1130	4.00	8070	24.0	3.84
	4	200	M	1320	4.00	11100	24.0	7.15
			F	1730	4.00	14900	24.0	237
			MF	1530	4.00	13000	24.0	122
Week 13	2	20	M	504	2.00	3120	24.0	7.61
			F	707	1.00	3320	24.0	2.29
			MF	595	1.00	3170	24.0	5.48
	3	60	M	856	4.00	7780	24.0	87.3
			F	1040	2.00	7080	24.0	116
			MF	859	2.00	7490	24.0	98.9
	4	200	M	1080	2.00	15500	24.0	471
			F	1450	4.00	15500	24.0	222
			MF	1190	4.00	15500	24.0	371

Note: Combined data is based on the analysis of the combined mean concentration data for both sexes.

M = Male; F = Female

^a Value is based on the mean concentration of one male and one female at the 24-hour collection time.

Stability and Homogeneity: Overall mean concentrations of all test article formulations prepared for dose administration on Day 1 of the dosing phase ranged from 105.7 to 106.9% of the nominal concentration. The analyzed formulations met the acceptance criteria indicating the formulations were homogeneous.

6.2.6 13-week Dog

39-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-5816 in Dogs with a 13-Week Interim Necropsy and a 4-Week Recovery

Study no.: TX-281-2009
 Study report location: electronic
 Conducting laboratory and location: (b) (4)
 Date of study initiation: September 27, 2012
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: GS-5816, 5816-XA-1, 95.9%

Key Study Findings: No adverse test article-related effects were noted following once-daily oral administration of GS-5816 for 13 weeks at doses up to 100 mg/kg/day in male and female dogs. The NOAEL for GS-5816 after 13 weeks of dosing is 100 mg/kg/day (C_{max} 2080 ng/mL and AUC_{0-t} 29,800 ng•hr/mL, sex-combined).

Methods

Doses:	Total: 0, 5, 20 and 100 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	2 ml/kg
Formulation/Vehicle:	[45% (v/v) propylene glycol and 15% (v/v) Kolliphor® HS-15 prepared in water, pH 2.0]
Species/Strain:	Beagle dogs
Number/Sex/Group:	3/sex/group
Age:	6-7 months old
Weight:	8.3 to 10.5 kg for males and 5.8 to 8.4 kg for females
Satellite groups:	none
Unique study design:	none
Deviation from study protocol:	none

Observations and Results

Mortality: Animals were checked twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress. No test article-related deaths occurred during the first 13 weeks of the study.

Clinical Signs: Detailed observations were conducted on Days 1 and 8 of the predose phase, prior to dosing on Day 1, and weekly (based on Day 1 of the dosing phase) during the dosing phase. Detailed observations were also conducted on the day of interim sacrifice (all surviving animals). There were no adverse clinical observations. Test article-related emesis was observed more frequently in males and females administered 100 mg/kg/day. The increase in vomitus was not considered adverse because it was not associated with any correlating effects on body weight, food consumption, other clinical observations, or clinical pathology indicative of inappetence or dehydration.

Body Weights: Recorded on Days 1 and 8 of the predose phase, prior to dosing on Day 1 of the dosing phase, and weekly thereafter (based on Day 1 of the dosing phase) during the dosing phase. Body weights were also recorded on the day of interim sacrifice (all surviving animals). No GS-5816-related alterations in mean body weight or mean body weight change were noted during the first 13 weeks of the study.

Feed Consumption: Measured quantitatively weekly during the dosing phase. No GS-5816-related effects occurred for food consumption. No GS-5816-related alterations in food consumption were noted during the first 13 weeks of the study.

Ophthalmoscopy: Performed on Day 7 of the predose phase and on Day 85 of the dosing phase. No visible lesions were noted. No visible abnormalities were noted during ophthalmic examinations conducted on Day 7 of the predose phase and Day 85 of the dosing phase.

ECG: Eight-lead ECG measurements were recorded on Day 5 of the predose phase and Day 89 of the dosing phase predose. No GS-5816-related changes in PR interval, QRS duration, QT interval, QTc interval, RR interval, or heart rate were observed on Day 5 or 89 of the dosing phase in animals administered 5, 20, or 100 mg/kg/day. No qualitative ECG abnormalities were attributed to administration of 5, 20, or 100 mg/kg/day of GS-5816.

Borderline first degree atrioventricular block (PR interval > 130 msec) was noted on Day 89 in one male given 5 mg/kg/day and one male given 100 mg/kg/day. In addition, infrequent nonconducted P waves, a form of second degree atrioventricular block was noted predose on Day 5 of the dosing phase in one female administered 100 mg/kg/day (Animal No. H08483) and 3.5 hours postdose on Day 5 of the dosing phase in a one female administered 5 mg/kg/day (Animal No. H08465). First degree atrioventricular block and infrequent nonconducted P waves are common arrhythmias in dogs, are considered normal variants, and were incidental findings.

Clinical Pathology: Blood samples were collected for hematology, coagulation, and clinical chemistry from fasted animals via a jugular vein on Days 5 and 9 of the predose phase and on the day of interim sacrifice (Day 92 of the dosing phase).

Hematology: No GS-5816-related changes were present.

Clinical Chemistry: No changes were noted.

Urinalysis: No changes.

Gross Pathology: No GS-5816-related macroscopic observations were present at 13-weeks.

Organ Weights: Organ weight parameters were increased for the prostate gland (only organ-to-body weight ratio was significant) of males at 100 mg/kg/day at the 13-week interim time point. The increased prostate gland weights occurred without a dose response or specific microscopic correlation. Additionally, the organ weight parameters for dogs administered 100 mg/kg/day were within the historical control ranges for prostate weights for Beagle dogs of similar ages and weights at (b) (4). The increase in prostate weights was considered a nonadverse finding of uncertain relationship to GS-5816.

Histopathology: Adequate Battery: yes Peer Review: yes

Histological Findings: No GS-5816-related microscopic observations were present at 13-weeks.

Toxicokinetics: Blood samples were collected via the jugular or cephalic vein on Days 1 and 85 of the dosing phase. Mean concentration-time profiles for males, females, and combined sexes showed exposure to GS-5816 increased with the increase in dose level

from 5 to 100 mg/kg/day on Day 1 and during Week 13 of the dosing phase (sponsor's table below). Increases in C_{max} and AUC_{0-t} were generally dose proportional between 5 and 20 mg/kg/day and less-than-dose proportional between 20 and 100 mg/kg/day. Values were generally similar on Day 1 and during Week 13 of the dosing phase, indicating no accumulation of GS-5816 after multiple dosing in dogs. Sex-based differences were <2-fold in GS-5816 mean C_{max} and AUC_{0-t} values.

Table 30: Mean Toxicokinetic Parameters for GS-5816 in Dog Plasma - Day 1 and Week 13 (Day 85)

Interval	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)	
Day 1	2	5	M	335	1.57	1880	22.3	5.47	
			F	231	1.57	1250	17.1	9.56	
			MF	283	1.57	1560	19.7	7.51	
	3	20	M	946	3.14	8610	24.0	33.5	
			F	1170	4.29	10900	24.0	31.9	
			MF	1060	3.71	9740	24.0	32.7	
	4	100	M	2370	7.11	32800	24.0	246	
			F	2040	5.33	25300	24.0	123	
			MF	2200	6.22	29000	24.0	185	
	Week 13	2	5	M	303	1.14	1500	22.3	6.47
				F	326	1.43	1720	24	5.97
				MF	314	1.29	1610	23.1	6.22
3		20	M	929	2.29	8700	24.0	96.3	
			F	1050	2.71	10500	24.0	54.3	
			MF	990	2.50	9600	24.0	75.3	
4		100	M	2090	5.78	28600	24.0	224	
			F	2060	6.44	31100	24.0	228	
			MF	2080	6.11	29800	24.0	226	

Note: Combined (male and female) data are based on combined mean data of both sexes.
F = Female; M = Male; N = Number; SD = Standard deviation.

Stability and Homogeneity: Overall mean concentrations of all test article formulations prepared for dose administration on Day 1 of the dosing phase ranged from 106.1 to 107.3% of the nominal concentration. The analyzed formulations met the acceptance criteria indicating the formulations were homogeneous.

6.2.7 26-week Rat

26-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-5816 in Rats with a 13-Week Interim Necropsy and a 4-Week Recovery Phase

Study no.: TX-281-2007

Study report location: electronic

Conducting laboratory and location: (b) (4)

Date of study initiation: October 2, 2012
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: GS-5816, 5816-XA-1, 95.9%

Key Study Findings: No GS-5816-related mortality; clinical observations; body weight, body weight changes, or food consumption changes; ophthalmic observations; or clinical and anatomic pathology findings were noted at doses up to 200 mg/kg/day for 13 or 26 weeks followed by a 4-week recovery period. The NOAEL for GS-5816 was 200 mg/kg/day (C_{max} of 1170 ng/mL and AUC_{0-t} of 12,200 ng*hr/mL, sexes combined).

Methods

Doses: 0, 20, 60 and 200 mg/kg/day
 Frequency of dosing: daily
 Route of administration: oral
 Dose volume: 5 ml/kg
 Formulation/Vehicle: [45% (v/v) propylene glycol and 15% (v/v) Kolliphor® HS-15 prepared in water, pH 2.0]
 Species/Strain: Rats/Hsd:Sprague Dawley
 Number/Sex/Group: 10/sex/group
 Age: 6-7 weeks old
 Weight: 186 to 223 g for males and 133 to 176 g for females
 Satellite groups: 9/sex/group
 Unique study design: none
 Deviation from study protocol: none

Observations and Results

Mortality: All animals were checked twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress. No GS-5816-related mortality occurred at the interim, terminal, or recovery sacrifice. Four toxicity animals from the dosing phase were sacrificed in moribund condition during the interim sacrifice interval, and one toxicity animal was sacrificed humanely following accidental injury. One control male from the dosing phase was found dead during the terminal sacrifice interval. The causes of unscheduled deaths were not test article-related and are presented in sponsor's table below.

Table 31: Cause of Unscheduled Deaths in 26- week Rat Study

Animal No.	Sex	Dose Level (mg/kg/day)	Study Day of Death	Status	Cause of Death/Moribund Condition
B50966	Male	0	146	FD	Nephroblastoma
B50990	Male	20	51	MS	Gavage-related
B51140	Female	60	26	MS	Gavage-related
B51122	Female	60	85	MS ^a	Accidental injury
B51164	Female	200	39	MS	Gavage-related
B51166	Female	200	76	MS	Mammary gland carcinoma

FD = Found dead; MS = Sacrificed in moribund condition

^a Animal No. B51122 was a humane sacrifice following accidental injury.

Clinical Signs: Cageside observations were conducted once daily during the predose and dosing phases, except on days when detailed observations were conducted. No GS-5816-related clinical observations were noted.

Body Weights: Body weights were recorded for all animals on Day 3 of the predose phase, prior to dosing on Day 1, and weekly (based on Day 1 of the dosing phase) thereafter. No GS-5816-related effects on body weight or body weight gain were noted.

Feed Consumption: Measured quantitatively for toxicity animals weekly during the dosing phase. No GS-5816-related effects on food consumption were noted.

Ophthalmic Findings: Examinations were performed once during the predose phase and for toxicity animals only on Day 86 of the dosing phase and on Day 177 (Week 26) of the dosing phase. No visible lesions were noted during ophthalmic examinations.

Clinical Pathology: For hematology, coagulation, and clinical chemistry blood and urine was collected on the day of sacrifice.

Hematology: No GS-5816-related changes were present in hematology test results.

Clinical Chemistry: No changes were noted

Urinalysis: Parameters were unaffected.

Gross Pathology: No GS-5816-related macroscopic observations were present at the interim, terminal, or recovery sacrifice.

Organ Weights: No GS-5816-related organ weight changes were present at the interim, terminal, or recovery sacrifice.

Histopathology: Adequate Battery: yes Peer Review: yes

Histological Findings: No GS-5816-related microscopic observations were present at the interim, terminal, or recovery sacrifice.

Toxicokinetics: Exposure to GS-5816 increased with the increase in dose level from 20 to 200 mg/kg/day (sponsor's table below). Increases in C_{max} and AUC_{0-t} values were generally less-than-proportional between all dose levels. Sex-based differences in plasma concentration of GS-5816 were less than 2-fold in C_{max} and AUC_{0-t} values. No accumulation of GS-5816 was observed after repeat dosing for 26 weeks.

Table 32: Toxicokinetic Parameters for GS-5816 in Rat Plasma - Day 1, Week 13 and Week 26

Interval	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)
Day 1	2	20	M	480	2.00	2960	23.1	12.0
			F	630	2.00	2950	27.9	12.0
			MF	555	2.00	3120	2.22 ^a	24.0 ^a
	3	60	M	917	4.00	6980	3.41	24.0
			F	1340	4.00	9170	4.27	24.0
			MF	1130	4.00	8070	3.84	24.0
	4	200	M	1320	4.00	11100	7.15	24.0
			F	1730	4.00	14900	237	24.0
			MF	1530	4.00	13000	122	24.0
Week 13	2	20	M	504	2.00	3120	7.61	24.0
			F	707	1.00	3320	2.29	24.0
			MF	595	1.00	3170	5.48	24.0
	3	60	M	856	4.00	7780	87.3	24.0
			F	1040	2.00	7080	116	24.0
			MF	859	2.00	7490	98.9	24.0
	4	200	M	1080	2.00	15500	471	24.0
			F	1450	4.00	15500	222	24.0
			MF	1190	4.00	15500	371	24.0
Week 26	2	20	M	499	2.00	3130	6.48	24.0
			F	800	2.00	3870	7.17	24.0
			MF	650	2.00	3500	6.76	24.0
	3	60	M	852	4.00	6690	82.0	24.0
			F	1160	2.00	6720	7.85	24.0
			MF	916	2.00	6700	44.9	24.0
	4	200	M	1110	4.00	11000	107	24.0
			F	1230	4.00	13400	171	24.0
			MF	1170	4.00	12200	139	24.0

F = Female; M = Male

Note: Combined data are based on analysis of the combined mean concentration data for both sexes.

^a Based on the mean concentration of one male and one female at the 24 hours collection time point.

Stability and Homogeneity: Overall mean concentrations of all test article formulations prepared for dose administration on Day 1 of the dosing phase ranged from 105.7 to 106.9% of the nominal concentration. The analyzed formulations met the acceptance criteria indicating the formulations were homogeneous.

6.2.8 39-week Dog

39-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-5816 in Dogs with a 13-Week Interim Necropsy and a 4-Week Recovery

Study no.: TX-281-2008

Study report location: electronic

Conducting laboratory and location: (b) (4)

Date of study initiation:	September 27, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-5816, 5816-XA-1, 95.9%

Key Study Findings: GS-5816-related clinical observations were limited to a slight increase in the incidence of vomitus in males and females administered 100 mg/kg/day. Food consumption was moderately lower in females administered 100 mg/kg/day. GS-5816-related effects on clinical pathology test results were limited to mildly decreased fibrinogen and globulin concentrations and mildly increased albumin:globulin ratio after 39 weeks in females administered 100 mg/kg/day. A transient increase in mean prostate weight at 100 mg/kg/day compared with concurrent controls was a nonadverse change without a microscopic correlate; this organ weight change was of uncertain relationship to GS-5816 administration at the interim Day 92 (Week 14) necropsy. The NOAEL for GS-5816 after 39 weeks of dosing is 100 mg/kg/day (C_{max} 2060 ng/mL and AUC_{0-t} 27,800 ng*hr/mL, sex-combined).

Methods

Doses:	Total: 0, 5, 20 and 100 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	2 ml/kg
Formulation/Vehicle:	[45% (v/v) propylene glycol and 15% (v/v) Kolliphor® HS-15 prepared in water, pH 2.0]
Species/Strain:	Beagle dogs
Number/Sex/Group:	9/sex/group (n=7 in mid/low dose groups)
Age:	6-7 months old
Weight:	8.3 to 10.5 kg for males and 5.8 to 8.4 kg for females
Satellite groups:	none
Unique study design:	none
Deviation from study protocol:	none

Observations and Results

Mortality: Animals were checked twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress. No test article-related deaths occurred. One male (100 mg/kg/day) and one control male were sacrificed in moribund condition on Days 16 and 18 and replaced on Day 17 and Day 19, respectively, of the dosing phase. Clinical observations and postmortem findings were consistent with gavage-related mortality and unrelated to GS-5816. One male (100 mg/kg/day) was sacrificed moribund on Day 232 of the dosing phase following a history of body weight loss, abdominal pain, and dehydration. The reason for the morbidity was vascular inflammation in the heart, spleen, thymus, and skin/subcutaneous. All remaining animals survived until the terminal sacrifice.

Clinical Signs: Cageside observations were conducted once daily during the predose, dosing, and recovery phases, except on days when detailed observations were conducted. In addition, on each dosing day, cageside observations were conducted 3 to 4 hours postdose (based on the last animal dosed/sex/group). Detailed observations were conducted on days 1 and 8 of the predose phase, prior to dosing on Day 1, and weekly (based on Day 1 of the dosing phase) during the dosing and recovery phases. Detailed observations were also conducted on the days of scheduled sacrifice. No adverse clinical observations were noted. Test article-related vomitus was observed occasionally but more frequently in males (31 occurrences versus 10 in controls) and females (13 occurrences versus 2 in controls) administered 100 mg/kg/day. The increase in vomitus was not considered adverse because it was not associated with any correlating effects on body weight, clinical observations, or clinical pathology indicative of inappetence or dehydration.

Body Weights: Body weights were recorded on days 1 and 8 of the predose phase, prior to dosing on Day 1 of the dosing phase, and weekly thereafter during the dosing and recovery phases. Body weights were also recorded on the days of scheduled sacrifice. No GS-5816-related alterations in mean body weight or mean body weight change were noted.

Feed Consumption: Food consumption was measured quantitatively weekly during the dosing and recovery phases. Food consumption in females administered 100 mg/kg/day was generally lower throughout the study. This was more notable beginning Days 99 through 106 of the dosing phase and continuing to the end of the recovery phase. This effect correlated with the occurrences of vomitus in females administered 100 mg/kg/day. The effect on food consumption did not correlate with any other observations and was not considered adverse.

Ophthalmoscopy: Animals were examined using an indirect ophthalmoscope on day 7 of the predose phase and on days 85 and 268 of the dosing phase by a board-certified veterinary ophthalmologist. The eyes were dilated with a mydriatic agent prior to examination. No visible abnormalities were noted during ophthalmic examinations during the predose phase and during Weeks 13 and 39 of the dosing phase.

ECG: Eight-lead ECG measurements were recorded on day 5 of the predose phase; on days 5, 89, and 271 of the dosing phase predose and approximately 3.5 hours postdose (based on the last animal dosed/sex/group); and on day 26 of the recovery phase. Routine quantitative measurements of ECGs were made on a single lead. The heart rate-corrected QT (QTc) interval was calculated using the Fridericia method. A qualitative review for rhythm abnormalities and disturbances of the collected ECGs was performed. No GS-5816-related changes in PR interval, QRS duration, QT interval, QTc interval, RR interval, or heart rate were observed on Days 5, 89, or 271 (Weeks 1, 13, and 39, respectively) of the dosing phase in animals administered 5, 20, or 100 mg/kg/day or on Day 26 of the recovery phase in animals administered 100 mg/kg/day.

An incidental statistical finding was noted in heart rate predose on Day 271 in males administered 5 mg/kg/day. Although mean heart rate was lower compared with controls predose on Day 271 in males administered 5 mg/kg/day, the value was similar to mean heart rate values on Day 5 of the predose phase. Furthermore, no statistically significant values were noted predose on Day 271 of the dosing phase in males administered 20 or 100 mg/kg/day or any other dosing phase day in males administered any dosage of GS-5816. Therefore, this change was an incidental finding and not attributed to GS-5816.

No qualitative ECG abnormalities were attributed to administration of 5, 20, or 100 mg/kg/day of GS-5816.

Borderline first degree atrioventricular block (PR interval > 130 msec) was noted sporadically on Days 89 (2 male dogs at 5 and 100 mg/kg) and 271 (2 male dogs at 100 mg/kg) of the dosing phase and Day 26 (1 male dog dosed 100 mg/kg) of the recovery phase. In addition, infrequent nonconducted P waves, a form of second degree atrioventricular block was noted predose on Day 5 of the dosing phase in one female administered 100 mg/kg/day (Animal No. H08483) and 3.5 hours postdose on Day 5 of the dosing phase in one female administered 5 mg/kg/day (Animal No. H08465). First degree atrioventricular block and infrequent nonconducted P waves are common arrhythmias in dogs, are considered normal variants, and were incidental findings.

Infrequent ventricular premature complexes were noted predose on Day 89 of the dosing phase in one male administered 20 mg/kg/day (Animal No. H08444). Ventricular premature complexes are an abnormal rhythm, but the observation of a few is considered within normal clinical limits.

Clinical Pathology: Blood samples were collected for hematology, coagulation, and clinical chemistry from fasted animals via a jugular vein on Days 5 and 9 of the predose phase and on the days of scheduled sacrifice (Days 92 and 274 of the dosing phase and Day 29 of the recovery phase).

Hematology: The only differences considered GS-5816-related were mildly decreased fibrinogen and globulin concentrations and mildly higher albumin:globulin ratio for females administered 100 mg/kg/day. These GS-5816-related effects were not apparent until Day 274 of the dosing phase; males were not similarly affected. The changes were not considered adverse because of their small magnitudes and absence of correlative findings.

Clinical Chemistry: No changes were noted.

Urinalysis: Urine samples were collected overnight in containers chilled on wet ice for urinalysis on Day 9 of the predose phase and on the days of scheduled sacrifice (Days 92 [Week 14] and 274 [Week 40] of the dosing phase and Day 29 of the recovery phase) from animals fasted overnight. No changes.

Gross Pathology: After 13 or 39 weeks of dosing (on Day 92 of the dosing phase), three animals/sex/group, having been fasted overnight, were anesthetized with sodium pentobarbital, exsanguinated, and necropsied. An examination of the external features of the carcass; external body orifices; abdominal, thoracic, and cranial cavities; organs; and tissues was performed. No GS-5816-related macroscopic observations were present at the interim, terminal, or recovery necropsy.

Organ Weights: No GS-5816-related organ weight changes were noted at interim, terminal, or recovery necropsy. Organ weight parameters were increased for the prostate gland (only organ:body weight ratio was significant) at the interim necropsy in males administered 100 mg/kg/day. Increased prostate gland weights at the interim necropsy were considered nonadverse and occurred without a dose response or specific microscopic correlation.

Histopathology: Adequate Battery: yes Peer Review: yes

Histological Findings: No GS-5816-related microscopic observations were present at the interim, terminal or recovery necropsy. Non-test article-related vascular inflammation (involving a single tissue) was present at the terminal necropsy in the epididymis of one male administered 20 mg/kg/day and at the recovery necropsy in the kidney, heart (coronary artery), and stomach of one control female and one male and female administered 100 mg/kg/day. Given the presence of vascular inflammation in a control animal, the common occurrence reported in literature and ^{(b) (4)} historical control data (up to 50% incidence), and the relatively prolonged study duration (13 or 39 weeks), the single incidences of vascular inflammation (minimal to slight) in animals from the terminal or recovery necropsies were considered incidental and not related to GS-5816.

Toxicokinetics: Blood samples were collected for hematology, coagulation, and clinical chemistry from fasted animals via a jugular vein on Days 1, 85 (Week 13), and 272 (Week 39) of the dosing phase predose (Days 85 and 272 only) and approximately 0.5, 1, 2, 4, 6, 8, 12, and 24 hours postdose. Animals were not fasted prior to collections. Exposure to GS-5816 increased with the increase in dose level from 5 to 100 mg/kg/day (sponsor's table below). Increases in C_{max} and AUC_{0-t} were generally dose-proportional between 5 and 20 mg/kg/day and less-than-dose proportional between 20 and 100 mg/kg/day. Sex-based differences were less than 2-fold, and no accumulation of GS-5816 was observed after multiple dosing.

Table 33: Mean Toxicokinetic Parameters for GS-5816 in Dog Plasma - Day 1, Week 13 and Week 39

Interval	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)
Day 1	2	5	M	335	1.57	1880	22.3	5.47
			F	231	1.57	1250	17.1	9.56
			MF	283	1.57	1560	19.7	7.51
	3	20	M	946	3.14	8610	24.0	33.5
			F	1170	4.29	10900	24.0	31.9
			MF	1060	3.71	9740	24.0	32.7
	4	100	M	2370	7.11	32800	24.0	246
			F	2040	5.33	25300	24.0	123
			MF	2200	6.22	29000	24.0	185
Week 13	2	5	M	303	1.14	1500	22.3	6.47
			F	326	1.43	1720	24	5.97
			MF	314	1.29	1610	23.1	6.22
	3	20	M	929	2.29	8700	24.0	96.3
			F	1050	2.71	10500	24.0	54.3
			MF	990	2.50	9600	24.0	75.3
	4	100	M	2090	5.78	28600	24.0	224
			F	2060	6.44	31100	24.0	228
			MF	2080	6.11	29800	24.0	226
Week 39	2	5	M	273	1.50	1500	24.0	3.30
			F	356	1.75	1950	24.0	6.82
			MF	314	1.63	1730	24.0	5.06
	3	20	M	859	2.50	6380	24.0	19.2
			F	1050	2.00	7030	24.0	20.2
			MF	955	2.25	6700	24.0	19.7
	4	100	M	1940	5.20	25500	24.0	297
			F	2170	6.33	29800	24.0	178
			MF	2060	5.82	27800	24.0	232

F = Female; M = Male.

Note: Combined (MF) data are based on the combined mean data of both sexes.

Stability and Homogeneity: Overall mean concentrations of all test article formulations prepared for dose administration on Day 1 of the dosing phase ranged from 106.1 to 107.3% of the nominal concentration. The analyzed formulations met the acceptance criteria indicating the formulations were homogeneous.

7 Genetic Toxicology

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

Study title: Bacterial Reverse Mutation Assay with a Confirmatory Assay with GS-5816

Study no.:

TX-281-2005

Study report location:

electronic

Conducting laboratory and location:

(b) (4)

Date of study initiation:

March 9, 2012

GLP compliance:

Yes

QA statement:

Yes

Drug, lot #, and % purity: GS-5816, 5126-182-28, 97.7%

Key Study Findings: GS-5816 was negative in the Bacterial Reverse Mutation Assay with a Confirmatory Assay when tested under the conditions of the test protocol up to dose precipitating concentrations and the 5000 µg/plate level.

Methods

Strains: *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and at the tryptophan locus of *Escherichia coli* (*E. coli*) strain WP2uvrA

Concentrations in definitive study: 16.0, 50.0, 160, 500, 1600, 3330, and 5000 µg/plate in the presence and absence of S9 metabolic activation

Basis of concentration selection: Dose range study

Negative control: dimethylsulfoxide (DMSO)

Positive control: 2-nitrofluorene, sodium azide, ICR-191, 4-nitroquinoline N-oxide, benzo[a]pyrene, 2-aminoanthracene

Formulation/Vehicle: The vehicle control article was dimethylsulfoxide (DMSO)

Incubation & sampling time: Overnight cultures were inoculated into flasks containing culturing broth and the flasks were placed in a shaker/incubator programmed to begin operation (shaking, 125 rpm; incubation, 37 degree C) so that overnight cultures were in late log phase when optical density (OD) monitoring began.

Methods: Treatments were performed by adding 100 µL tester strain and 50 µL of test or vehicle control article to 2.5 mL of molten selective top agar (maintained at 45 degree C). After the required components had been added, the mixture was vortexed and overlaid onto the surface of 25 mL minimal bottom agar in a 15 x 100 mm petri dish. After the overlay solidified, the plates were inverted and incubated for 52 hours at 37 degree C. Plates not evaluated immediately were held at 2 to 8 degree C until such time that colony counting and bacterial background lawn evaluation could take place.

Study was valid.

Results: There were no GS-5816 treatment-related increases in mean revertant frequencies compared to vehicle control values with or without S9. Results of the confirmatory test essentially reproduced what was determined in the initial test and indicate a negative response. All vehicle control values were within the acceptable range defined by historical control data and positive controls showed robust response to treatment meeting criteria for a valid study.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes with GS-5816

Study no.: TX-281-2006
Study report location: electronic
Conducting laboratory and location: (b) (4)
Date of study initiation: March 9, 2012
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: GS-5816, 5126-182-28, 97.7%

Key Study Findings: GS-5816 under the conditions of this test protocol is negative for inducing chromosomal aberrations in cultured human lymphocytes without and with metabolic activation when tested up to dose precipitating concentrations and the 500 µg/mL limit dose.

Methods

Cell line: cultured human peripheral blood lymphocytes
Concentrations in definitive study: 500, 350, 245, 172, 120, 84, 58.8, 41.2, and 28.8 µg/mL for approximately 24 hours without metabolic activation and 500, 350, 245, 172, 120, and 84.0 µg/mL for 3 hours with metabolic activation. All cultures were harvested approximately 24 hours from the initiation of treatment.
Basis of concentration selection: Dose range study
Negative control: DMSO
Positive control: Mitomycin C (MMC) and cyclophosphamide (CP)
Formulation/Vehicle: The vehicle control article was dimethylsulfoxide (DMSO)
Incubation & sampling time: In the confirmatory chromosomal aberrations assay, the treatment period was approximately 24 hours without metabolic activation and 3 hours with metabolic activation

Methods: For the assay without metabolic activation, 2 days after culture initiation, cells were incubated at 37 degree C with the test article at predetermined concentrations, vehicle and positive controls for approximately 24 hours with 0.1 µg/mL Colcemid added for the last 2 hours of incubation. The cultures were then harvested. For the assay with metabolic activation, 2 days after culture initiation, cultures were incubated at 37 degree C for 3 hours in the presence of the test article at predetermined concentrations, vehicle and positive controls, and the S9 activation mix. The cultures were then washed with

phosphate-buffered saline. The cells were incubated, for the rest of the culture period up to the time of harvest, with 0.1 µg/mL Colcemid® during the last 2 hours of treatment. The cultures were then harvested (approximately 24 hours after initiation of treatment).

Study was valid.

Results: The 500 µg/mL high dose showed a 0% reduction in the mitotic index compared with the concurrent vehicle control cultures (sponsor's table below). Precipitate at dosing was observed starting at the 84.0 µg/mL treatment level. Precipitate remained in cultures through wash in the 245, 350, and 500 µg/mL cultures. There were no significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication observed in any GS-5816 treatment level analyzed. There were no relevant reductions in the mitotic indices of any GS-5816 treated culture compared to concurrent vehicle controls. There were no significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication in any GS-5816 treatment level analyzed. The vehicle control cultures were in the historical control range for cells with chromosomal aberrations and the positive control cultures had significant increase in cells with chromosomal aberrations as compared with the vehicle control cultures. The 500 µg/mL high doses selected for analysis in the assays showed precipitate in cultures and met the high dose limit.

Table 34: Confirmatory Assessment of Toxicity (GS-5816) for Chromosomal Aberrations Assay

Treatment		Dose	Units	% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Index Reduction
Vehicle Control	DMSO	10.0	µL/mL	9.4	9.9	9.7	0
Positive control	CP	20.0 ^a	µg/mL	5.2	5.5	5.4	44
		25.0	µg/mL	5.9	5.1	5.5	43
		40.0	µg/mL	0.7	3.3	2.0	79
Test Article		84.0 ^b	µg/mL	11.2	11.6	11.4	0
		120 ^b	µg/mL	9.4	10.1	9.8	0
		172 ^b	µg/mL	11.4	11.1	11.3	0
		245 ^c	µg/mL	9.8	10.0	9.9	0
		350 ^c	µg/mL	10.3	9.7	10.0	0
		500 ^c	µg/mL	9.7	10.1	9.9	0

CP = cyclophosphamide; DMSO = dimethylsulfoxide.

^a Precipitate observed at harvest.

^b Precipitate observed at dose.

^c Precipitate observed at dose and wash.

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

Study title: Rat Micronucleus Assessment Following 2-Week Oral

Administration of GS-5816

Study no: TX-281-2003
Study report location: Electronic
Conducting laboratory and location: (b) (4)
Date of study initiation: March 7, 2012
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: GS-5816, 5126-180-38, 91.6%

Key Study Findings: No GS-5816 related effects were noted following oral administration for 2 weeks at doses up to 200 mg/kg/day to male and female rats. GS-5816 did not induce statistically significant increases in micronucleated PCEs.

Methods

Doses in definitive study: 20, 60 and 200 mg/kg/day
Frequency of dosing: Daily for 14 days
Route of administration: Oral Gavage
Dose volume: 10 mL/kg
Formulation/Vehicle: 45% (v/v) propylene glycol, 15% (v/v) Solutol HS-15 prepared in reverse osmosis water, pH 2.0
Species/Strain: SD Rats
Number/Sex/Group: 5
Satellite groups: TK group
Basis of dose selection: Dose range study
Negative control: Vehicle
Positive control: Cyclophosphamide

Results: There were no GS-5816-related effects. GS-5816 showed no signs of bone marrow cytotoxicity (no decreases in the PCE:NCE ratios). GS-5816 did not induce biologically relevant increases in micronucleated PCEs at any dose level.

The vehicle control micronucleated PCE value of approximately 0.06% was within the vehicle historical control range showing a normal background level in this test. The positive control, cyclophosphamide, induced a statistically significant increase in micronucleated PCEs, confirming the validity of the assay.

Therefore, GS-5816 was evaluated as negative in the rat bone marrow micronucleus assay when tested up to 200 mg/kg/day and under the conditions of this assay.

8 Reproductive and Developmental Toxicology

8.1 Fertility and Early Embryonic Development

Study title: Oral Gavage Study of Fertility and Early Embryonic Development to Implantation with GS-5816 in Rats

Study no.: TX-281-2012
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 18 June 2013
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: GS-5816, PP-1013-2001, 98.2%

Key Study Findings: Oral administration of GS-5816 to male and female rats prior to mating and until termination (males) or through early gestation (females) at 0, 20, 60, or 200 mg/kg/day had no effect on reproduction. Based on these results, the NOAEL for fertility and early embryonic development in rats was 200 mg/kg/day.

Methods

Doses: 0 (vehicle control), 20, 60, or 200 mg/kg/day via oral gavage
Frequency of dosing: once daily
Dose volume: 5 ml/kg
Route of administration: oral
Formulation/Vehicle: (45% [v/v] propylene glycol and 15% [v/v] Kolliphor HS-15 prepared in water, pH of 2.0.
Species/Strain: Male and female CrI:CD(SD) rats
Number/Sex/Group: 22/group/sex
Satellite groups: None
Study design: Females were dosed for at least 14 days prior to mating, throughout the mating period and through Gestation Day (GD) 7. On GD 13, females were necropsied and the uterus of each was examined for the number of live and dead fetuses and resorptions, and ovaries were examined for the number of corpora lutea. Males were dosed for at least 28 days prior to mating, during the mating period and through the day prior to termination after at least 10 weeks of dosing, and evaluated for reproductive capacity.
Deviation from study protocol: None

Observations and Results

Mortality: Animals were checked twice daily for mortality, abnormalities, and signs of pain or distress. No GS-5816-related mortality was observed.

Clinical Signs: Cageside observations were performed for each animal at approximately 1 hour postdose, during the first 2 weeks of dosing. Unscheduled observations were recorded, and detailed observations were performed daily until study termination. During the dosing phase detailed observations were performed prior to dosing. No clinical observations were considered GS-5816-related in males or females.

Body Weight: Body weights were recorded on Gestation Day (GD) 0, 3, 7, 10, and 13. There were no test article-related effects on body weight for males or females during the pre-mating phase. GS-5816 had no effect on female body weight or body weight gain throughout gestation.

Feed Consumption: Food consumption was measured weekly. There were no GS-5816-related effects on food consumption for males or females over the assessed time period.

Toxicokinetics: Not reported.

Dosing Solution Analysis: Formulations prepared for the first day and Week 3, 7, and 10 of dosing ranged from 94.4% to 101.5%; results were within the acceptance criteria of $\pm 10\%$ of the respective theoretical concentrations.

Necropsy: On GD 13, confirmed-mated females were sacrificed by carbon dioxide inhalation followed by exsanguination. Any grossly abnormal cervical, thoracic, or abdominal viscera were noted. The uterus from each gravid animal was excised and examined for the number and placement of live fetuses, the number of early and late resorptions, and any abnormalities. The uterine contents were then discarded. The right and left ovaries from each gravid female were examined for the number of corpora lutea. For apparently non-gravid females, the uterus was pressed between two glass slides and examined for implantation sites. After at least 10 weeks of dosing, all males were weighed, anesthetized with carbon dioxide inhalation followed by exsanguination. A necropsy was performed on each animal. No macroscopic findings noted were considered GS-5816-related.

Reproductive Indices: Animals from respective groups were mated by placing one female in the breeding cage of a male from the same dose group. A record of mating pairs was maintained. Once mating had occurred, the males and females were separated. The mating phase was a total of two weeks, with females changing to a new (proven) male for the second week if they did not mate after the first week. During mating, a daily inspection was made for the presence of a retained copulatory plug. Females not found with retained copulatory plugs were evaluated for vaginal sperm by lavage. The day sperm or plug(s) was observed was designated as GD 0. GS-5816 did not affect female reproductive performance.

At the scheduled sacrifice the first ten surviving males and any males that failed to impregnate at least one female partner from each dose group were evaluated for reproductive capacity. Sperm motility and sperm concentration were evaluated using the computer-assisted sperm analysis system IVOS in the epididymal fluid. Male reproductive performance was not affected by GS-5816 administration; mating, fertility, and fecundity indices were similar in all groups.

Estrous Cycle: Daily vaginal lavage was used to determine the stage of estrous for two weeks prior to dose initiation and for the first two weeks of dosing prior to the initiation of mating. No GS-5816-related effects on estrous cycle were observed. The number of cycles and mean cycle lengths were similar across all groups.

Cesarean Section: There were no GS-5816-related effects on any cesarean section parameters examined, including effects on numbers of corpora lutea, implantation sites, resorptions, or number of live fetuses.

Organ Weights: No GS-5816-related effects on organ weights and organ-to-body weight percentages were observed in males or females.

Sperm Motility & Concentration: No GS-5816-related effects on sperm motility were observed in males. There were no effects of GS-5816 on sperm concentration.

8.2 Embryonic Fetal Development

Study title: An Oral (Gavage) Study of the Effects of GS-5816 on Embryo-Fetal Development in CD-1 Mice

Study no.:	TX-281-2032
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	12 December 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-5816, 201407220267, 98.7%

Key Study Findings: No maternal or embryo/fetal developmental toxicity was observed at dosages up to 1000 mg/kg/day. A dosage level of 1000 mg/kg/day was considered to be the (NOAEL) for maternal toxicity and embryo/fetal development in mice.

Methods	
Doses:	0, 30, 100, or 1000 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 ml/kg
Route of administration:	Oral

Formulation/Vehicle: 0.2% [w/v] hydroxypropyl methylcellulose [HPMC] and 0.2% [v/v] Tween® 20 in deionized water

Species/Strain: Crl:CD-1 mice

Number/Sex/Group: 25/group. Additional 6-36/ for TK

Satellite groups: None

Study design: mated Crl:CD-1 mice (25/group) were dosed during organogenesis (gestation days [GD] 6-15.

Deviation from study protocol: None

Observations and Results

Mortality and Clinical Signs: All mice were observed twice daily, once in the morning and once in the afternoon, for moribundity and mortality. Individual clinical observations were recorded daily from GD 0 through 18 (prior to dose administration during the treatment period). Animals were also observed for signs of toxicity between 3-4 hours following dose administration. No mortality or test article-related clinical findings were noted. One and 2 females in the vehicle control and 30 mg/kg/day groups, respectively, delivered on GD 18.

Body Weight: Individual maternal body weights were recorded on GD 0 and 6-18 (daily) for the embryo/fetal development phase and on GD 0 and 6-15 (daily) for the toxicokinetic phase. Group mean body weights were calculated for each of these days. Mean body weight changes were calculated for each corresponding interval and also for GD 6-9, 9-12, 12-16, 6-16, and 16-18 for the embryo/fetal development phase animals. There were no test article-related effects on body weight or body weight change.

Feed Consumption: Individual food consumption was recorded on GD 0 and 6-18 (daily). No effect on food consumption was observed in GS-5816-treated dams.

Toxicokinetics: Blood samples (as much as possible) for toxicokinetics were collected on GD 6 and 15 from all animals in the vehicle control group at 4 hours following dose administration and from animals in the test article-treated groups at 0 (pre-dose, GD 15 only), 1 (GD 6 only), 2, 4, 8, 12, and 24 hours after dose administration. Exposure to GS-5816 generally increased with the increase in dosage level from 10 to 1000 mg/kg/day (sponsor's table below). The increases in C_{max} and AUC_{0-t} were less than dose proportional between the 10 and 1000 mg/kg/day dosage levels. Mean concentrations of GS-5816 were generally similar after multiple doses when compared to a single dose. GS-5816 was readily absorbed, with T_{max} values ranging from 1.00 to 4.00 hours on GD 6 and from 2.00 to 4.00 hours on GD 15. Values for C_{max} and AUC_{0-t} were generally similar on GD 6 and GD 15, indicating no accumulation of GS-5816 after multiple doses in pregnant mice.

Table 35: Toxicokinetic Parameters for GS-5816 in the Plasma of Pregnant Mice: GD 6 and 15

Interval	Dose Group	Dose Level (mg/kg/day)	C _{max} (ng/mL)	AUC _{0-t} (ng•hr/mL)
GD 6	2	10	2100	7550*
	3	30	4650	22,700*
	4	100	9240	56,300
	5	1000	13,500	156,000
GD 15	2	10	1730	6820
	3	30	4070	22,100
	4	100	6560	43,700
	5	1000	10,300	93,000

GD = Gestation day

* = T_{last} is less than 24 hours

Dosing Solution Analysis: All homogeneity and concentration verification results met acceptance criteria.

Necropsy: Laparohysterectomies and macroscopic examinations were performed blind to treatment group. All females (including the females that delivered) were euthanized on GD 18 by carbon dioxide inhalation. The thoracic, abdominal, and pelvic cavities were opened by a ventral mid-line incision, and the contents were examined. The uterus and ovaries were then exposed and excised. The number of corpora lutea on each ovary was recorded (using magnification). The trimmed uterus was weighed and opened, and the number and location of all fetuses, early and late resorptions, and the total number of implantation sites were recorded. The placentae were also examined. There were no test article-related macroscopic observations in any animals at scheduled GD 18 necropsy. There were no effects of GS-5816 on reproductive performance. Female no. 3350 (vehicle control) and female nos. 3441 and 3473 (30 mg/kg/day) delivered on GD 18, these females had no remarkable macroscopic findings.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.):

All animals produced viable litters and cesarean section data were similar across vehicle control and GS-5816-treated groups.

Offspring (Malformations, Variations, etc.): Fetal examinations were performed blind to treatment group. The detailed external examination of each fetus included, but was not limited to, an examination of the eyes, palate, and external orifices, and each finding was recorded. Crown-rump measurements and degrees of autolysis were recorded for late resorptions, a gross external examination was performed (if possible), and the tissues were discarded. Following examination of the fetuses, no external, visceral, or skeletal variations or malformations were attributed to GS-5816 exposure.

Study title: Oral Gavage Study for Effects on Embryo-Fetal Development with GS-5816 in Rats

Study no.: TX-281-2013
Study report location: DARRTS
Conducting laboratory and location: [REDACTED] (b) (4)
Date of study initiation: 25 July 2013
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: GS-5816, PP-1013-2001, 98.5%

Key Study Findings: Oral administration of GS-5816 to pregnant rats during the period of organogenesis at 20, 60, or 200 mg/kg/day showed no effects on maternal or embryofetal toxicity, and the NOAEL for developmental toxicity in rats was 200 mg/kg/day.

Methods

Doses: 0, 20, 60, or 200 mg/kg/day
Frequency of dosing: Once daily
Dose volume: 5 ml/kg
Route of administration: Oral
Formulation/Vehicle: (45% [v/v] propylene glycol and 5% [v/v] Kolliphor HS-15 prepared in reverse osmosis [RO] water, pH 2.0
Species/Strain: Crl:CD(SD) rats
Number/Sex/Group: 22/group
Satellite groups: None
Study design: Time-mated female Crl:CD(SD) rats were assigned to four groups. GS-5816 at 20, 60, or 200 mg/kg/day by once daily oral gavage on GD 6 through GD 17 at a dose volume of 5 mL/kg.
Deviation from study protocol: None

Observations and Results

Mortality: Each animal was observed twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress. All animals on study survived to scheduled termination on GD 21.

Clinical Signs: On GD 0, the supplier provided documentation as to whether mated females appeared normal. This data was maintained in the raw data records. Daily cageside observations were performed on all animals in the a.m. and postdose observations were made for each animal on GD 6-17 at approximately 4 hours postdose based on the last animal dosed per group. Detailed observations beginning on GD 4 were made daily for each animal. During the dosing phase, detailed observations were performed prior to dosing. No clinical observations were considered test article-related.

Body Weight: Animals were weighed on GD 0, 4, 6, 8, 10, 12, 14, 16, 18, and 21, as appropriate. The animal supplier provided the GD 0 body weights for entry into the computer data collection system. There were no test article-related effects on body weight or body weight change.

Feed Consumption: Beginning on GD 4, food consumption was measured at body weight intervals. No effect on food consumption was observed in GS-5816-treated dams.

Toxicokinetics: Blood samples were collected from the Toxicokinetic animals (TX-281-2009) via a jugular vein on GD 6 and GD 17: Control (three/group) and 1st three/GS-5816-treated Group, predose (GD 17 only) and approximately 4 and 12 hours postdose; 2nd three/ GS-5816-treated group, approximately 0.5, 2, and 24 hours postdose; last three/ GS-5816-treated group: approximately 1 and 8 hours postdose. Exposure to GS-5816 increased with the increase in dose level from 20 to 200 mg/kg/day (sponsor's table below). The increases in C_{max} and AUC_{0-t} were roughly dose proportional between the 20 and 60 mg/kg/day dose levels and less than dose proportional between the 60 and 200 mg/kg/day dose levels. GS-5816 was readily absorbed, with T_{max} values ranging from 4.00 to 8.00 hours on GD 6 and a value of 4.00 hours on GD 17. Values for C_{max} and AUC_{0-t} were slightly higher on GD 17 than on GD 6.

Table 36: Mean Toxicokinetic Parameters for GS-5816 in Plasma of Pregnant Rats: GD 6 and 17 (TX-281-2009)

Interval	Dose Group	Dose Level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng·hr/mL)
GD 6	2	20	308	4.00	2170
	3	60	880	4.00	6840
	4	200	1300	8.00	12100
GD 17	2	20	550	4.00	3830
	3	60	1120	4.00	9300
	4	200	1750	4.00	17100

Dosing Solution Analysis: All homogeneity and concentration verification results met acceptance criteria.

Necropsy: On GD 21, rats were sacrificed by carbon dioxide inhalation followed by exsanguination. Uterine contents were examined. Any grossly abnormal cervical, thoracic, or abdominal viscera were noted. Abnormalities of the placenta or amniotic sac were described. The uterus from each gravid animal was excised, weighed, and

examined for the number and placement of live and dead fetuses, the number of early or late resorptions, and any abnormalities. The uterus was not reweighed after the contents were removed. The right and left ovaries from each gravid female were examined for the number of corpora lutea. The uterus from the nonpregnant female was pressed between two glass slides and examined for implantation sites. There were no test article-related macroscopic observations in any animals at scheduled GD 21 necropsy. There were no effects of GS-5816 on reproductive performance.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.):

All animals produced viable litters and cesarean section data were similar across vehicle control and GS-5816-treated groups.

Offspring (Malformations, Variations, etc.): Each fetus (live or dead) was weighed and examined for external abnormalities. Live fetuses were sacrificed via injection by an appropriate barbiturate. Approximately one half of all fetuses from each litter were processed for visceral examination. Findings were judged to be variations or malformations. Malformations are developmental deviations which (1) are gross structural changes, (2) are incompatible with life, or (3) may affect the quality of life. Variations are structural deviations which are thought to have no effect on body conformity or the well-being of the animal. Following examination of the fetuses, no external, visceral, or skeletal variations or malformations were attributed to GS-5816 exposure.

Study title: Oral Gavage Study for Effects on Embryo-Fetal Development and Toxicokinetics with GS-5816 in Rabbits

Study no.:	TX-281-2014
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	22 July 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-5816, PP-1013-2001, 98.2%

Key Study Findings: Oral administration of GS-5816 to pregnant NZW rabbits (GD 7-20) resulted in a single test article-related death at 300 mg/kg/day. There was no evidence of fetal toxicity at any dose administered. The NAOEL for maternal toxicity was 100 mg/kg/day and the NOAEL for embryo-fetal development was 300 mg/kg/day (GD20 C_{max} and AUC_{0-t}: 218 ng/mL and 2060 ng.hr/mL, respectively).

Methods

Doses:	0, 30, 100, or 300 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 ml/kg
Route of administration:	Oral

Formulation/Vehicle: 0.5% (w/v) hydroxypropyl methylcellulose (HPMC), 0.1% (v/v) Tween 20 and 0.9% (v/v) benzyl alcohol in reverse osmosis (RO) water

Species/Strain: Time-mated female Hra:(NZW)SPF rabbits

Number/Sex/Group: 25/group

Satellite groups: None

Study design: Time-mated female rabbits were assigned to four groups (25/group) and administered either the vehicle control or GS-5816 at 30, 100, or 300 mg/kg/day by once daily oral gavage on GD 7 through GD 20 at a dose volume of 10 mL/kg.

Deviation from study protocol: None

Observations and Results

Mortality: Each animal was observed twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress. One animal (Animal No. F01359) in the 300 mg/kg/day group was euthanized in moribund condition on GD 21; the death was considered test article-related. The animal had prior clinical signs of thin appearance, few/no feces, rough hair coat, general debilitation and weight loss (~400 g) from GD 13-21 and low food consumption. All other animals on study survived to scheduled termination on GD 29.

Clinical Signs: On GD 0, the supplier provided documentation as to whether mated females appeared normal. This data was maintained in the raw data records. Daily cageside observations were performed on all animals in the a.m. and postdose observations were made for each animal on GD 7-20 at approximately 4 hours postdose based on the last animal dosed per group. Detailed observations beginning on GD 4 were made daily for each animal. During the dosing phase, detailed observations were performed prior to dosing. With the exception of the single moribund animal, there were no additional clinical findings related to GS-5816 exposure.

Body Weight: Animals were weighed on GD 0, 4, 7, 9, 11, 13, 15, 18, 21, 24, 27, and 29, as appropriate. The animal supplier provided the GD 0 body weights for entry into the computer data collection system. There were no test article-related effects on body weight or body weight change.

Feed Consumption: Beginning on GD 4, food consumption was measured at body weight intervals. No effect on food consumption was observed in GS-5816-treated dams.

Toxicokinetics: Blood samples (approximately 0.5 mL) were collected via medial auricular artery on GD 7 and GD 20. Samples were collected predose (on GD 20 only) and approximately 1, 2, 4, 8, 12 and 24 hours postdose of the dosing phase. GS-5816 was readily absorbed, with mean T_{max} values ranging from 3.33 to 4.10 hours on GD 7 and a value of 4.00 hours on GD 20. Mean C_{max} and AUC_{0-t} were generally 2.3- to 6.6-fold higher on GD 20 than on GD 7 in pregnant rabbits (sponsor's table below).

Table 37: Mean Toxicokinetic Parameters for GS-5816 in Plasma of Pregnant Rabbit: GD 7 and 20

Interval	Dose Group	Dose Level (mg/kg/day)		C _{max} (ng/mL)	T _{max} (hr)	AUC _{0-t} (ng-hr/mL)	T _{last} (hr)	C _{last} (ng/mL)
GD 7	2	30	Mean	NA	NA	NA	NA	NA
			SD	NA	NA	NA	NA	NA
			N	1	1	1	1	1
	3	100	Mean	107	3.33	739	20.0	6.60
			SD	20.7	1.15	206	6.93	3.78
			N	3	3	3	3	3
	4	300	Mean	66.7	4.10	442	24.0	4.80
			SD	NA	NA	NA	NA	NA
			N	2	2	2	2	2
GD 20	2	30	Mean	NA	NA	NA	NA	NA
			SD	NA	NA	NA	NA	NA
			N	1	1	1	1	1
	3	100	Mean	258	4.00	1940	24.0	11.5
			SD	171	0	915	0	6.42
			N	3	3	3	3	3
	4	300	Mean	218	4.00	2060	24.0	39.2
			SD	NA	NA	NA	NA	NA
			N	2	2	2	2	2

NA Not applicable (Note: Mean and SD values not calculated as only one animal was pregnant.)

Dosing Solution Analysis: All homogeneity and concentration verification results met acceptance criteria.

Necropsy: Cesarean sections were conducted on GD 29. Rabbits were sacrificed by administration of Beuthanasia® followed by exsanguination. Uterine contents were examined. The uterus from each gravid animal was excised, weighed, and examined for the number and placement of live and dead fetuses, the number of early or late resorptions, and any abnormalities. The uterus was not reweighed after the contents were removed. The right and left ovaries from each gravid female were examined for the number of corpora lutea. No macroscopic findings were considered related to GS-5816 exposure. Discolored uterus was observed in one 100 mg/kg/day animal only and was considered spontaneous. There were no effects of GS-5816 on reproductive performance. Several does were not pregnant (2, 6, 3 and 3 in the 0, 30, 100 and 300 mg/kg/day groups, respectively) but this was unrelated to GS-5816 treatment due to the absence of a dose-response and similar occurrence in control animals.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.):

There were no GS-5816 related effects on any cesarean section parameter examined. In addition, all of the fetuses were viable in all groups at cesarean section.

Offspring (Malformations, Variations, etc.): Each fetus (live or dead) was weighed and examined for external abnormalities. Live fetuses were sacrificed. Approximately one half of all fetuses from each litter were processed for visceral examination. Findings were judged to be variations or malformations. Malformations are developmental deviations which (1) are gross structural changes, (2) are incompatible with life, or (3) may affect the quality of life. Variations are structural deviations which are thought to have no effect on body conformity or the well-being of the animal. There were no GS-5816-related effects on fetal external, visceral, and skeletal variations or malformations.

8.3 Prenatal and Postnatal Development

Study title: An Oral (Gavage) Study of the Effects of GS-5816 on Pre- and Postnatal Development, Including Maternal Function, in Rats

Study no.:	TX-281-2027
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	17-Mar-2014
GLP compliance:	Yes
QA statement:	Yes (final report)
Drug, lot #, and % purity:	GS-5816, PP-1013-2001, 98.2%

Key Study Findings: No test article-related effects were noted at any dosage level during the study. 200 mg/kg/day was considered to be the NOAEL for F0 maternal systemic toxicity, F1 neonatal/developmental toxicity, F1 parental systemic toxicity, F1 reproductive toxicity, and F2 neonatal/early postnatal toxicity of GS-5816 when administered orally to rats. The NOAEL corresponds to C_{max} values of 1670 ng/mL and 29.7 ng/mL and AUC_{0-t} values of 13900 ng·h/mL and 583 ng·h/mL, for F0 females (LD 10) and F1 pups (PND 10), respectively.

Methods

Doses:	0 (vehicle control), 20, 60, 200 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	Oral gavage
Route of administration:	5 ml/kg
Formulation/Vehicle:	45% propylene glycol, 15% Kolliphor HS-15 in deionized water, pH 2.0
Species/Strain:	Rat/Crl:CD(SD) Sprague Dawley
Number/Sex/Group:	25 females/group
Satellite groups:	3-9/ females group

Study design:

- GS-5816 administered to pregnant females from gestation day (GD) 6 to lactation day (LD) 20 and sacrificed on LD21. F₁ pups not selected for F₁ generation sacrificed on PND21.
- F₁ generation split into subsets A or B (see Sponsor table below). F₁ pups not selected for breeding phase sacrificed after attainment of developmental landmarks.
- Laparohysterectomies performed on GD 20 (F₂ generation morphological assessment) for F₁ animals selected for breeding phase (reproductive assessment). F₁ males sacrificed.

Deviation from study protocol: None that affected the integrity or conclusions of the study.

Observations and Results

F0 (Pre-and Postnatal Development Phase)

Survival: All rats were observed twice daily, once in the morning and once in the afternoon, for moribundity and mortality. There were no test article-related effects on survival at any dosage level. One F0 female (no. 11885) in the 60 mg/kg/day group was euthanized *in extremis* on LD 5 following clinical observations of piloerection, decreased defecation, a thin body, red material around the urogenital area, and a swollen urogenital area during the daily examinations on the day of euthanasia. Due to the single occurrence and a lack of a dose response, the moribundity of this animal was not attributed to test article administration. All other F0 maternal animals in the vehicle control, 20, 60, and 200 mg/kg/day groups survived to the scheduled necropsy.

Clinical Signs: Individual clinical observations were recorded daily (prior to test article administration during the treatment period) for each F0 female from GD 0 until necropsy. All animals were also observed for signs of toxicity approximately 1.5 hours following dose administration each day. Females expected to deliver were also observed twice daily during the period of expected parturition and for dystocia (prolonged labor, delayed labor, or other difficulties). No test article-related clinical findings were noted for surviving F0 females at any dosage level.

Body Weights: Individual maternal body weights were measured on GD 0, 6, 9, 12, 15, 18, and 20 (both phases) and on LD 0 (when possible), 1, 4, 7, 10, 14, 17, and 21 (pre- and postnatal development phase) or LD 0 (when possible), 1, 4, 7, and 10 (toxicokinetic phase). Group mean body weights were calculated for each of these days. Group mean body weight changes were calculated for the pre- and postnatal development phase for each corresponding interval of gestation and lactation, and also for GD 6-20 and for LD 1-21. Mean body weights and body weight gains were unaffected by test article administration during gestation or lactation.

Food Consumption: Individual maternal food consumption was recorded on GD 0, 6, 9, 12, 15, 18, and 20 and on LD 1, 4, 7, 10, 14, 17, and 21. Food intake was reported as g/animal/day and g/kg/day for the corresponding intervals of gestation and lactation, and also for GD 6-20 and LD 1-21. Mean food consumption, evaluated as g/animal/day and g/kg/day, was unaffected by test article administration during gestation or lactation.

Gestation Length and Parturition: All females were allowed to deliver naturally and rear their young until euthanasia (LD 11, toxicokinetic phase) or weaning (LD 21, pre- and postnatal development phase). During the period of expected parturition, the females were observed twice daily for initiation and completion of parturition and for signs of dystocia. No test article-related effects were noted on mean gestation lengths or the process of parturition at any dosage level.

Macroscopic Examination: All surviving females with viable pups on LD 21 or that did not deliver (post-mating day 25) were euthanized by carbon dioxide inhalation. A gross necropsy was performed for each of these females; the thoracic, abdominal, and pelvic cavities were opened and the contents were examined. For females that delivered, the numbers of former implantation sites (the attachment site of the placenta to the uterus) were recorded. The number of unaccounted-for sites was calculated for each female that delivered by subtracting the number of pups born from the number of former implantation sites observed. For females that failed to deliver, a pregnancy status was determined, and specific emphasis was placed on anatomic or pathologic findings that may have interfered with pregnancy. In the 60 mg/kg/day group, F0 female no. 11885 was euthanized *in extremis* on LD 5. Macroscopically, this female was noted with discolored adrenal glands, reddened renal cortico-medullary junction, distended urinary bladder with dark red contents, and discolored vagina with dark red contents. The dark red contents in the urinary bladder and vagina correlated with clinical findings of red material around the urogenital area noted at the daily examinations.

No internal findings were observed at any dosage level for females that failed to deliver or that survived to the scheduled necropsy on LD 21.

Toxicokinetics: F0 maternal and F1 pup blood samples (approximately 0.5 mL each) for toxicokinetics were collected on GD 6 and LD 10 from test article-treated groups at the following time points: prior to dose administration (LD 10 only) and at approximately 0.5, 1, 2, 4, 8, 12, and 24 hours following dose administration. In addition, a single blood sample (approximately 0.5 mL each) was collected from the vehicle control group at approximately 4 hours following dose administration. The mean concentration-time profiles for F0 females show that exposure to GS-5816 generally increased with the increase in dose level from 20 to 200 mg/kg/day (sponsor's table below). The increases in the F0 C_{max} and AUC_{0-t} values were approximately dose proportional between 20 to 60 mg/kg/day and less than dose proportional with further increase in the dose level to 200 mg/kg/day. Values for C_{max} and AUC_{0-t} in F0 generation females were generally similar on GD 6 and LD 10, indicating no accumulation of GS-5816 after multiple doses.

The mean concentration-time profiles for F1 males, females, and combined sexes show that exposure to GS-5816 generally increased with the increase in maternal dose level from 20 to 200 mg/kg/day on PND 10 (sponsor's table below). The increases in F1 C_{max} values were generally dose proportional while F1 AUC_{0-t} values were greater than dose proportional between the 20 to 200 mg/kg/day maternal dose levels. Gender-based differences were less than 2-fold in GS-5816 C_{max} and AUC_{0-t} values. Values for AUC_{0-t} on LD 10 in F0 generation females were >20 fold higher than in F1 generation males and females.

Table 38: Toxicokinetic Parameters for GS-5816 in Plasma of Maternal Rats: GD 6 and LD 10

Interval	Dose Group	Dose Level (mg/kg/day)	Generation	Sex	C _{max} (ng/mL)	T _{max} (h)	AUC _{0-t} (ng·h/mL)	M:O Ratio AUC _{0-t}
GD 6	2	20	F ₀	F	503	2.00	2790	NA
	3	60		F	750	4.00	6500	NA
	4	200		F	1180	4.00	10200	NA
LD 10	2	20	F ₀	F	402	2.00	2540	NA
	3	60		F	970	4.00	7940	NA
	4	200		F	1670	4.00	13900	NA
PND 10	2	20	F ₁	M	3.79	12.0	36.1	70.3
				F	3.80	12.0	34.6	73.3
				MF	3.80	12.0	34.0	74.7
	3	60	F ₁	M	11.8	12.0	186	42.6
				F	12.2	12.0	193	41.2
				MF	12.0	12.0	190	41.9
	4	200	F ₁	M	31.8	0	617	22.5
				F	27.5	0	548	25.3
				MF	29.7	0	583	23.8

NA Not applicable.

Note: Combined (MF) data is based on the analysis of the combined mean concentration data for both F₁ sexes.

Dosing Solution Analysis: The analyzed dosing formulations were within (b) (4) SOP range for solutions (90% to 110%). The test article was not detected in the vehicle formulation that was administered to the vehicle control group (Group 1).

F1 Litter Data (Pre-and Postnatal Development Phase)

Survival: Each litter was examined daily for survival. The mean number of F1 pups born, live litter size, percentage of males per litter at birth, and postnatal survival between birth and PND 0 (relative to number born), PND 0-1, 1-4 (pre-selection), 4 (post-selection)-7, 7-14, 14-21, and from birth to PND 4 (pre-selection), and PND 4 (post-selection) to PND 21 were unaffected by the F0 maternal test article administration at all dosage levels.

General Physical Condition: To reduce variability among the litters, 8 pups per litter, 4 per sex when possible, were randomly selected on PND 4. Litters were examined daily for survival and any adverse changes in appearance or behavior. Each pup received a clinical examination on PND 1, 4, 7, 10, 14, 17, and 21 (pre- and postnatal development phase) or PND 1, 4, 7, and 10 (toxicokinetic phase). The general physical condition (defined as the occurrence and severity of clinical findings) of all F1 pups in this study was unaffected by F0 maternal test article administration due to the absence of noteworthy clinical findings for pups at all dosage levels.

Offspring Body Weights: Pups were individually weighed on PND 1, 4, 7, 10, 14, 17, and 21 (pre- and postnatal development phase) or PND 1, 4, 7, and 10 (toxicokinetic phase). Mean F1 pup body weights and body weight changes in the 20, 60, and 200 mg/kg/day group males and females were unaffected by F0 maternal test article administration throughout the postnatal period.

Necropsy of F1 Pups Found Dead: The numbers of F1 pups (litters) found dead from PND 0 through the selection of the F1 generation were 15(9), 14(9), 13(10), and 13(8) in the vehicle control, 20, 60, and 200 mg/kg/day groups, respectively. No internal macroscopic findings that could be attributed to F0 maternal administration to the test article were noted at the necropsies of F1 pups that were found dead.

F1 Developmental Landmarks, Sensory Function and Behavioural Testing (Pre- and Postnatal Development Phase):

Balanopreputial Separation: Each male pup (2 per litter, if possible) was observed for balanopreputial separation beginning on PND 35. The day on which balanopreputial separation was first observed was recorded for each pup. Mean ages of attainment of balanopreputial separation and mean body weights at the age of attainment were unaffected by F0 maternal test article administration.

Vaginal Patency: Each female pup (2 per litter, if possible) was observed for vaginal perforation beginning on PND 25. The day on which the vaginal lumen was first observed to open was recorded for each pup. Mean ages of attainment of vaginal patency and mean body weights at the age of attainment were unaffected by F0 maternal test article administration.

Auditory Startle Response: An auditory startle response test was performed on 1 rat/sex/litter (from 25 litters/group, if possible) assigned to Subset A on PND 20 and 60. Administration of 20, 60, and 200 mg/kg/day had no significant effect on auditory startle responsiveness conducted as a longitudinal assessment with selected F1 animals evaluated on PND 20 and again at sexual maturity (PND 60).

Motor Activity: Motor activity was assessed for 1 rat/sex/litter (from 25 litters/group, if possible) assigned to Subset A on PND 21 and 61. Motor activity patterns (total activity as well as ambulatory activity counts) in F1 animals were unaffected by F0 maternal test

article administration at all dosage levels when evaluated on PND 21 and 61 (as animals approached sexual maturity).

Biel Maze Swimming Trials: Beginning on PND 22 and PND 62, swimming ability and learning and memory were assessed for 1 rat/sex/litter (from 25 litters/group, if possible) using a water-filled 8-unit T-maze. Swimming ability on day 1 of the Biel maze assessment (PND 22 or 62) was similar between the vehicle control and 20, 60, and 200 mg/kg/day groups, with the following exception. A significantly ($p=0.010$) shorter mean escape time was noted for the 20 mg/kg/day group males compared to the vehicle control group on PND 62. However, the difference did not occur in a dose-related manner and a shorter mean time to escape was not considered to be toxicologically relevant; therefore, the shorter mean escape time was not attributed to F0 maternal test article administration.

F1 Generation (Pre-and Postnatal Development Phase)

Clinical observation and survival: All of the selected F1 animals were observed twice daily for any adverse clinical findings. Clinical examinations were performed weekly until necropsy. No test article-related effects on survival were noted for males and females in the F1 generation at any dosage level. Female no. 11839-16 in the 200 mg/kg/day group was found dead on LD 1 and viable pups were subsequently euthanized. There were no remarkable clinical or macroscopic findings or noteworthy changes in mean body weight for this female prior to the day of death. Since the mortality at 200 mg/kg/day occurred in a single animal with no remarkable findings, this death was not considered test article-related.

No clinical findings related to F0 maternal treatment to the test article were noted during the F1 generation at the weekly examinations.

Body Weights: F1 males were weighed weekly following weaning until necropsy. No effects on mean F1 weekly body weights, body weight gains, and cumulative body weight gains were noted in the 20, 60, and 200 mg/kg/day groups that could be attributed to F0 maternal test article administration.

Reproductive Performance: Vaginal lavages were performed daily and the slides were evaluated microscopically to determine the stage of the estrous cycle of each F1 female for 10 consecutive days before cohabitation and continuing until evidence of mating was observed or until the end of the mating period. F1 male and female reproductive parameters are presented in the sponsor's following table. No effects on F1 reproductive performance were observed at any dosage concentration that could be attributed to F0 maternal administration to the test article.

Table 39: Reproductive Performance in Rats

Parameter	Dosage Level (mg/kg/day)				(b) (4) HC ^a
	0	20	60	200	Mean (Range)
Male Mating Index (%) ^b	91.7(22)	100.0 (24)	91.7 (22)	92.0 (23)	95.6 (84.0-100.0)
Female Mating Index (%) ^b	100.0 (24)	100.0 (24)	95.8 (23)	96.0 (24)	98.0 (92.0-100.0)
Male Fertility Index (%) ^b	87.5 (21)	87.5 (21)	87.5 (21)	84.0 (21)	90.2 (60.0-100.0)
Female Fertility Index (%) ^b	95.8 (23)	87.5 (21)	91.7 (22)	88.0 (22)	92.9 (60.0-100.0)
Male Copulation Index (%) ^b	95.5 (21)	87.5 (21)	95.5 (21)	91.3 (21)	93.9 (71.4-100.0)
Female Conception Index (%) ^b	95.8 (23)	87.5 (21)	95.7 (22)	91.7 (22)	93.6 (65.2-100.0)
Estrous Cycle Length (days) (n)	4.2 (22)	4.2 (22)	4.1 (21)	4.1 (25)	4.3 (4.0-5.0)
Pre-Coital Interval (days) (n)	3.7 (24)	3.1 (24)	3.4 (23)	3.8 (24)	3.2 (2.3-4.8)

^a = (b) (4) historical control data

^b = Presented as percentage confirmed, with number of animals confirmed in parentheses

Gestation Length and Parturition: All females were allowed to deliver naturally and rear their young to PND 4. During the period of expected parturition, the females were observed twice daily for initiation and completion of parturition and for signs of dystocia. No test article-related effects were noted on mean gestation lengths or the process of parturition at any dosage level.

Macroscopic Examination: A gross necropsy was performed for each of these females; the thoracic, abdominal, and pelvic cavities were opened and the contents were examined. For females that delivered, the numbers of corpora lutea and former implantation sites (the attachment site of the placenta to the uterus) were recorded. The number of unaccounted-for sites was calculated for each female that delivered by subtracting the number of pups born from the number of former implantation sites observed. For females that failed to deliver, a pregnancy status was determined, and specific emphasis was placed on anatomic or pathologic findings that may have interfered with pregnancy. No test article-related effects on survival were noted for F1 animals at any dosage level. Female no. 11839-16 in the 200 mg/kg/day group was found dead on LD 1; at necropsy, this female had an enlarged placenta and retained 13 dead fetuses (with no apparent malformations) *in utero*. No internal findings attributed to F0 maternal test article administration were observed at the scheduled necropsies of F1 males and females at any dosage level.

F2 Litter data (Pre-and Postnatal Development Phase):

Each litter was examined daily for survival. The mean number of F2 pups born, live litter size, percentage of males per litter at birth, and postnatal survival between birth and PND 0 (relative to number born), PND 0-1 and 1-4, and from birth to PND 4 were unaffected by the F0 maternal test article administration at all dosage levels.

General Physical Condition: Litters were examined daily for survival and any adverse changes in appearance or behavior. Each pup received a clinical examination on PND 1 and 4. The general physical condition (defined as the occurrence and severity of clinical findings) of all F2 pups in this study were unaffected by F0 maternal test article administration.

Offspring Body Weights: Pups were individually weighed on PND 1 and 4. Mean F2 pup body weights and body weight changes in the 20, 60, and 200 mg/kg/day group males and females were unaffected by F0 maternal test article administration throughout the postnatal period (PND 1-4).

Necropsy: Pups that survived to PND 4 were euthanized by an intraperitoneal injection of sodium pentobarbital and discarded following a clinical examination. No internal findings that could be attributed to F0 maternal administration to the test article were noted at the necropsies of F2 pups that were found dead or euthanized *in extremis*.

Three pups from 1 litter (no. 11839-16) in the 200 mg/kg/day group were euthanized due to death of the dam on LD/PND 1. Aside from the absence of milk in the stomach for all 3 pups, no internal findings were noted at necropsy.

9 Special Toxicology Studies

Neutral Red Uptake Phototoxicity Assay of GS-5816 in Balb/c 3T3 Mouse Fibroblasts (TX-281-2015)

To assess the phototoxicity of GS-5816 in Balb/c 3T3 mouse fibroblasts, cells were exposed to 5 J/cm² of UVA and 21 mJ/cm² of UVB from a xenon arc solar simulator equipped with a Schott WG 320 filter and GS-5816 at concentrations up to the solubility limit of 40.3 mg/L. Promethazine was used as the positive control. GS-5816 demonstrated potential phototoxicity by the Photoirritancy Factor (PIF; >266) and Mean Photo Effect (MPE; 0.625). GS-5816 was not cytotoxic. Promethazine cytotoxicity and phototoxicity criteria were met indicating that the assay was valid (sponsor's table below).

Table 40: Phototoxicity Potential in Mice

Test Material	IC ₅₀ (mg/L) -UVR (cytotoxicity)	IC ₅₀ (mg/L) +UVR (phototoxicity)	Photoirritancy Factor (PIF)	Mean Photo Effect (MPE)	Phototoxic Potential
Promethazine	57.09	1.133	50.400	0.443	Phototoxic
GS-5816	-	0.151	>266.364	0.625	Phototoxic

- Not Applicable

GS-5816: Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (Individual animal approach) (TX-281-2041)

To assess the skin sensitization potential of GS-5816 the local lymph node assay examined whether GS-5816 induced proliferation of lymphocytes in the lymph nodes. The lymphocyte proliferation can offer an index of the potency of a sensitizing substance through incorporation of radiolabelled Thymidine. A test substance is regarded as a sensitizer if the Sensitization Index (SI) is 3 or more in any of the concentrations tested. The test substance, GS-5816, was prepared for administration as a series of graded concentrations in the vehicle, by direct dilution. The main study

comprised three treated groups, each comprising five female mice receiving GS-5816 at concentrations of 10, 25 or 50% w/v. Similarly constituted groups received the vehicle or positive control substance (25% v/v hexyl cinnamic aldehyde). The mice were treated by daily application of 25 µL of the appropriate concentration or 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. The response was expressed as radioactive disintegrations per minute per lymph node (dpm/node) and as the ratio of ³HTdR incorporation into LNC of test nodes relative to that recorded for control nodes (test/control ratio), termed as Stimulation Index (SI).

All animals survived to the terminal sacrifice. No GS-5816-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 2.4, 2.3 and 2.8 respectively which indicates that GS-5816 did not show the potential to induce skin sensitization. The SI for the positive control substance hexyl cinnamic aldehyde was 32.5, which demonstrates the validity of this study. Therefore, GS-5816 is not regarded as a potential skin sensitizer.

A Multiple Dose Phototoxicity Study to Determine the Effects of Oral Administration of GS-5816 on Skin in Pigmented Rats (TX-281-2016)

GS-5816 was administered to Long-Evans pigmented female rats via oral gavage at dose levels of 0, 20, 60, or 200 mg/kg/day for three consecutive days. The objectives of the study were to determine the potential phototoxic effects of GS-5816 on the skin with 4 hours of exposure to radiation (60 mins. in positive control). 8-Methoxypsoralen (8-MOP) served as a positive control. All rats survived to scheduled euthanasia, and no body weight changes were considered related to GS-5816 administration. Three daily administrations of GS-5816 at doses as high as 200 mg/kg/day followed by a single exposure (4 hrs.) to approximately 10 J/cm² of UVA and approximately 145 mJ/cm² of UVB from a xenon arc solar simulator did not result in any skin reactions indicative of phototoxicity. A single administration of the comparator article 8-MOP elicited cutaneous signs of phototoxicity and validated the assay. GS-5816 was detected in the 4 hour samples (time of UVR exposure) in a dose-related manner (see sponsor's table). GS-5816 was considered non-phototoxic to rats.

Table 41: GS-5816 Plasma Concentration following 4 hours of Radiation Exposure in Pigmented Rats

Group	GS-5816 Dose (mg/kg/day)	Mean GS-5816 Levels (ng/mL)	
		Pre-Dose (0 hour)	Post-Dose (4 hour)
6	0 ¹	– ²	– ²
7	20	– ²	240
8	60	3.13	846
9	200	6.95	1266

1. Administered Vehicle Control Article
2. Below limit of detection (< 2.00 ng/mL)

The Bovine Corneal Opacity and Permeability Assay (BCOP) (TX-281-2039)

To evaluate the ocular corrosivity/severe irritancy potential of GS-5816 the Bovine Corneal Opacity and Permeability Assay (BCOP) was performed. Corneas were treated (4 hour application) in triplicate with either the test substance, positive control (imidazole) or negative control (0.9% sodium chloride solution). Corneal opacity and permeability were measured and combined to give an In Vitro Irritancy Score (IVIS) which was used to assign an in vitro irritancy hazard classification category for prediction of the ocular irritation potential of the test substance. If the test substance was not identified as either Category 1 (IVIS >55) or No Category (IVIS ≤ 3), additional testing would be required for classification or labelling purposes. Throughout the assay the corneas were examined for opaque spots or other irregularities. Following treatment with test substance, GS-5816, the corneas were noted as clear. The corneas treated with the positive control, imidazole, were opaque, and corneas treated with the negative control, 0.9% saline, were clear. GS-5816 elicited an IVIS of 3.4 ± 0.6 with a 4 hour incubation and in accordance with the criteria (no prediction can be made), additional testing is not required (sponsor’s table below).

Table 42: IVIS of GS-5816 in the Bovine Corneal Opacity and Permeability Assay

Sample	Opacity ± SD	Permeability ± SD	In vitro irritancy Score ± SD	In vitro classification
GS-5816	3.333 ± 0.577	0.001 ± 0.001	3.4 ± 0.6	No prediction can be made
Imidazole	103.667 ± 14.731	3.376 ± 0.465	154.3 ± 13.0	Category 1
0.9% Saline	1.333 ± 1.528	0.029 ± 0.026	Not applicable	Not applicable

GS-5816: Skin Irritation to the Rabbit (TX-281-2040)

To assess the skin irritation potential of GS-5816 in rabbits, approximately 0.5 g GS-5816 was dermally applied to intact exposed skin sites on three animals (4 hrs.). An additional site was similarly treated without test substance and acted as a control. After

four days, there was no sign of toxicity or ill health in any rabbit, and no dermal reaction was observed. The Primary Irritation Index was calculated to be 0.0; GS-5816 was classified as 'non-irritant.'

10 Integrated Summary and Safety Evaluation

Eplclusa™, a once daily fixed-dose combination (FDC) tablet containing velpatasvir and sofosbuvir, is intended for the treatment of chronic HCV genotypes 1-6 infection in adult patients. Velpatasvir (VEL, GS-5816) is a specific inhibitor of NS5A of HCV that has displayed potent inhibition of HCV replication *in vitro*. Sofosbuvir (SOF, GS-7977) is a nucleotide prodrug of 2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate that is converted intracellularly to the active uridine triphosphate (GS-461203) within tissues. GS-461203 is a specific inhibitor of NS5B of HCV that has displayed potent inhibition of HCV replicon RNA replication *in vitro*. SOF (Sovaldi, in a combination antiviral treatment regimen with ribavirin) was approved for marketing in the U.S. in December 2013 (refer to NDA-204,671), and as a component of a FDC with ledipasvir (Harvoni) in October 2014 (refer to NDA-205,834). Refer to the Pharmacology/Toxicology reviews for NDA 204,671 and NDA 205,834 for a detailed summary of SOF nonclinical data (as well as Table 44 in this review for updated exposure margins).

Pharmacokinetics:

The steady state volume of distribution (V_{ss}) following intravenous infusion of VEL was ~1.6 L/kg in rats, dogs and monkeys, values greater than total body water volume, with mean residence times (MRT) from ~1 to 6 hours. VEL was shown to be highly bound to plasma protein (>99.5%), and have low systemic plasma clearance rates at 0.3 to 0.9 L/hr/kg (with terminal half-lives of ~2.4 to 5.5 hours) in the nonclinical species. In comparing the pharmacokinetics of intravenous to oral administration of VEL, VEL showed moderate oral bioavailability (~25-30% in rats, monkeys and dogs) with T_{max} values of ~6 hours at the highest feasible exposure following oral dosing in each model. Higher doses could not be used due to low intrinsic solubility of GS-5816 and saturation of absorption. Nonetheless, adequate circulating VEL exposure levels were achieved in the toxicology studies with terminal half-lives of ~2 to 9 hours.

Oral administration of VEL resulted in wide tissue distribution in rodents, including the gall bladder (and bile), liver, adrenal gland, pancreas, kidney, small intestine, and the eye (harderian gland and uveal tract). VEL was rapidly eliminated from most tissues and mainly excreted in the bile/feces within 24 hours, except from the eye which maintained VEL exposure at 168 hours postdose. Additional assessments in pigmented rats revealed binding with melanin-containing tissues (including the eye), but VEL did not reach meaningful concentrations in the eye (<1% of absorbed dose). Despite this association, follow-up studies in rats and rabbits suggest that VEL was neither phototoxic or an ocular irritant. Although several minor metabolites were identified, unchanged parent drug was the predominant circulating component (in mice, rats, dogs and human subjects) as well as the primary drug component in feces, with the major route of VEL elimination as biliary excretion (<0.3% excreted in urine).

The oral FDC of VEL/SOF doubled the clinical exposure of SOF (and its main circulating metabolite, GS-331007) which appears to be the result of increased intestinal absorption of SOF due to VEL inhibition of intestinal efflux transporters. In this respect, VEL showed the potential to inhibit intestinal P-gp, BCRP, and OATP2B1 at concentrations achievable during absorption. The permeability of SOF across Caco-2 cell monolayers *in vitro* is increased in the presence of inhibitors of these transporters (specifically P-gp and BCRP). The increase in SOF exposure due to this effect did not significantly alter the safety margins associated with SOF, as shown in Table 44. VEL had no inhibitory or activating effects on the activities of common drug metabolizers, including CYP450s, AhR, or PXR, so it is unlikely to affect commonly coadministered drugs.

Safety Pharmacology:

No significant effects on neurologic (modified Irwin test) or respiratory parameters (plethysmography) were observed in male rats following single oral doses of VEL up to 200 mg/kg (estimated C_{max} ~1.53 $\mu\text{g}/\text{mL}$ and AUC_{0-t} ~13.0 $\mu\text{g}\cdot\text{hr}/\text{mL}$ for GS-5816), providing an ~4-fold rat to human VEL exposure multiple at the recommended FDC (SOF/VEL: 400/100 mg) dose. These exposure values (Day 1) were obtained from the 13-week toxicology study in rats (study #TX-281-2007). In addition, no significant cardiovascular effects on hemodynamic or electrocardiographic parameters were noted for up to 25 hours post-dose in telemetry-monitored male dogs given single oral doses of VEL up to 100 mg/kg (estimated C_{max} ~2.2 $\mu\text{g}/\text{mL}$ and AUC_{0-t} ~29.0 $\mu\text{g}\cdot\text{hr}/\text{mL}$ for GS-5816), providing an ~9-fold dog to human VEL exposure multiple at the recommended FDC dose. These exposure values (Day 1) were obtained from the 13-week toxicology study in dogs (study #TX-281-2009). GS-5816 did not significantly inhibit hERG current *in vitro* at the maximal feasible concentration (6.5 μM). No significant increase in the number of cardiovascular events was observed in clinical trials with the FDC.

Repeat-Dose Toxicology Studies:

No clear target organs of toxicity were identified in repeat-dose toxicology studies in mice, rats and dogs administered VEL doses of up to 1500, 200 and 100 mg/kg/day for 1, 6 and 9 months, respectively. Therefore, no specific overlapping toxicity of potential significant clinical concern was identified in animals administered VEL or SOF alone. Due to the saturation of VEL exposure in the optimized vehicle, these were the highest doses tested in the nonclinical species.

VEL-related effects in mice were limited to the highest dose examined (1500 mg/kg/day). These findings included non-adverse effects on hematology parameters in males and non-adverse increases in organ weights in females. GS-5816 exposure (C_{max} : 16.3 $\mu\text{g}/\text{mL}$; AUC_{0-24} : 220.0 $\mu\text{g}\cdot\text{hr}/\text{mL}$) in mice at this dose level was ~68-fold higher than that in humans at the recommended FDC dose.

In rats, the observed effects were generally not related to VEL exposure and considered incidental or accidental. These effects were limited to unscheduled sacrifices/deaths at all doses (n=5) and in a control rat. The cause of deaths ranged from incidences of gavage-related errors to a commonly observed tumor (mammary gland carcinoma) in a single rat. Based on the absence of any proliferative changes in

the mammary gland of other high-dose male and female rats after 13 weeks and 26 weeks of VEL administration and the reported incidence of mammary gland carcinomas in similarly aged female rats, this finding was not considered VEL-related. The VEL exposure at the NOAEL (200 mg/kg: C_{max} 1.2 $\mu\text{g}/\text{mL}$ and AUC_{0-24} 12.2 $\mu\text{g}\cdot\text{hr}/\text{mL}$) for rats was ~4-fold higher than that in humans at the recommended FDC dose.

In dogs, VEL-related effects were limited to the highest dose examined (100 mg/kg/day) but not considered adverse. A slight increase in emesis at 100 mg/kg/day, and moderately lower food consumption in females were not associated with effects on body weight, clinical observations, or clinical pathology indicative of inappetence or dehydration. Mildly decreased fibrinogen and globulin concentrations and mildly increased albumin:globulin ratio after 39 weeks in females administered 100 mg/kg/day were also absent of correlative findings. A transient increase in mean prostate weight at the 13-week interim sacrifice was without a microscopic correlate and was not observed in dogs that completed 39-weeks of dosing. Idiopathic polyarthritis, which has been found to spontaneously develop in Beagle dogs, was noted in a single male from the high dose group. The VEL exposure at the NOAEL (100 mg/kg: C_{max} 2.0 $\mu\text{g}/\text{mL}$ and AUC_{0-24} 27.8 $\mu\text{g}\cdot\text{hr}/\text{mL}$) for dogs was ~9-fold higher than that in humans at the recommended FDC dose.

Genetic Toxicology and Carcinogenicity:

VEL was not mutagenic or clastogenic as tested in the Ames assay up to 5000 $\mu\text{g}/\text{plate}$, the *in vitro* chromosomal aberration assay in human peripheral blood lymphocytes up to 500 $\mu\text{g}/\text{mL}$, and the *in vivo* rat micronucleus assay up to day 14 of 200 mg/kg/day. A 6-month rasH2 transgenic mouse study and a 2-year rat carcinogenicity study with VEL are on-going.

Reproductive Toxicity Studies:

VEL exposure was not associated with effects on fertility. Daily oral doses of VEL to rats for 14 days (females) or 28 days (males) prior to cohabitation and during cohabitation had no effects on male or female reproductive performance, on the estrous cycle or sperm, or on embryo/fetal viability. The NOEL for fertility and early embryonic development in rats is 200 mg/kg/day (AUC_{0-t} 17.1 $\mu\text{g}\cdot\text{hr}/\text{mL}$; TX-281-2003), the highest dose tested in the study. When compared to human VEL exposure following administration of the FDC, the margin of exposure for VEL is ~5-fold.

VEL exposure was not associated with effects on embryo-fetal development. Developmental toxicity studies were conducted in mice, rats, and rabbits. Mice were examined, in addition to rats, due to the inability to achieve higher exposures in the rat and to provide an adequate exposure margin of safety compared to humans. In mice and rats, there were no effects on maternal or embryo-fetal development, and the NOEL was 1000 mg/kg/day (GD15 AUC_{0-24} 93 $\mu\text{g}\cdot\text{hr}/\text{mL}$) for mice and 200 mg/kg/day (GD17 AUC_{0-24} 17.1 $\mu\text{g}\cdot\text{hr}/\text{mL}$) for rats, the highest doses tested. In rabbits, a single test article-related death was noted in the high-dose group of 300 mg/kg/day. While no cause of death was identified, the animal had prior clinical signs of thin appearance, few/no feces, rough hair coat, general debilitation and weight loss (~400 g) from GD 13-21 and low food consumption. The NOEL for maternal toxicity was 100 mg/kg/day and the NOEL for embryo-fetal development was 300 mg/kg/day (GD20 AUC_{0-24} 1.94 $\mu\text{g}\cdot\text{hr}/\text{mL}$).

and 2.06 $\mu\text{g}\cdot\text{hr}/\text{mL}$, respectively). At the developmental NOELs, VEL exposures in the mouse, rat, and rabbit were approximately 29-, 5- and 0.6-fold compared with the clinical exposure of the FDC.

VEL maternal exposure was not associated with effects on pre- and postnatal development. VEL at doses up to 200 mg/kg/day in rats had no maternal effects, and no effects on behavior, reproduction, or development of the offspring. One F0 female in the 60 mg/kg/day group was euthanized *in extremis* on LD 5 following clinical observations of piloerection, decreased defecation, a thin body, red material around the urogenital area, and a swollen urogenital area. One F1 female in the 200 mg/kg/day group was found dead on LD1; pups were viable, but 13 dead fetuses were still present. No remarkable clinical findings or noteworthy changes were identified prior to death. These occurrences were likely not attributable to test article administration, and no other noteworthy findings were observed in the differing phases. VEL maternal (F0) exposure (LD 10 AUC_{0-24} 13.9 $\mu\text{g}\cdot\text{h}/\text{mL}$) at the maternal and F1 offspring NOEL in the pre- and postnatal development study was ~5-fold higher than the mean clinical exposure with the FDC.

The tables below summarize the calculated exposure multiples for the respective toxicity studies comparing the plasma VEL and SOF exposures in animals to the human exposure after Epclusa™ administration.

Table 43 Summary of Systemic Exposure Margins for Velpatasvir

Species	Study Type/ Duration/ Toxicity	Dose (mg/kg)	Approximate Exposure Margin Based on VEL AUC*
Mouse	1-month RD	1500 (NOAEL)	68
	EFD	1000	29
Rat	6-month RD/ Fertility/ EED	200 (NOAEL)	4
Rat (pregnant)	EFD	200 (NOEL)	4
Rabbit (pregnant)	EFD	300 (NOAEL)	<1
Dog	9-month RD	100 (NOAEL)	9

*AUC_{tau} in HCV-infected human subjects given FDC (VEL/SOF): 3253.1 ng*hr/mL at 400/100 mg/day. RD=repeat-dose toxicology study; EFD=embryo-fetal development study; EED=early embryonic development; NOEL=no effect level; NOAEL=no adverse effect level.

Table 44: Updated Summary of Systemic Exposure Multiples for Sofosbuvir (modified from original Table included in NDA-204,671)

Species	Study Type/ Duration/ Toxicity	Dose (mg/kg)	Approximate Exposure Margin Based on GS- 331007 AUC*	Approximate Exposure Margin Based on SOF (GS-7977) AUC ^a
Mouse	3-month RD	100	2(♂) 6(♀)	NA
	2-yr Carcinogenicity	600(♀) 200(♂) (NOEL)	15 3	NA
Rat	Lethality & heart toxicity	2000 (NOEL) ^a	14	NA
	6-month RD, Fertility & EED	500 (NOAEL)	5	NA
	2-yr Carcinogenicity	750 (NOEL)	8	NA
Dog	GI toxicity	500	14	62'
	9-month RD	100 (NOAEL)	6	14'

*AUC_{tau} in HCV-infected human subjects given FDC (VEL/SOF): GS-331007- 14186.8 ng*hr/mL at 400/100 mg/day.

ªAUC_{tau} in HCV-infected human subjects given FDC (VEL/SOF): SOF-1622.9 ng*hr/mL at 400/100 mg/day.

Using AUC from the 3 month repeat dose study.

ª7 day Postmarketing Requirement Study to address cardiotoxicity observed in original NDA submission.

RD=repeat-dose toxicology study; EFD=embryo-fetal development study; EED=early embryonic development; NOEL=no effect level; NOAEL=no adverse effect level.

11 Appendix/Attachments

**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: 208-341

Supporting document/s:

Supporting Document	Sponsor Submission Date	CDER Received Date
3	10/28/15	10/28/15
9	1/11/16	1/11/16
15	2/16/16	2/16/16

Product: Sofosbuvir/Velpatasvir Fixed Dose Combination

Indication: treatment of chronic HCV infection in adults

Applicant: Gilead Sciences Inc.

Review Division: Division of Antiviral Products

Reviewer: Mark W. Powley, Ph.D.

Supervisor/Team Leader: Hanan Ghantous, Ph.D., DABT

Division Director: Debra B. Birnkrant, M.D.

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Disclaimer

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1 Executive Summary

1.1 Introduction

Gilead Sciences Inc. has submitted an NDA to support the fixed dose combination therapy of sofosbuvir (SOF) and velpatasvir (VEL; GS-5816) for treating chronic HCV infection in adults. The proposed dosing regimen includes 400 mg/day SOF + 100 mg VEL for 12 weeks.

This review focuses on qualification of organic impurities, residual solvents, elemental impurities, and degradants. Regulatory decisions utilize recommendations from ICH M7 “Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk”, ICH Q3A(R2) “Impurities in New Drug Substances”, ICH Q3B(R2) “Impurities in New Drug Products”, ICH Q3C(R5) “Impurities: Guideline for Residual Solvents”, and ICH Q3D “Guideline for Elemental Impurities”.

Overall, proposed specifications (or lack of specifications) are considered acceptable from a pharmacology/toxicology perspective.

2 Qualification of Sofosbuvir Drug Substance

Proposed specifications for sofosbuvir organic impurities, residual solvents, and elemental impurities (b) (4) accepted under NDA#204-671.

3 Qualification of Velpatasvir Drug Substance

3.1 Specified Impurities

3.1.1 Organic Impurities

The qualification of specified organic impurities in the VEL drug substance is based on results from general toxicology studies, experimental Ames assay data, and/or assessments of mutagenic potential using (quantitative) structure-activity relationship [(Q)SAR].

General Toxicology – Specified impurities were present in the drug lots used in 2-week and 26-week rat studies (TX-281-2003, TX-281-2007, and TX-281-2042; reviewed by Dr. Pritam Verma). Using NOAELs established in these studies, qualified impurity levels are adequate to support the proposed specifications. Summary information is provided below.

Table 45. Velpatasvir drug substance organic impurity specifications

Organic Impurity	Toxicology Study Content	NOAEL	Qualified % ^a	Proposed Specification
(b) (4)	(b) (4) % ^b , (b) (4) % ^c	200 mg/kg/day	(b) (4) % ^b , (b) (4) % ^c	≤ (b) (4) %
	(b) (4) % ^c	200 mg/kg/day	(b) (4) % ^c	≤ (b) (4) %
	% ^c	200 mg/kg/day	% ^c	≤ (b) (4) %
	% ^b	200 mg/kg/day	% ^b	≤ (b) (4) %
	(b) (4) % ^b , (b) (4) % ^c	200 mg/kg/day	(b) (4) % ^b , (b) (4) % ^c	≤ (b) (4) %
	(b) (4) % ^d	200 mg/kg/day	(b) (4) % ^d	≤ (b) (4) %
	% ^b	200 mg/kg/day	% ^b	≤ (b) (4) %
	% ^b	200 mg/kg/day	% ^b	≤ (b) (4) %
	% ^b	200 mg/kg/day	% ^b	≤ (b) (4) %
	% ^b	200 mg/kg/day	% ^b	≤ (b) (4) %
individual, unspecified	-	-	% ^e	≤ (b) (4) %
total	-	-	-	≤ (b) (4) %

^a qualified level = [% impurity x non-clinical dose (mg/kg/day)] / [body surface area conversion factor (i.e., 6.2 for rats) x maximum clinical dose (i.e., (b) (4) mg/kg/day for 50 kg body weight)]

^b from Study no. TX-281-2042 (2-week study in rats)

^c from Study no. TX-281-2003 (2-week study in rats)

^d from Study no. TX-281-2007 (26-week study in rats)

^e ICH Q3A(R2) qualification threshold for drugs with maximum daily dose ≤ 2 g

Genotoxicity – The VEL (b) (4) are covered by negative experimental results for the parent drug (i.e., Ames assay, *in vitro* chromosomal aberration assay, *in vivo* micronucleus assay; reviewed by Dr. Pritam Verma). Recommended testing for impurities with exposures exceeding the ICH Q3A qualification threshold (i.e., lower of 0.15% or 1.0 mg/day) include a genetic toxicology evaluation consisting of Ames and *in vitro* mammalian cell assays. In contrast, ICH M7 indicates that initial genetic toxicology qualification can be limited to (Q)SAR predictions of mutagenic potential for impurities with exposures ≤ 1 mg/day. Although the highest proposed specification yields a clinical exposure of (b) (4) mg/day, the specification is for a combination of 2 co-eluting impurities (i.e., ≤ (b) (4) % for (b) (4)). Because proposed specifications for any individual impurity do not clearly exceed (b) (4) mg/day, (Q)SAR predictions of mutagenic potential are considered sufficient for qualification.

Per the Sponsor, “(Q)SAR and expert knowledge were used to predict the outcome of the microbial reverse mutation assay for velpatasvir potential and/or actual impurities”. Because the (Q)SAR models only account for 2D structure, evaluation of stereoisomers was excluded in cases where predictions were available for parent compounds. The Sponsor’s (Q)SAR evaluation included Derek Nexus (v3.01 and v4.10) and Leadscope Model Applier (v1.7.4, v1.8.3, and v2.03).

Table 46. (Q)SAR summary for specified velpatasvir drug substance organic impurities

Impurity (b) (4)	Derek Nexus	Leadscope Model Applier		Overall
		<i>Salmonella</i>	<i>E.coli/TA102</i>	
	negative	negative	negative	negative
	negative	negative	negative	negative
	negative	OD	OD	negative ^a
	negative	OD	OD	negative ^a
	negative	OD	OD	negative ^a
	negative	OD	OD	negative ^a
	negative	OD	OD	negative ^a
	negative	OD	OD	negative ^a
	negative	OD	OD	negative ^a

OD = out of domain for the model; no prediction provided

^a expert knowledge used to support negative prediction (see description in text below)

Although predictions were only provided by 1 system for several impurities, (Q)SAR results are acceptable due to the following:

1. Use of a single (Q)SAR system is acceptable during the ICH M7 guideline implementation period (i.e., 18 months following the guideline publication date).
2. VEL was also out of domain for Leadscope Model Applier. Per the Sponsor:

“Structures in this group contain (b) (4). These changes were not considered significant to their mutagenic potential, and no additional structural alerts were identified.”

Based on the Sponsor’s (Q)SAR assessment, all specified impurities are deemed to lack mutagenic potential.

3.1.2 Residual Solvents

With the exception of (b) (4), residual solvents in the VEL drug substance are listed in the ICH Q3C(R5) guideline. While the proposed specification for (b) (4) and there is minimal toxicological concern. Proposed specifications for the remaining listed solvents are (b) (4). Summary information is provided below.

Table 47. Velpatasvir drug substance residual solvent specifications

Residual Solvents	ICH Q3C(R5) Limit		Proposed Specification	
	Concentration Limit (Option 1)	PDE (Option 2)	Concentration	Clinical Exposure ^a
(b) (4)				

^a based on VEL clinical dose of 100 mg/day

^b risk assessment provided below

(b) (4) – The most appropriate source of data to calculate a permissible daily exposure (PDE) comes from an oral developmental toxicity study in rats ((b) (4)). In this study, female Wistar rats were administered oral doses of (b) (4) mg/kg/day of (b) (4) from Gestation Day 6 through 15. Control animals received water. Mortality (n =3), decreased body weight gain, and decreased food consumption were observed in dams at the high-dose. A single high/mid-dose dam died. Fetal effects included decreased body weight and length, delayed ossification, enlargement of cerebral ventricles and subarachnoid space, anophthalmia, hydrocephalus and hydronephrosis. These effects were generally limited to the high-dose, although enlargement of cerebral ventricles was noted in the low/mid-, high/mid-, and high-dose groups as well. The author concluded drug-related fetal effects occurred only in the presence of maternal toxicity at the high-dose and assigned an NOAEL = (b) (4) mg/kg/day.

The European Chemical Agency (ECHA) registered substance database reports that (b) (4) is non-mutagenic in 2 experimental Ames assays. Each study evaluated effects in Salmonella strains TA98, TA100, TA1535, and TA1537 with and without metabolic activation at top doses exceeding 5000 µg/plate.

The NOAEL supports a PDE = (b) (4) mg/day (calculation below) or (b) (4)% of the maximum recommended clinical dose of VEL.

$$\text{PDE} = \text{NOAEL} \times 50 \text{ kg (body weight)} / (\text{F1} \times \text{F2} \times \text{F3} \times \text{F4} \times \text{F5})$$

$$\text{NOAEL} = (b) (4) \text{ mg/kg/day}$$

$$\text{F1} = 5 \text{ (extrapolation from rats to humans)}$$

$$\text{F2} = 10 \text{ (account for variability between individuals)}$$

$$\text{F3} = 1 \text{ (entire period of organogenesis)}$$

$$\text{F4} = 10 \text{ (severe toxicity)}$$

$$\text{F5} = 1 \text{ (NOAEL used)}$$

$$= (b) (4) \text{ mg/kg/day} \times 50 \text{ kg} / (5 \times 10 \times 1 \times 10 \times 1)$$

$$= (b) (4) \text{ mg/day}$$

References



3.1.3 Elemental Impurities

None

3.2 Unspecified Impurities

In addition to the (Q)SAR systems described in section 3.1.1, CASE Ultra (v1.5.2.0) was included in the evaluation of 2 unspecified impurities. (b) (4) were identified by the Sponsor as potentially mutagenic impurities. (b) (4) was shown to be experimentally mutagenic in TA98 and TA100 with and without metabolic activation (Study no. TX-281-2033; study not reviewed). Through the manufacturing process, these potential impurities are controlled to levels below the appropriate TTC (i.e., default TTC of (b) (4) $\mu\text{g}/\text{day}$). Therefore, these impurities are not included in the drug substance specifications.

The chemistry reviewer (Dr. Sithamalli Chandramouli) requested a pharmacology/toxicology opinion regarding the potential mutagenicity of (b) (4) and clarification of the Sponsor's conclusions that (b) (4) lacks mutagenic potential. The following sections address these issues.

(b) (4) – The ECHA registered substance database reports that (b) (4) is non-mutagenic in 2 experimental Ames assays. In the first study, the chemical was negative when tested in *Salmonella* strains TA98, TA100, TA1535, and TA1537 as well as *E. coli* WP2 *uvrA* with and without metabolic activation. A second study evaluated mutagenicity in *Salmonella* strains TA98, TA100, TA1535, TA 1537, and TA1538 with and without metabolic activation. Both studies included a top dose of 5000 $\mu\text{g}/\text{plate}$.

References:

(b) (4)

(b) (4) – This impurity (structure shown below) was originally considered potentially reactive due to the presence of an (b) (4) group. While the molecule was predicted to be non-mutagenic by Derek Nexus, it was out of domain for Leadscope Model Applier. (b) (4)

(b) (4)

It is worth noting that (b) (4) based on Ames assays conducted with highly cytotoxic test articles. Although the chemistry reviewer indicated that concern with clinical exposures was low due to the reactive nature of the compound, (b) (4) was not supported by the available data. The following correspondence was forwarded to the Sponsor on 2/2/16:

“The Division agrees that (b) (4) poses little clinical risk based on the minimal degree of carryover. However, the Division would like to emphasize that the mutagenic potential of this impurity has not been adequately addressed.” (b) (4)

“the rationale is based on insufficient information. The data used to support (b) (4) is taken from studies conducted in a limited number of bacterial tester strains and/or tested at extremely low doses (i.e., due to cytotoxicity). Given the suboptimal nature of these studies, it is not possible to conclude that (b) (4) lack reactive potential. In the absence of additional information, we recommend that (b) (4) be classified as Class 3.”

The Sponsor submitted the following response on 2/16/16:

“Although cytotoxicity seems to be common for the (b) (4), cytotoxicity of a compound resulting in (b) (4) according to ICH S2(R1) and OECD 471 guidances. Therefore, despite the limitations of supporting information of the (b) (4) given the additional supporting evidence found in the literature.”

A review of data for 8 potentially relevant structural analogs showed that experimental Ames testing was severely limited by cytotoxicity (data summarized in the Appendix). As a result, a majority of these analogs were only tested at doses \leq (b) (4) $\mu\text{g}/\text{plate}$ with S9 and \leq (b) (4) $\mu\text{g}/\text{plate}$ without S9. This is substantially lower than the ICH S2(R1) and OECD test guideline 471 recommend top dose of 5000 $\mu\text{g}/\text{plate}$ for soluble, non-toxic test articles. While cytotoxicity can be used for establishing the highest dose tested in the Ames assay, the OECD test guideline points out that (b) (4)

Therefore, the currently available

experimental data (b) (4) does not support assignment of (b) (4) impurity.

4 Qualification of the Sofosbuvir/Velpatasvir Drug Product

4.1 Degradants

Proposed specifications for SOF degradants (b) (4) accepted under NDA#204-671. VEL degradant proposed specifications are supported by data described in Section 3.1 and are summarized below.

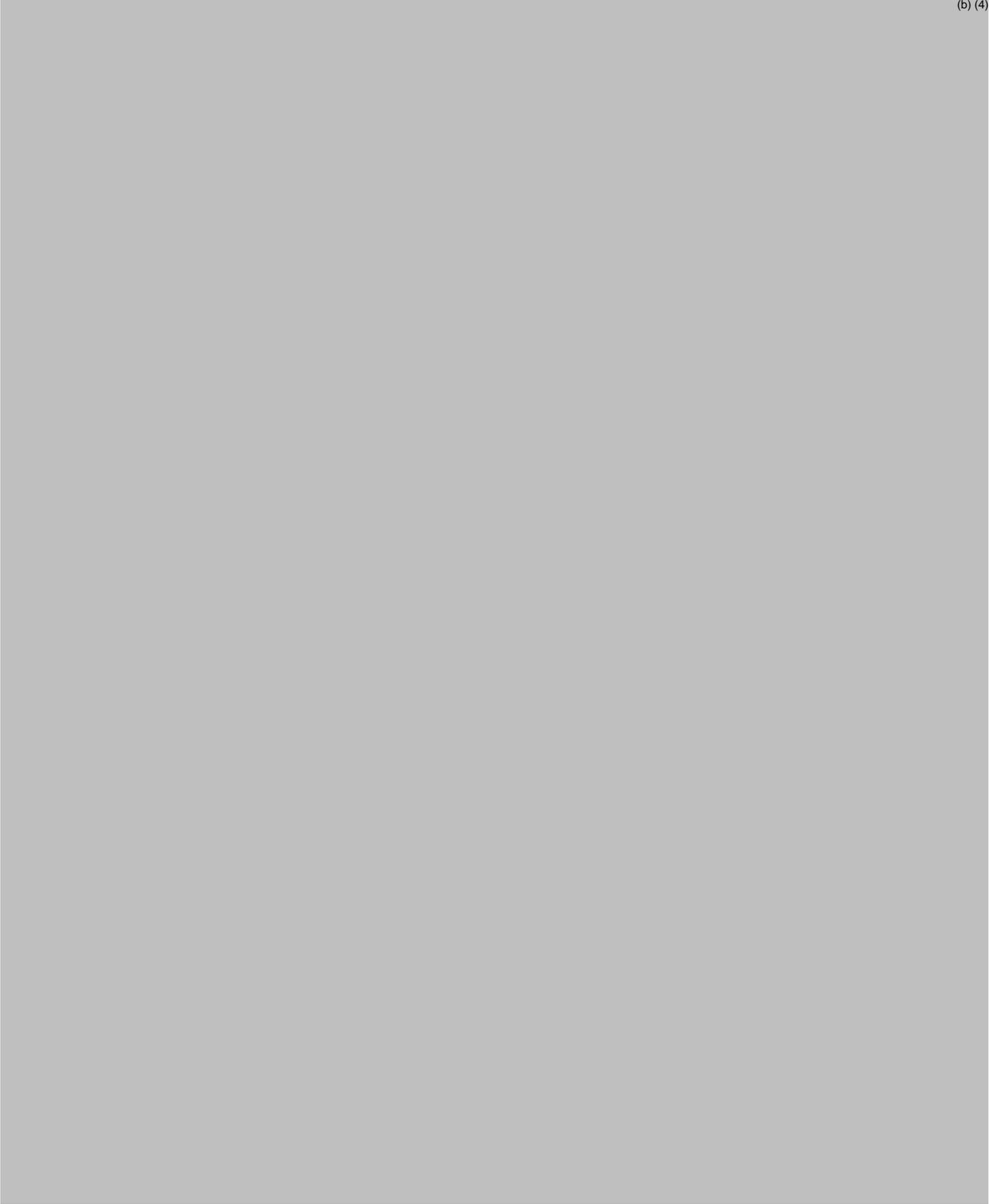
Table 48. Velpatasvir drug product degradant specifications

Degradant	Qualified Levels	Proposed Specification	
		Release	Shelf Life
Velpatasvir (b) (4) individual, unspecified total	(b) (4) % (b) (4) % (b) (4) % % % % (b) (4) % ^a -	< (b) (4) % < % < % < % < % < % < % < (b) (4) %	< (b) (4) % < % < % < % < % < % < % < (b) (4) %

^a ICH Q3B(R2) qualification threshold for drugs with maximum daily dose 10 to 100 mg

Appendix

(b) (4)



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/s/

JOHN H DUBINION
03/28/2016

HANAN N GHANTOUS
03/28/2016

I concur with Dr. Dubinion's conclusion to approve the marketing of Epclusa.

Comments on NDA 208341 Epclusa

From: A Jacobs, AD

Date 3/1/16

1. I concur that there are no pharm-tox approval issues.
2. I have made some suggestions to the reviewer and they have been addressed as appropriate.

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

/s/

ABIGAIL C JACOBS
03/24/2016