CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

205834Orig1s000

PHARMACOLOGY REVIEW(S)

Comments on N205,834 Harvoni: ledipasvir and sofosbuvir fixed-dosed combination

Sofosbuvir was previously approved as part of a different combination

1. I concur that there are no pharm-tox approval issues, and that the pregnancy category of B is appropriate.

2. I conveyed a comment to the reviewer that he will address as appropriate.

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/s/

ABIGAIL C JACOBS
07/15/2014

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:	205,834
Supporting document/s:	001, 022
Applicant's letter date:	February 8, 2014
CDER stamp date:	February 10, 2014
Product:	Harvoni™ is a fixed-dose combination (FDC) tablet of ledipasvir (LDV), an HCV NS5A inhibitor, and sofosbuvir (SOF), an HCV nucleotide analog NS5B polymerase inhibitor
Indication:	Harvoni™ is indicated for the treatment of chronic hepatitis C (CHC) genotype 1 infection
Applicant:	Gilead Sciences
Review Division:	Division of Antiviral Products
Reviewer:	Christopher Ellis, Ph.D.
Supervisor/Team Leader:	Hanan Ghantous, Ph.D., DABT
Division Director:	Debra Birnkrant, M.D.
Project Manager:	Linda Onaga, M.P.H.

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1 Executive Summary

1.1 Introduction

Harvoni™, a once daily fixed-dose combination (FDC) tablet containing ledipasvir and sofosbuvir, is intended to be indicated for treatment of chronic hepatitis C virus (HCV) genotype 1 infection in adult patients. Ledipasvir (LDV, GS-5885) 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 (as a component of a combination antiviral treatment regimen) was approved for marketing in the U.S. in December 2013 (refer to NDA-204,671).

The nonclinical safety profile of LDV 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 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 LDV have been conducted, while rat and mouse carcinogenicity studies with LDV are currently in progress. With the exception of rodent 2-year carcinogenicity studies that were reviewed with this application, nonclinical safety studies for SOF to support the FDC were reviewed previously. Refer to the Pharmacology/Toxicology review for NDA-204,671 for a detailed summary of SOF nonclinical data (as well as Table 41 in this review for updated exposure multiples).

1.2 Brief Discussion of Nonclinical Findings

LDV had moderate oral bioavailability (~30-50% in rats, monkeys and dogs) with T_{max} values of ~4-5 hours, despite low intrinsic aqueous solubility and saturation of absorption that nonetheless resulted in adequate circulating LDV exposure levels in toxicology studies. LDV was bound highly to plasma protein (>99.8%) and was distributed widely to tissues including gall bladder (and bile), liver, adrenal, salivary, thyroid, pituitary, pancreas, adipose tissue (brown), kidney (in mice and/or rats) and the uveal tract of the eye in pigmented rats, but did not accumulate to any great extent in tissues (mean residence times ~6-13 hours). Although several minor metabolites were identified, unchanged parent drug is the predominant circulating component (in mice, rats, dogs and human subjects) as well as the primary component in feces, with a major route of LDV elimination occurring as biliary excretion of unchanged parent (in rats and dogs).

No clear target organs of toxicity were identified in repeat-dose toxicology studies in mice, rats and dogs administered LDV doses of up to 300, 100 and 30 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 LDV or SOF alone. However, a potential LDV-related mild hepatobiliary toxicity signal (not considered adverse and not clearly dose dependent) was noted, with slight increases in ALP and/or ALT associated with increased liver/gall bladder weight (high-dose males only) without correlating histopathology changes observed in mice following oral administration of LDV at up to 300 mg/kg/day ($AUC_{0\text{-}24hr}$~164 & 271 μg.h/ml for GS-5885 in females and males, respectively). In addition, minimal to slight random foci of hepatocyte necrosis (males) and bile duct hyperplasia (males and females) were noted in rats following oral administration of LDV at up to 100 mg/kg/day ($AUC_{0\text{-}24hr}$~56 μg.h/ml for GS-5885). These non-adverse hepatobiliary findings were observed at GS-5885 AUC exposure ~8- and 30-fold higher, in rats and mice respectively, than that in humans at the recommended LDV dose. In addition, slight increases in cholesterol and triglycerides were noted in rats at 100 mg/kg/day. In dogs, no clear clinically relevant LDV-related findings were observed following oral administration of LDV at up to 30 mg/kg/day ($AUC_{0\text{-}24hr}$~41.3 & 80.3 μg.h/ml for GS-5885 in males and females, respectively), resulting in GS-5885 AUC exposure ~9-fold higher than that in humans at the recommended LDV dose.

The average number of corpora lutea, implantations and viable embryos were reduced slightly in rats following administration of LDV at 100 mg/kg/day (estimated $AUC_{0\text{-}24hr}$~25.1 μg.h/ml for GS-5885) and were associated with non-adverse maternal toxicity findings consisting of slight body weight loss and reduced food consumption. The NOEL for female fertility and early embryonic development is considered to be 30 mg/kg/day (estimated $AUC_{0\text{-}24hr}$~12.1 μg.h/ml for GS-5885). At 30 and 100 mg/kg/day, GS-5885 AUC exposure is estimated to be ~2 and 3.4-fold higher, respectively, than that in humans at the recommended LDV dose.

1.3 Recommendations

1.3.1 Approvability

Yes, the sponsor provided sufficient nonclinical safety information on ledipasvir in support of approval for marketing in the U.S. Sofosbuvir (as a component of a combination antiviral treatment regimen) was approved for marketing in the U.S. in December 2013.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The Pharmacology/Toxicology portion of the sponsor's draft product label with reviewer suggested modifications (designated by ~~strikethrough~~ or ***bold italics)*** is included below.

8.1 Pregnancy

Pregnancy Category B

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

Animal Data

Ledipasvir: No effects on fetal development have been observed in rats and rabbits at the highest doses tested. In the rat and rabbit, AUC exposure to ledipasvir was ***approximately*** (b) (4) ~~4~~- and 2-fold, respectively, the exposure in humans at the recommended clinical dose.

(b) (4)

Sofosbuvir: No effects on fetal development have been observed in rats and rabbits at the highest doses tested. In the rat and rabbit, AUC exposure to the predominant circulating metabolite GS-331007 increased over the course of gestation from approximately 3- to 6-fold and 7- to (b) (4) ***7***-fold the exposure in humans at the recommended clinical dose, respectively.

8.3 Nursing Mothers

It is not known whether [TRADENAME] and its metabolites are present in human breast milk. When administered to lactating rats, ledipasvir was detected in the plasma of suckling rats likely due to (b) (4) ***the presence*** of ledipasvir (b) (4) ***in*** milk. Ledipasvir had no ***clear*** effects on the nursing pups. The predominant circulating metabolite of sofosbuvir (GS-331007) was the primary component observed in the milk of lactating rats, without effect on nursing pups. (b) (4)

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for [TRADENAME] and any potential adverse effects on the breastfed child from the drug or from the underlying maternal condition. (b) (4)

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis and Mutagenesis

Ledipasvir: Ledipasvir was not genotoxic in a battery of ***in vitro*** or ***in vivo*** assays, including bacterial mutagenicity, chromosome aberration using human peripheral blood lymphocytes and ***in vivo*** rat micronucleus assays.

Carcinogenicity studies of ledipasvir in mice and rats are ongoing.

Sofosbuvir: Sofosbuvir was not genotoxic in a battery of *in vitro* or *in vivo* assays, including bacterial mutagenicity, chromosome aberration using human peripheral blood lymphocytes and *in vivo* mouse micronucleus assays.

(b) (4)

-Two-year carcinogenicity studies in mice and rats were conducted with sofosbuvir. Mice were administered doses of up to 200 mg/kg/day in males and 600 mg/kg/day in females, while rats were administered doses of up to 750 mg/kg/day in males and females. No increase in the incidence of drug-related neoplasms were observed at the highest doses tested in mice and rats, resulting in AUC exposure to the predominant circulating metabolite GS-331007 of approximately 4- and 18-fold (in mice) and 8- and 10-fold (in rats), in males and females respectively, the exposure in humans at the recommended clinical dose.

Impairment of Fertility

Ledipasvir: Ledipasvir had no adverse effects on mating and fertility. In female rats, the mean number of corpora lutea and implantation sites were (b) (4) reduced ***slightly*** at maternal exposures ***approximately*** (b) (4)***3***-fold the exposure in humans at the recommended clinical dose. At the ***highest dose levels without*** (b) (4) effect***s*** ~~level~~, AUC exposure to ledipasvir was approximately (b) (4)***5***- and (b) (4)***2***-fold, in males and females~~,~~ respectively, the (b) (4) exposure ***in humans*** at the recommended clinical dose.

Sofosbuvir: Sofosbuvir had no effects on embryo-fetal viability or on fertility when evaluated in rats. At the highest dose tested, AUC exposure to the predominant circulating metabolite GS-331007 was approximately 5-fold the exposure in humans at the recommended clinical dose.

13.2 Animal Toxicology and/or Pharmacology

(b) (4)

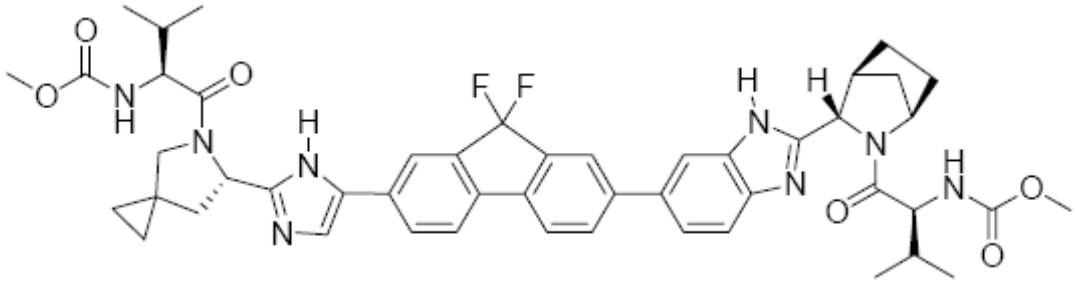
Sofosbuvir: Heart degeneration and inflammation were observed in rats following GS-9851 (a stereoisomeric mixture containing approximately 50% sofosbuvir) doses of 2,000 mg/kg/day for up to 5 days. At this dose, AUC exposure to the predominant ***circulating*** metabolite GS-331007 is approximately 1(b)(4)***7***-fold higher than human exposure at the recommended clinical dose. No heart degeneration or inflammation was observed in mice, rats or dogs in studies up to 3 months, 6 months or 9 months at GS-331007 AUC exposures approximately 2(b)(4)***4***-, 5- or 1(b)(4)***7***-fold ***higher***, respectively, (b)(4) than human exposure at the recommended clinical dose. ***In addition,*** no heart degeneration or inflammation was observed in ***rats following sofosbuvir doses of up to 750 mg/kg/day in*** the 2-year carcinogenicity stud***y***~~ies~~ at GS-331007 AUC exposure~~s~~ approximately (b)(4) 9-fold the ***exposure in humans at the*** recommended clinical dose.

2 Drug Information

2.1 Drug

2.1.1 Ledipasvir

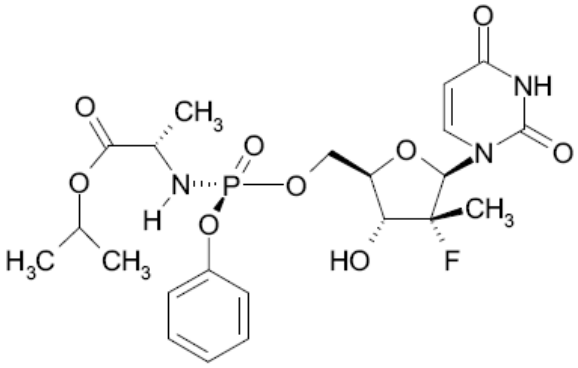
CAS Registry Number	1256388-51-8
Generic Name	Ledipasvir (LDV)
Code Name	GS-5885
Chemical Name	Methyl [(2*S*)-1-{(6*S*)-6-[5-(9,9-difluoro-7-{2-[(1*R*,3*S*,4*S*)-2-{(2*S*)-2-[(methoxycarbonyl) amino]-3-methylbutanoyl}-2-azabicyclo [2.2.1]hept-3-yl]-1*H*-benzimidazol-6-yl}-9*H*-fluoren-2-yl)-1*H*-imidazol-2-yl]-5-azaspiro [2.4]hept-5-yl}-3-methyl-1-oxobutan-2-yl] carbamate (IUPAC)
Molecular Formula/Molecular Weight	$C_{49}H_{54}F_2N_8O_6$//889.00 g/mol
Structure	



Pharmacologic Class	HCV NS5A inhibitor

2.1.2 Sofosbuvir

CAS Registry Number	1190307-88-0
Generic Name	Sofosbuvir (SOF)
Code Name	GS-7977 (PSI-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 (IUPAC)
Molecular Formula/Molecular Weight	$C_{22}H_{29}FN_3O_9P$/529.46 g/mol
Structure	



Pharmacologic Class	HCV nucleotide analog NS5B polymerase inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND- (b) (4) IND-106,739 and NDA-204,671 (for SOF) and IND-115,268 (for LDV & SOF FDC).

2.3 Drug Formulation

LDV/SOF fixed dose combination (FDC) tablets are orange, (b) (4) film-coated, diamond shaped tablets containing 90 mg of LDV and 400 mg of SOF (refer to Sponsor Table below).

Table 1: Composition of LDV/SOF FDC tablets

Component	Composition (% w/w)	Unit Formula (mg/tablet)	Reference Quality Standards	Function
Intragranular				
Sofosbuvir[a]	40.0	400.0	In-house	Active Ingredient
Ledipasvir[b,c,d,e]	9.0	90.0	In-house	Active Ingredient
Copovidone[d,e]	(b) (4)		USP, Ph. Eur.	(b) (4)
(b) (4			USP, Ph. Eur.	
Lactose Monohydrate[a,d]			NF, Ph. Eur.	
Microcrystalline Cellulose			NF, Ph. Eur.	
Croscarmellose Sodium			NF, Ph. Eur.	
Collodial Silicon Dioxide			NF, Ph. Eur.	
Magnesium Stearate			NF, Ph. Eur.	
(b) (4)				
			NF, Ph. Eur.	
			NF, Ph. Eur.	
			NF, Ph. Eur.	
			--	
			In-house	
			USP, Ph. Eur.	
				(b) (4)

2.4 Comments on Novel Excipients

Not applicable. All excipients are compendial.

2.5 Comments on Impurities/Degradants of Concern

Qualification assessment of residual solvents, metals and LDV-related impurities (specified and unspecified process intermediates, degradants, starting materials etc.) was conducted by Dr. Mark Powley. Refer to the Pharmacology/Toxicology review of NDA-204,671 for the qualification assessment of SOF. The qualification of specified impurities in the LDV drug substance is based on results from general repeat-dose toxicology studies, assessment of potential mutagenicity using (quantitative) structure-activity relationship [(Q)SAR] models and/or phototoxicity evaluation (for (b) (4) Repeat-dose toxicology studies in rats were conducted using multiple LDV batches containing various levels of LDV-related impurities, with no indication that the impurities present in these batches altered the toxicity profile of LDV. In addition, numerous LDV impurities were evaluated by (Q)SAR analyses and are expected to be non-mutagenic. Finally, a 3-day multiple dose ocular phototoxicity study was conducted in male Long Evans (LE) rats administered LDV or LDV (spiked w/ (b) (4) % (b) (4) given a specific cause for concern identified regarding the presence of a potentially reactive impurity/ photodegradant ((b) (4) that (like LDV) absorbs ultraviolet (UV) light and (likely) accumulates in the uveal tract of the eye in pigmented LE rats. This study was considered negative (refer to Section 10). Overall, the proposed LDV specifications are considered acceptable from a pharmacology/toxicology perspective.

2.6 Proposed Clinical Population and Dosing Regimen

LDV/SOF FDC is to be indicated for treatment (single tablet once a day) of chronic hepatitis C (CHC) genotype 1 infection for 12 to 24 weeks in adult patients.

2.7 Regulatory Background

NDA-204,671 (for SOF in combination w/ other agents) was approved on December 6, 2013, while (b) (4) IND-115,268 (for LDV/SOF FDC) were opened on April 23, 2010 and July 1, 2012, respectively.

3 Studies Submitted

3.1 Studies Reviewed

Study Title	Study #
<u>Safety Pharmacology</u>	
Effects of GS-5885 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells	PC-256-2008

Cardiovascular Safety Pharmacology Evaluation of GS-5885 Administered by Oral Gavage to Male Telemetry-Instrumented Conscious Dogs	PC-256-2005
Respiratory Safety Pharmacology Evaluation of GS-5885 Using Head-Out Plethysmography following Oral Gavage Administration to Male Rats	PC-256-2006
Central Nervous System Safety Pharmacology Evaluation of GS-5885 Following Oral Gavage Administration to Male Rats	PC-256-2007
<u>Analytical Method Validation</u>	
Abbreviated Validation of a Method for the Determination of GS-5885 in Mouse Plasma by HPLC with MS/MS Detection	BA-256-2009
Validation of a Method for the Determination of GS-5885 in Mouse Plasma by HPLC with MS/MS Detection	BA-256-2011
Validation of a Method for the Determination of GS-5885 in Rat Plasma by HPLC with MS/MS Detection	BA-256-2002
Partial Validation of a Method for the Determination of GS-5885 in Rat Plasma (K_3EDTA) by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)	BA-256-2007
Partial Validation of a Method for the Assessment of the Impact of Co-administered Drugs on the Quantitation of GS-5885 in Rat Plasma (K_3EDTA) by Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS)	BA-256-2008
Abbreviated Validation of a Method for the Determination of GS-5885 in Rabbit Plasma by HPLC with MS/MS Detection	BA-256-2004
Abbreviated Validation of a Method for the Determination of GS-5885 in Dog Plasma by HPLC with MS/MS Detection	BA-256-2003
Validation of a Method for the Determination of GS-5885 in Dog Plasma (K_3EDTA) by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)	BA-256-2005
Partial Validation of a Method for the Assessment of the Impact of Co-administered Drugs on the Quantitation of GS-5885 in Dog Plasma (K_3EDTA) by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)	BA-256-2006
<u>ADME/Pharmacokinetics</u>	
Permeability of GS-5885 across Caco-2 Cell Monolayers	AD-256-2108
Pharmacokinetics of GS-5885 Following Single Oral Doses to Male and Female CD-1 Mice	AD-256-2135
Determination of the Pharmacokinetics of GS-5885 After a Single Oral Gavage Dose to Male 001178-W (wild type) Mice	AD-256-2131
Pharmacokinetics of GS-5885 in Sprague-Dawley Rats	AD-256-2102
Pharmacokinetics of GS-5885 Following Single Ascending Oral Doses in Sprague-Dawley Rats	AD-256-2116

Pharmacokinetics of GS-5885 Following Oral Suspension Doses to Non-fasted Male SD Rats	AD-256-2129
Pharmacokinetic Study of GS-5885 in Female Rabbits	AD-256-2152
Pharmacokinetics of GS-5885 in Male beagle dogs	AD-256-2103
Pharmacokinetics of GS-5885 in Cynomolgus Monkeys	AD-256-2104
In Vitro Determination of GS-5885 Protein Binding by Equilibrium Dialysis	AD-256-2094
In Vitro Metabolic Stability of GS-5885 in Hepatic Subcellular Fraction from CD-1 Mice	AD-256-2138
In Vitro Metabolic Stability of GS-5885 in Hepatic Subcellular Fractions from Human, Dog, Rat and Monkey and in Cryopreserved Human Hepatocytes	AD-256-2095
Pharmacokinetics, Absorption, Distribution, and Excretion of ^{14}C-GS-5885 Following Oral Administration to Mice	AD-256-2136
Pharmacokinetics, Absorption, Distribution, and Excretion of ^{14}C-GS-5885 Following Oral Administration to Rats	AD-256-2083
Pharmacokinetics, Absorption, and Excretion of ^{14}C-GS-5885 Following Oral Administration to Intact and Bile Duct-Cannulated Dogs	AD-256-2127
Pharmacokinetics of GS-5885 in Bile-Duct Cannulated Beagle Dogs	AD-256-2105
Profiling and Identification of Metabolites in Selected Plasma, Urine, and Feces Samples from Mice After Oral Administration of ^{14}C-GS-5885	AD-256-2137
Profiling and Identification of Metabolites in Selected Plasma, Bile, and Feces Samples from Rats after Oral Administration of ^{14}C-GS-5885	AD-256-2084
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Dogs after Oral Administration of ^{14}C-GS-5885	AD-256-2128
<u>General Toxicology</u>	
2-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-5885 in Rats	TX-256-2003
2-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-5885 in Dogs	TX-256-2004
4-Week Oral Gavage Dose Range-Finding Toxicity and Toxicokinetic Study with GS-5885 in Model 001178-W (Wild-Type), CByB6F1-Tg(HRAS)2Jic Mice	TX-256-2018
26-Week Oral Gavage Toxicity and Toxicokinetic Study of GS-5885 in Rats with a 13-Week Interim Necropsy and a 4-Week Recovery Phase	TX-256-2008

39-Week Oral Gavage Toxicity and Toxicokinetic Study of GS-5885 in Dogs with a 13-Week Interim Necropsy and a 4-Week Recovery Phase	TX-256-2009
<u>Genetic Toxicology</u>	
Bacterial Reverse Mutation Assay with a Confirmatory Assay with GS-5885	TX-256-2005
Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes with GS-5885	TX-256-2006
In Vivo Rat Bone Marrow Micronucleus Assay with GS-5885	TX-256-2007
<u>Carcinogenicity</u>	
TWENTY-FOUR MONTH ORAL GLP CARCINOGENICITY STUDY OF PSI-7977 (GS-7977) IN MICE	TX-334-2002
TWENTY-FOUR MONTH ORAL GLP CARCINOGENICITY STUDY OF PSI-7977 (GS-7977) IN RATS	TX-334-2001
<u>Reproductive and Developmental Toxicology</u>	
Study of Fertility and Early Embryonic Development to Implantation of GS-5885 Administered by Oral (Gavage) in Rats	TX-256-2017
Oral Gavage Dose Range-Finding Developmental Toxicity and Toxicokinetic Study with GS-5885 in Rats	TX-256-2011
Oral Gavage Study for Effects on Embryo-Fetal Development with GS-5885 in Rats	TX-256-2012
Oral Gavage Dose Range-Finding Developmental Toxicity and Toxicokinetic Study with GS-5885 in Rabbits	TX-256-2010
Oral Gavage Study for Effects on Embryo-Fetal Development with GS-5885 in Rabbits	TX-256-2013
An Oral (Gavage) Study of the Effects of GS-5885 on Pre- and Postnatal Development, Including Maternal Function in Rats	TX-256-2020
<u>Toxicology Studies (other)</u>	
2-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-5885 and GS-5885-02 in Rats	TX-256-2014

2-Week Oral Gavage Qualification Toxicity and Toxicokinetic Study with GS-5885 in Male Rats	TX-256-2035
Phototoxicity Evaluation of GS-5885-02 Administered as a Single Oral (Gavage) Dose in Hairless Mice	TX-256-2015
A Multiple Dose Phototoxicity Study to Determine the Effects of Oral (Gavage) Administration of GS-5885 and GS-5885 + (b) (4) on Eyes in Pigmented Rats	TX-256-2038
A 13-week Oral Gavage Toxicity and Toxicokinetic Study of GS-9190, GS-9451 and GS-5885 in the Rat	TX-248-2001
A 13-week Oral (Capsule) Toxicity and Toxicokinetic Study of GS-9190, GS-9256 and GS-5885 in the Beagle Dog	TX-248-2002
GS-5885-03: Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (Individual animal approach)	TX-256-2031
GS-5885-03: The Bovine Corneal Opacity and Permeability Assay (BCOP)	TX-256-2029
GS-5885-03: Skin Irritation to the Rabbit	TX-256-2030

3.2 Studies Not Reviewed

All nonclinical safety and PK/ADME studies were reviewed. Note: *In vitro* PK drug interaction studies utilizing human matrices (cells, microsomes etc.) were reviewed by the clinical pharmacology reviewer.

3.3 Previous Reviews Referenced

Some GS-5885 nonclinical safety studies, including safety pharmacology, ADME, repeat-dose toxicology, genetic toxicology, combination toxicology and reproductive toxicology studies have been reviewed by Dr. Verma or Dr. Lansita to support the NDA, and are summarized (as appropriate) in sections of this review, with complete reviews of pivotal studies included within the review text. With the exception of the rodent 2-year carcinogenicity studies, nonclinical safety studies for GS-7977 (SOF) to support the NDA were reviewed previously (refer to Pharmacology/Toxicology review for NDA-204,671).

4 Pharmacology

4.1 Primary Pharmacology

Ledipasvir (LDV, GS-5885) is a specific inhibitor of nonstructural protein 5A (NS5A) of hepatitis C virus (HCV) that has displayed potent inhibition of HCV replication *in vitro* (EC_{50} values of 0.053 nM against genotype 1a and 0.004 nM against

genotype 1b replicons). 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*. Refer to clinical virology reviews for a detailed description and review of the primary pharmacology data for LDV (submitted with this NDA) and SOF (reviewed in NDA-204,671).

4.2 Secondary Pharmacology

No significant pharmacologic activity of LDV against other viruses and no specific concerns regarding the *in vitro* cytotoxicity profile of LDV were noted. Potential molecular targets of LDV were screened using radioligand binding assays against a panel of 68 mammalian ion channels and receptors (study# PC-256-2020). Significant inhibition of the sodium ion channel (site 2) was observed (IC_{50}~210 nM). The clinical significance of this inhibition is considered minimal given the low unbound clinical C_{max} (~ 0.8 nM) of LDV at therapeutic exposure levels.

4.3 Safety Pharmacology

Note: The following GS-5885 study reviews were taken directly from the original IND-(b) (4) review by Dr. Pritam Verma. Refer to Pharmacology/Toxicology review of NDA-204,671 for SOF data.

1. Effects of GS-5885 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (PC-256-2008)

Based on the limit of the solubility of GS-5885 in the hERG vehicle, a nominal top dose concentration of 0.6 µM was chosen for this study. However, since measured concentrations of GS-5885 at 0.3 and 0.6 µM were not within ± 10% of the nominal concentrations, measured concentrations (0.25 and 0.50 µM) are reported below.

GS-5885 inhibited hERG current by 0.3 ± 0.2% (mean ± SEM, n = 3) at 0.25 µM and 0.8 ± 0.2% at 0.50 µM versus 0.5 ± 0.2% (n = 3) in the control cells. The decrease in hERG current at 0.25 and 0.50 µM was not statistically significant when compared to vehicle control values. The IC50 for the inhibitory effect of GS-5885 on hERG potassium current was not calculated but was estimated to be greater than 0.50 µM. Conclusion: The test article was found to inhibit hERG current at higher concentrations.

2. Cardiovascular Safety Pharmacology Evaluation of GS-5885 Administered by Oral Gavage to Male Telemetry-Instrumented Conscious Dogs (PC-256-2005)

The potential cardiovascular effects of GS-5885 administered by oral gavage were evaluated in non-naïve telemetry-instrumented conscious male Beagle dogs. Four male dogs were administered a single dose via oral gavage of all dosages using a modified Latin square design with a 3-day washout period between each dose administration. Each animal received dose preparations containing vehicle control article, or GS-5885 at 3, 10, or 30 mg/kg in a dose volume of 2.0 mL/kg. Evaluation of cardiovascular

effects was based on electrocardiography and hemodynamic parameters collected pre-dose and up to 25 hours post-dose.

No mortality or change in clinical condition was observed following any dosage of GS-5885 or vehicle control article. All electrocardiograms (ECGs) were qualitatively and quantitatively within normal limits and no test article-related arrhythmias were observed. No GS-5885-related effects on any quantitative ECG parameters, including PR, QRS duration, QT, or corrected QT interval (QTc, using Fridericia correction) were observed. There were no effects on hemodynamic or intra-abdominal temperature measurements. The NOEL for GS-5885 is 30 mg/kg, the highest dose tested.

Following doses of 3, 10, and 30 mg/kg GS-5885, plasma concentrations of GS-5885 ranged from 207 to 380 ng/mL, 1140 to 1350 ng/mL, and 1930 to 2960 ng/mL, respectively, at 4.5 hours post dose.

3. Respiratory Safety Pharmacology Evaluation of GS-5885 Using Head-Out Plethysmography following Oral Gavage Administration to Male Rats (PC-256-2006)

The potential effects of orally administered GS-5885 on respiratory function as indicated by tidal volume, respiration rate, and minute volume were evaluated in male rats. Four groups (8 animals/group) received a single dose of 0 (vehicle), 10, 30, or 100 mg/kg of GS-5885 and respiratory parameters were measured continuously for 6 hours post-dose and a single 30-minute average at approximately 24 hours post-dose.

No mortality or clinical signs of toxicity related to GS-5885 were observed. One animal given 10 mg/kg GS-5885 was found dead at approximately 5.75 hours post dose. Cause of death was attributed to equipment malfunction and not to test article administration. No GS-5885-related effects were noted on tidal volume, respiration rate, or minute volume. Based on these results, the NOEL for GS-5885 on respiratory function was considered to be 100 mg/kg, the highest dose tested.

4. Central Nervous System Safety Pharmacology Evaluation of GS-5885 Following Oral Gavage Administration to Male Rats (PC-256-2007)

The potential neurological effects of GS-5885 when administered as a single dose via oral gavage were evaluated in male rats. Four groups (6 animals/group) received a single dose of 0 (vehicle), 10, 30, or 100 mg/kg of GS-5885. Evaluation of neurological effects was based on behavioral observations collected pre-dose and at intervals up to 24 hours post-dose using a modified Irwin battery of neurological assessments, including home-cage, hand-held, open-field, and elicited response observations. In addition, general measures of toxicity consisting of mortality, clinical signs, and body temperature were evaluated.

All animals survived until scheduled sacrifice and no effects related to GS-5885 were observed for clinical signs, body temperature, or for the home-cage, hand-held, open-

field, or elicited components of the modified Irwin battery at any dose level. The NOEL for the evaluation of neurological effects in male rats was considered to be 100 mg/kg GS-5885, the highest dose tested.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption, distribution, metabolism and excretion of ledipasvir (LDV, GS-5885) have been studied in numerous *in vitro* and *in vivo* studies. A summary of the significant findings from studies in mice, rats, rabbits, dogs and monkeys is presented below. Refer to Pharmacology/Toxicology review for NDA-204,671 for SOF summary data.

Absorption:

The oral bioavailability of LDV was estimated to be ~33, 41 and 53% in rats, monkeys and dogs, respectively, following administration by gavage in vehicle [5% ethanol, 45% PEG 400 and 50% citrate buffer (pH 3.0)] (refer to Sponsor Table below). Oral T_{max} values for LDV in rats, monkeys and dogs ranged from ~4 to 5 hours. LDV has low intrinsic aqueous solubility (<1 µg/mL at pH 7.0) and low solubility in simulated intestinal fluid (25 µg/mL at pH 6.5 & 230 µg/mL at pH 5.0). This low solubility, as well as saturation of absorption, limited the exposure obtained during toxicology studies with less than proportional increases in exposure observed following oral administration at higher doses in mice, rats, rabbits and dogs. Despite this low solubility and saturation of absorption, absorption of LDV from the GI tract was sufficient to achieve adequate circulating LDV exposure levels in the toxicology studies submitted in these species.

Although no nonclinical combination PK/ADME studies were conducted with SOF and LDV, increases in SOF (and its main circulating metabolite, GS-331007) exposure were observed clinically when administered as a FDC w/ LDV (compared to w/o LDV). This increased exposure of SOF and metabolites appears to be the result of increased intestinal absorption of SOF due to LDV inhibition of intestinal transporters, as evidenced *in vitro* by measuring the effect of LDV on the bidirectional permeability of SOF using Caco-2 cell monolayers. No significant safety issues for this increased exposure were identified, with exposure multiples recalculated and included in Table 41.

Table 2: Summary of plasma PK parameters of LDV in rats, dogs and monkeys following oral gavage administration

Species	Dose[a] (mg/kg)	t_{max} (h)	C_{max} (nM)	$t_{1/2}$ (h)	$AUC_{(0-\infty)}$ (nM•h)	%F
Sprague-Dawley Rat	2.5	4.7 ± 1.2	356 ± 111	4.7 ± 0.3	4114 ± 813	32.5 ± 6.7
Beagle Dog	0.5	4.0 ± 3.5	156 ± 28	6.9 ± 1.4	2138 ± 501	53.0 ± 12.4
Cynomolgus Monkey	1.0	4.0 ± 0.0	197 ± 30	8.6 ± 1.3	2734 ± 240	41.1 ± 3.6

a Vehicle contained 5% ethanol, 45% PEG 400 and 50% citrate buffer (pH 3.0, 50 mM)

1 nM LDV = 0.889 ng/mL

Source: m4.2.2.2, AD-256-2102, AD-256-2103, and AD-256-2104

Distribution:

In rats, dogs and monkeys, the steady state volume of distribution (V_{ss}) was 2.7, 1.2, and 2.2 L/kg, respectively, values greater than total body water volume, with mean residence times (MRT) of ~6, 9 and 13 hours, respectively (refer to Sponsor Table below). In addition, LDV was bound highly to plasma protein in all species (>99.8% bound), including CD-1 mice, Sprague-Dawley rats, Beagle dogs, Cynomolgus monkeys and humans, as determined by equilibrium dialysis at 2 & 10 μM (study #AD-256-2094). These data with LDV appear reasonable given its hydrophobic structure and low intrinsic aqueous solubility.

Table 3: Summary of plasma PK parameters of LDV in rats, dogs and monkeys following intravenous administration

Species	$AUC_{(0-\infty)}$ (nM•h)	CL (L/h/kg)	V_{ss} (L/kg)	$t_{½}$ (h)	MRT (h)
Sprague-Dawley Rat	2373 ± 203	0.43 ± 0.04	2.7 ± 0.1	4.7 ± 0.6	6.2 ± 0.3
Beagle Dog	1401 ± 243	0.13 ± 0.02	1.2 ± 0.1	7.4 ± 0.8	9.2 ± 1.4
Cynomolgus Monkey	3374 ± 97	0.17 ± 0.00	2.2 ± 0.4	10.3 ± 1.2	12.9 ± 2.1

1 nM LDV = 0.889 ng/mL

Source: m4.2.2.2, AD-256-2102, AD-256-2103, and AD-256-2104

Tissue distribution of LDV and related metabolites was examined using quantitative whole body autoradiography in male CD-1 mice following a single 20 mg/kg dose of ^{14}C-LDV (study #AD-256-2136). ^{14}C-LDV-derived radioactivity distributed to most tissues by 3 hours post-dose, with the highest maximum concentrations of radioactivity observed in the GI tract, gall bladder, liver, harderian gland and kidney. LDV does not appear to cross the blood-testes and blood-brain barriers significantly in mice since only low levels of radioactivity were measured in testes and trace amounts detected in the brain. Tissue distribution was also examined using similar methods in male pigmented [Long-Evans (LE)] and non-pigmented [Sprague-Dawley (SD)] rats following a single 10 mg/kg dose of ^{14}C-LDV (study #AD-256-2083). Differences

between pigmented and non-pigmented rats were observed, most notably in the eye uveal tract. Concentration of LDV and related metabolites in the eye uveal tract in LE rats peaked at 8 hours post-dose (at ~4 times that in plasma) and was still detectable at 168 hours post-dose (at ~32% that at 8 hours). Although a slightly increased distribution of LDV to pigmented versus non-pigmented skin was observed in these rats, direct binding of LDV to melanin appears unlikely. In Sprague-Dawley rats, concentration of LDV and related metabolites in liver were ~17 and 23 times that in plasma 1 and 4 hours post-dose, at 24 hours was ~15% that at 1 hour and was below the level of detection by 168 hours. In addition to liver, the highest concentrations were measured in the GI tract, bile, adrenal, salivary, thyroid, pituitary and harderian glands, pancreas, adipose tissue (brown) and kidney (refer to Table below). LDV and related metabolite concentrations were less than 50% of plasma (at all time-points) in only bone, brain, epididymis, eye and spinal cord. At 168 hours post-dose, LDV and related metabolites were almost completely absent from tissues and could only be detected in the adrenal and pituitary glands as well as the kidney.

Table 4: Selected[*] tissue to plasma ratios of LDV and related metabolites from male Sprague-Dawley rats administered a single 10 mg/kg dose of ^{14}C-LDV.

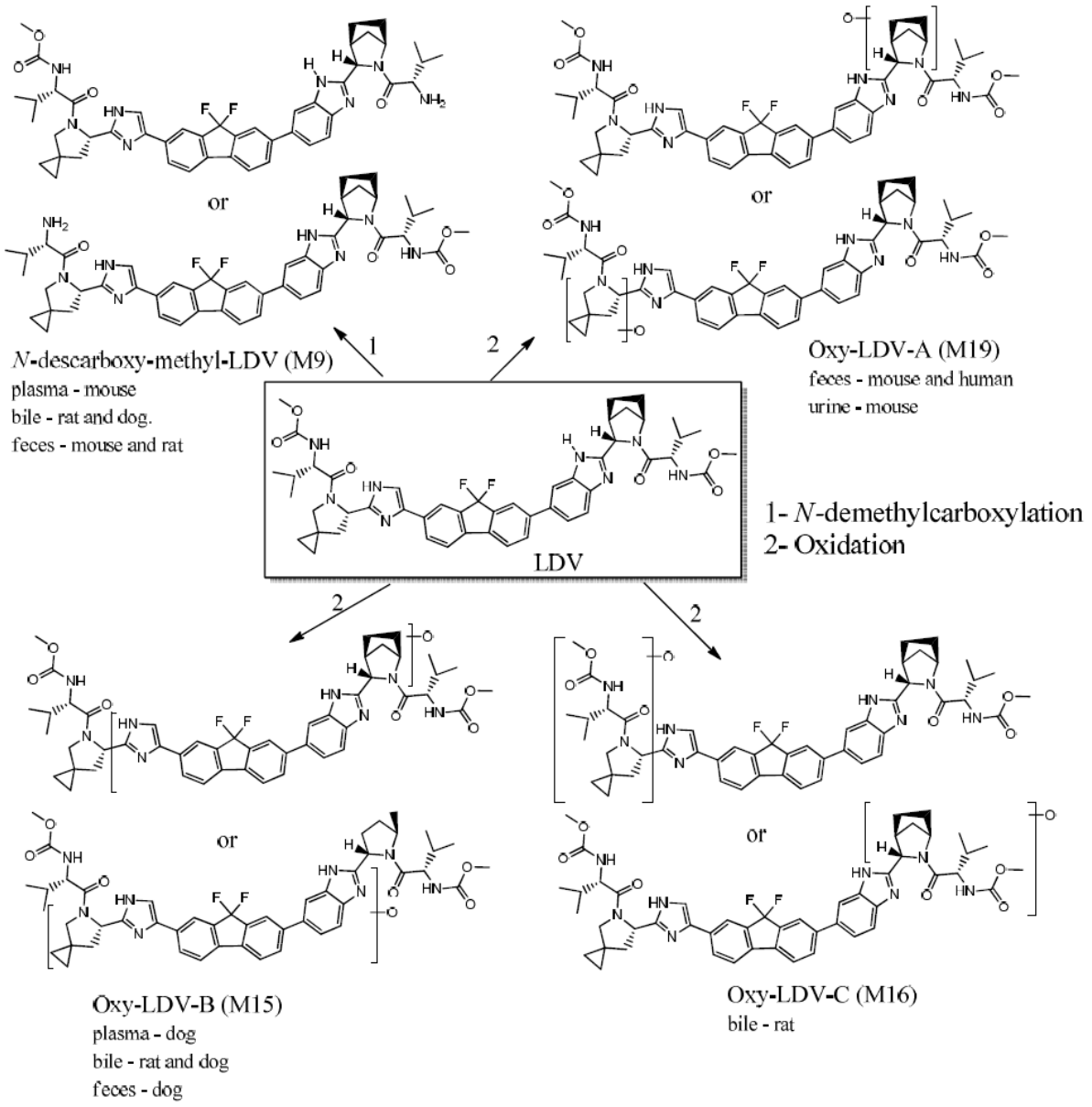
Tissue	2 hrs post-dose	8 hrs post-dose	24 hrs post-dose
Bile	32	26	29
Liver	20	20	17
Adrenal Gland	8	17	29
Renal Cortex	7	13	22
Renal Medulla	6	6	20
Harderian gland	NA	9	34
Spleen	4	6	10
Salivary Gland	3	9	9
Pancreas	4	9	6
Thyroid Gland	5	8	5
Adipose (brown)	4	7	5
Pituitary Gland	3	5	8
Urinary Bladder	9	2	ND
Urine	6	ND	ND
Diaphragm	3	4	4
Heart (myocardium)	4	4	3
Lung	2	4	3
Lacrimal gland (exorbital)	NA	6	5
Lacrimal gland (intraorbital)	NA	4	6
Prostate gland	NA	4	5
Thymus	NA	4	5
Lymph Node(s)	NA	4	5
Skin (non-pigmented)	NA	2	3
Skeletal Muscle	NA	3	ND

[*]non-GI tract tissues with ratios >2-fold; ND=not determined due to insufficient data; NA=not applicable since <2-fold

Neonatal rats were exposed to LDV via milk consumption from lactating rats administered LDV once daily by oral gavage from Gestation Day (GD) 6 through Lactation Day (LD) 10 (study #TX-256-2020, refer to Section 9.3). Briefly, LDV was administered at dose levels up to 100 mg/kg/day to F_0 female animals. Exposure to LDV in the F_1 neonate rats increased greater than dose proportionally as the maternal dose level increased from 10 to 100 mg/kg/day. The $AUC_{0\text{-}24h}$ values (sexes combined) on LD 10 in the F_1 neonate rats at these dose levels ranged from ~0.6 to 9.8 µg•h/mL, which were ~25% of the exposure in corresponding F_0 female rats. Although tissue distribution of LDV was not evaluated, neonatal rats were exposed to significant amounts of LDV via lactation. In addition, placental transfer of LDV and metabolites was not specifically evaluated in rodents.

Metabolism:

LDV was stable metabolically, as assessed *in vitro* in hepatic microsomes from mice, rats, dogs, monkeys and humans and in cryopreserved human hepatocytes. Unchanged parent drug was the major circulating component, representing ~87 to 98% of total plasma AUC following oral administration of ^{14}C-LDV to CD-1 and rasH2 mice (study# AD-256-2137), intact and bile duct cannulated rats (study# AD-256-2084), intact and bile duct cannulated dogs (study# AD-256-2128) and healthy human subjects. Unchanged parent drug was not only the major component in feces (accounting for greater than 80% of the total) in mice, rats, dogs, and humans, but also accounted for ~44% and 80% of the radioactivity recovered in bile from rats and dogs, respectively. Less than 1% of the total dose was recovered in urine from all nonclinical species. Several minor metabolites were identified in plasma, feces, bile and/or urine, primarily formed via oxidation and *N*-demethylcarboxylation (refer to Sponsor Figure and Table below).



Note: The metabolites and pathways are proposed based on general knowledge of metabolism and do not imply definitive pathways. Direct experimentation has not been performed.

Source: m4.2.2.4, AD-256-2084, AD-256-2128, AD-256-2138 and m5.3.3.1, GS-US-256-0108

Figure 1: Proposed biotransformation pathway of LDV

Table 5: Percent of total plasma AUC for LDV and metabolites following oral administration of ^{14}C-LDV

Compound	CD-1 Mouse[a]	rasH2 Mouse[a]	Sprague-Dawley Rat[a]	Beagle Dog[a]	Human[a,b]
M1	ND	ND	4.63	ND	0.981
M3	ND	ND	0.78	ND	ND
M9	1.12	0.12	ND	ND	ND
M10	ND	ND	0.91	ND	ND
M12	ND	ND	ND	ND	0.283
M15	ND	ND	ND	5.29	ND
LDV	96.9	97.2	87.1	87.5	98.3
Total	98.0	97.3	93.4	92.8	99.6

f AUC = area under the plasma concentration-time curve from time zero to 24 hours post dose in mice, dogs and human or 12 hours post dose in rats.

g Data from the mean of individual human subjects (n = 8).

ND = not detected

Source: m4.2.2.4, AD-256-2137, AD-256-2084, AD-256-2128 and m5.3.3.1, GS-US-256-0108

Excretion:

The systemic clearance (CL) of LDV was low in all species tested (less than 12% of the hepatic blood flow) with terminal half-lives of ~5, 7, and 10 hours in rats, dogs and monkeys, respectively (refer to Table 3). Approximately 93 to 96% of the administered LDV-derived radioactivity was excreted in feces from mice, rats and dogs. In addition, it was estimated that ~86% and 98% of the absorbed dose was eliminated via biliary excretion in rats and dogs, respectively, so biliary excretion of unchanged parent compound was a major route of elimination for LDV.

PK Drug Interactions:

Refer to the clinical pharmacology review for a detailed description and review of the PK drug interaction-related data for LDV and SOF.

5.2 Toxicokinetics

Review of this data is included in Section 6.

6 General Toxicology

6.1 Single-Dose Toxicity

Not conducted

6.2 Repeat-Dose Toxicity

Note: The following study reviews were summarized from the original IND-(b) (4) review by Dr. Pritam Verma. Refer to Pharmacology/Toxicology review for NDA-204,671 for SOF data.

Study title: 2-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-5885 in Rats

Study no.:	TX-256-2003
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 23, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-5885, 3542-189-3 and 90.8%

Key Study Findings: GS-5885 administered once daily by oral gavage to male and female rats for 14 days was well-tolerated at dose levels up to 100 mg/kg/day. Mean body weight gain was similar across all groups during Week 2 of the dosing phase, but remained slightly decreased over the entire dosing period. The NOAEL was considered to be 100 mg/kg/day (Day 14 C_{max} and AUC_{0-24} of 2,200 and 1,947 ng/mL, and 34,464 and 25,083 ng•hr/mL, in males and females, respectively).

Table 6: Mean plasma TK parameters for GS-5885 in rats following 1 or 14 days of GS-5885 administration at 10, 30 or 100 mg/kg dose levels

Dose Group	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (hr)	C_{last} (ng/mL)	T_{last} (hr)	AUC_{0-t} (ng·hr/mL)	AUC_{0-24} (ng·hr/mL)
				Day 1				
2	10	M	328	6.00	45.8	24.0	4556	4556
		F	303	4.00	18.3	24.0	3659	3659
3	30	M	981	4.00	207	24.0	12430	12430
		F	782	4.00	94.8	24.0	8978	8978
4	100	M	1630	8.00	568	24.0	23200	23200
		F	1149	6.00	335	24.0	17104	17104
				Day 14				
2	10	M	464	4.00	13.4	48.0	7592	6493
		F	414	2.00	2.20	48.0	4739	4490
3	30	M	1273	4.00	40.7	48.0	21730	18218
		F	1099	4.00	8.45	48.0	12826	12054
4	100	M	2200	4.00	99.1	48.0	41388	34464
		F	1947	8.00	10.8	48.0	29598	25083

Table from sponsor

Study title: 2-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-5885 in Dogs

Study no.:	TX-256-2004
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 16, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-5885, 3542-189-3 and 90.8%

Key Study Findings: Test article-related adverse effects were body weight loss and lower food consumption during Week 1 of the dosing phase in several animals given 30 mg/kg/day; however, this dose level was well-tolerated when nutritional supplementation was provided in the form of canned food during Week 2 of the dosing phase. Large cerebral ventricles noted at trimming in one female given 30 mg/kg/day correlated with the microscopic finding of minimal dilatation of ventricles. Based on these findings, the NOAEL was considered to be 10 mg/kg/day (Day 14 C_{max} and AUC_{0-24} of 2,407 and 2,330 ng/mL and 37,403 and 35,382 ng.hr/mL in males and females, respectively).

Table 7: Mean plasma TK parameters for GS-5885 in dogs following 1 or 14 days of GS-5885 administration at 3, 10 or 30 mg/kg dose levels

Dose Group	Dose Level (mg/kg/day)	Sex		C_{max} (ng/mL)	T_{max} (hr)	C_{last} (ng/mL)	T_{last} (hr)	AUC_{0-24} (ng·hr/mL)
				Day 1				
2	3	M	Mean	626	3.33	123	24.0	7819
			SD	94	1.15	53	0	1875
			N	3	3	3	3	3
		F	Mean	528	4.00	141	24.0	8099
			SD	168	0	62	0	2524
			N	3	3	3	3	3
3	10	M	Mean	1433	6.00	465	24.0	21574
			SD	316	5.29	342	0	9645
			N	3	3	3	3	3
		F	Mean	1477	5.33	527	24.0	24589
			SD	157	2.31	14	0	2143
			N	3	3	3	3	3
4	30	M	Mean	5860	2.67	2223	24.0	67615
			SD	2922	1.15	935	0	43636
			N	3	3	3	3	3
		F	Mean	3350	4.00	1025	24.0	50577
			SD	377	0	186	0	6048
			N	3	3	3	3	3
				Day 14				
2	3	M	Mean	755	3.33	170	24.0	10225
			SD	122	1.15	90	0	3815
			N	3	3	3	3	3
		F	Mean	686	4.00	112	24.0	8132
			SD	192	0	45	0	2235
			N	3	3	3	3	3
3	10	M	Mean	2407	3.33	791	24.0	37403
			SD	853	1.15	333	0	15552
			N	3	3	3	3	3
		F	Mean	2330	4.67	705	24.0	35382
			SD	569	3.06	250	0	7747
			N	3	3	3	3	3
4	30	M	Mean	7173	3.00	3107	24.0	121015
			SD	1787	1.73	913	0	34549
			N	3	3	3	3	3
		F	Mean	4867	4.00	1217	24.0	75354
			SD	2515	0	1114	0	51562
			N	3	3	3	3	3

Table from sponsor

Note: The following study review was taken directly from the original IND- (b) (4) review by Dr. Janice Lansita.

Study title: 4-Week Oral Gavage Dose Range-Finding Toxicity and Toxicokinetic Study with GS-5885 in Model 001178-W (Wild-Type), CByB6F1-Tg(HRAS)2Jic Mice

Study no.:	TX-256-2018
Study report location:	Gilead Sciences, Inc. 333 Lakeside Drive Foster City, California 94404
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	18 June 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-5885-02, RS-A-5885-02-4. 99.6%

Key Study Findings

In the 4-week oral gavage study in CByB6F1-(Tg(HRAS)2Jic wild-type littermates) mice at doses of 0, 20, 60 and 300 mg/kg, there were no GS-5885-related adverse findings. The NOAEL was the highest dose of 300 mg/kg/day, since no toxicity was observed.

Methods

Doses:	0, 20, 60, 300 mg/kg
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Vehicle: 0.2% Tween 20 (v/v), 0.2% (w/v) hydroxypropyl methylcellulose, and 0.9% (v/v) benzyl alcohol in reverse osmosis water
Species/Strain:	Model 001178-W (wild type), CByB6F1-Tg(HRAS)2Jic mice
Number/Sex/Group:	10
Age:	7 weeks old at study initiation
Weight:	19.2 to 29.5 g for males and 14.5 to 22.6 g for females
Satellite groups:	TK - 6/sex control group, 39/sex/group
Unique study design:	None
Deviation from study protocol:	No impact deviations.

Observations and Results

Mortality

All animals survived to the end of the study with the exception of one TK female that was found dead on Day 15. The death was not considered to be test article related since no clinical observations were seen in this animal and no macroscopic findings were observed during necropsy.

Clinical Signs

No test article related clinical signs were observed.

Body Weights and Food Consumption

Test article related increases in mean body weight were seen in high dose males (5.2%) on Day 29 compared with controls. Mean body weight gain was increased in high dose males (77.3%) and females across dose groups (19.2%-50%). The increase in mean absolute body weight and mean body weight gain in high dose males correlated with increased mean food consumption in high dose males (up to 20% from Day 15-22) compared to controls.

Ophthalmoscopy

Not applicable

ECG

Not applicable

Hematology

Increases in mean reticulocyte counts (high dose males - 21.4%, high dose females - 18.9%) and white blood cell counts (high dose females - 35.6%) were observed but were not considered to be toxicologically significant.

Clinical Chemistry

An increase in mean ALP was observed in high dose males (51.9%) and high dose females (19.5%) which may correlate with an increase in liver/gall bladder weights in high dose males. Mean ALT was slightly elevated in mid- and high-dose males (26.7%) and moderately elevated in low-dose (53.6%) and high-dose females (64.3%). The sponsor did not consider these changes to be adverse. It is possible that the ALP (males and females), ALT (males and females) and liver/gall bladder weight (males only) changes are indicative of mild liver toxicity (summarized below). However, since no correlating histopathology changes were seen in the liver these findings were not considered to be adverse.

Table 8: Summary of ALP, ALT and Liver/Gall Bladder Weight Mean % Change Relative to Mean Control Values in High Dose (300 mg/kg) Male and Female Mice

Parameter	Mean % Change Relative to Controls	% Change Relative to Controls
	High Dose Males	High Dose Females
ALP	51.9%	19.5%
ALT	26.7%	64.3%
Liver/Gall Bladder Weights	23% Absolute 15% Relative to Brain 21% Relative to Body	No significant change.

Urinalysis

Not applicable

Gross Pathology

No test article-related gross pathology findings were observed. Gavage-related macroscopic findings were seen in one high dose male (Animal No. A76303) and included a raised area in the distal esophagus serosal membrane (or serosa), semisolid material in the thoracic cavity, and multiple tan adhesions of all lung lobes.

Organ Weights

An increase in liver/gall bladder weights in high dose males (absolute - 23%, relative to body weight - 15%, and relative to brain weight - 21%) and slight increases in liver/gall bladder weights in females (absolute - 9%, relative to body weight - 8%, and relative to brain weight - 9%) were seen. Additional organ weight changes in high dose males included decreased seminal vesicle weights (absolute - 19%, relative to body weight - 25%, and relative to brain weight - 21%). These changes were not considered to be toxicologically significant since there were no correlating toxicities identified by clinical pathology and/or histopathology.

Histopathology

Adequate Battery
Yes
Peer Review
No
Histological Findings
Histopathology was performed on the control and high dose groups. Microscopic pathology findings were seen in the esophagus and were described as minimal to slight regeneration and/or degeneration of the esophageal muscularis in control and GS-5885 treated animals; the sponsor attributed the findings to the gavage procedure. However, there was a slight increase in the incidence of regeneration of the esophageal muscularis in high dose females compared with controls: 7 high dose females vs. 3 control females. This apparent change may be a result of physical trauma related to the gavage procedure (i.e., if the test article was more viscous or difficult to administer compared with the vehicle) since test-article related findings in the esophagus were not reported in previously conducted toxicity studies. Chronic cardiac inflammation of the heart and pleura of the lung in one high dose male were also attributed to the gavage procedure and correlated with macroscopic findings in the lung and thoracic cavity. Finally, an additional male animal showed cardiac inflammation described as focal chronic inflammation of the epicardial fat and epicardium (minimal severity) was considered to be an incidental finding. Since cardiac findings were not observed in previously conducted toxicity studies in rats and dogs with GS-5885, this interpretation appears to be reasonable.

Special Evaluation

None

Toxicokinetics

The TK of GS-5885 in male and female mice was characterized on Day 1 and Day 28

(see Sponsor Table below). T_{max} ranged from 2-8 hours on Day 1 and 2-4 hours on Day 28. C_{max} and AUC overall increased with dose less than dose-proportionally (with the exception of females between 20 and 60 mg/kg) on Day 1 and Day 28. Slight accumulation was seen in high dose males on Day 28 compared with Day 1 for C_{max} (2X) and AUC (1.4X). High dose males showed higher C_{max} (96%) and AUC values (65%) compared with females on Day 28 across dose groups.

Table 9. Mean Toxicokinetic Parameters of GS-5885 in the 4-week Oral Gavage Study in Wild Type Mice (TX-256-2018)

Dose Level (mg/kg/day)	Sex	C_{max} (µg/mL)		AUC_{0-24} (µg·h/mL)	
		Day 1	Week 4	Day 1	Week 4
20	Male	4.06	3.77	48.7	38.4
60	Male	6.70	6.71	85.0	83.6
300	Male	13.4	26.2	198	271
20	Female	2.60	2.61	28.0	16.7
60	Female	6.55	7.54	69.1	56.3
300	Female	11.0	13.4	161	164

Dosing Solution Analysis

The homogeneity and concentration verification analyses met the acceptance criteria of the study. The stability analyses met the acceptance criteria at day 15 however, were out of specification for two conditions at 100 mg/ml- the 25 hour room temperature and 11-day refrigerated intervals. The out of specification results did not impact the study since the 15 day stability results established stability across dose concentrations and temperatures.

Note: The following study review was taken directly from the original IND-(b) (4) review by Dr. Pritam Verma.

Study title: 26-Week Oral Gavage Toxicity and Toxicokinetic Study of GS-5885 in Rats with a 13-Week Interim Necropsy and a 4-Week Recovery Phase

Study no.: TX-256-2008
Study report location: Electronic
Conducting laboratory and location: (b) (4)
Date of study initiation: 06 July 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: GS-5885, 5885-XB-1 and 97.7%

Key Study Findings: GS-5885 administered once daily via oral gavage to rats for at least 26 weeks was well-tolerated at dose levels up to 100 mg/kg/day. Therefore, the NOAEL for GS-5885 was 100 mg/kg/day (mean Week 26 C_{max} of 3,228 ng/mL and AUC_{0-24} of 56,008 ng·hr/mL; sexes combined).

Methods

Doses:	0, 10, 30 and 100 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral
Dose volume:	5 ml/kg
Formulation/Vehicle:	[45% propylene glycol and 15% Solutol HS-15 prepared in 40% pH-adjusted reverse osmosis water (pH 2.5 ± 0.1) (v/v/v)].
Species/Strain:	Rats/Crl:CD(SD)
Number/Sex/Group:	10/sex/group
Age:	6-7 weeks old
Weight:	170 to 324 g for males and 145 to 252 g for females
Satellite groups:	5/sex/group
Unique study design:	None
Deviation from study protocol:	None

Observations and Results

Mortality: All toxicity animals designated for the interim and terminal phase sacrifice survived to their respective scheduled sacrifice, except four females given 100 mg/kg/day. Two of these deaths were attributed to gavage error and two were of undetermined cause.

Toxicity group female No. B88091 given 100 mg/kg/day was sacrificed on Day 57 and was observed with red hair coat (front legs/facial and perineal regions), particularly on Days 55, 56, and 57; few feces; general debilitation on Day 57; and a 15% body weight loss during Days 50 to 57. No remarkable clinical pathology, macroscopic or microscopic observations were made for this animal. The cause of death was undetermined and of uncertain relationship to treatment.

Toxicity group female No. B88103 given 100 mg/kg/day was sacrificed on Day 134 and was observed with red hair coat (head/front legs), particularly on Days 131, 133, and 134; yellow hair coat on Days 133 and 134; few feces; general debilitation on Day 134; and a 13% body weight loss from Days 127 to 134. The only notable clinical pathology finding was increased absolute neutrophil count. The only noteworthy microscopic findings for this animal were slight ulceration/erosion and epithelial hyperplasia of the nonglandular stomach; which are common microscopic findings in debilitated rats and were not test-article related. The cause of death was undetermined and of uncertain relationship to treatment.

Clinical Signs: Clinical signs were limited to a slightly increased incidence of red hair coat around the perioral region, nose, and/or mouth in males given ≥ 30 mg/kg/day and

in females given 100 mg/kg/day, and struggling during dosing during the first few weeks of dosing for multiple females given 100 mg/kg/day. Red hair coat was not observed during the recovery phase.

Body Weights: After Week 1 of dosing, mean body weights for males given 100 mg/kg/day were significantly lower (8%) compared with controls, and mean body weight gain was lower for males and females given 100 mg/kg/day (decreased 45 and 29% versus controls, respectively). At Week 26, lower mean body weight (non-significantly decreased 9% versus control) and mean body weight gain (significantly decreased 16% versus control) was noted in males given 100 mg/kg/day. During the recovery phase, body weight gain for males given 100 mg/kg/day was comparable with controls.

At Week 26, there were no notable effects on body weight or body weight gain in females given 100 mg/kg/day, and no changes in body weight in males or females treated with 10 and 30 mg//kg/day GS-5885.

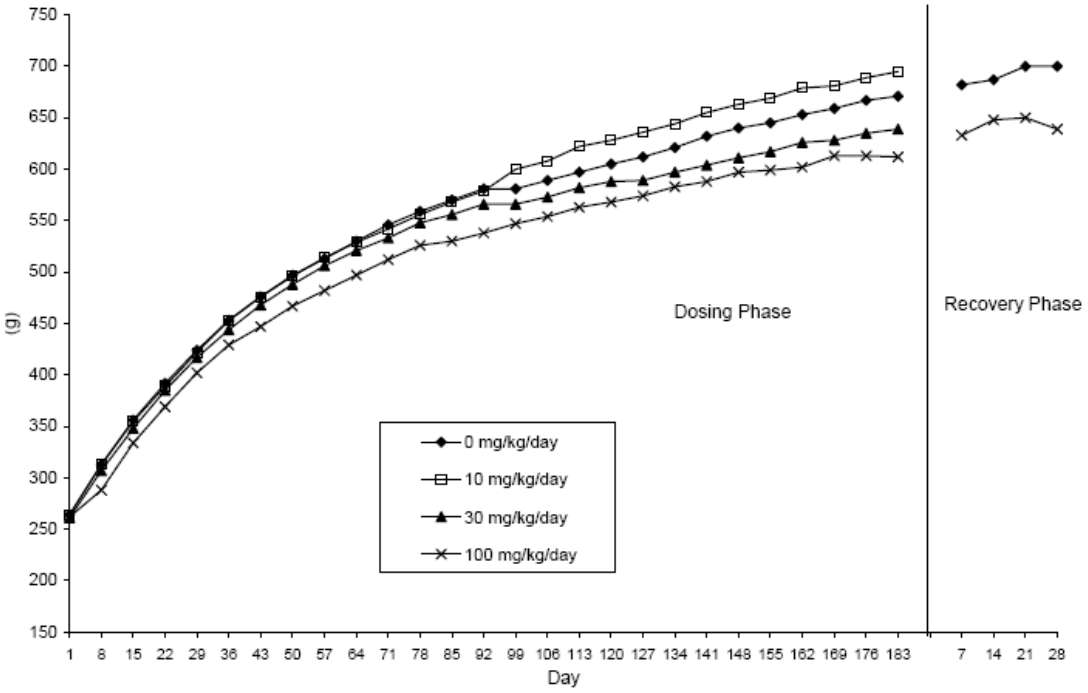


Figure 2: Mean Body Weight Data – Males

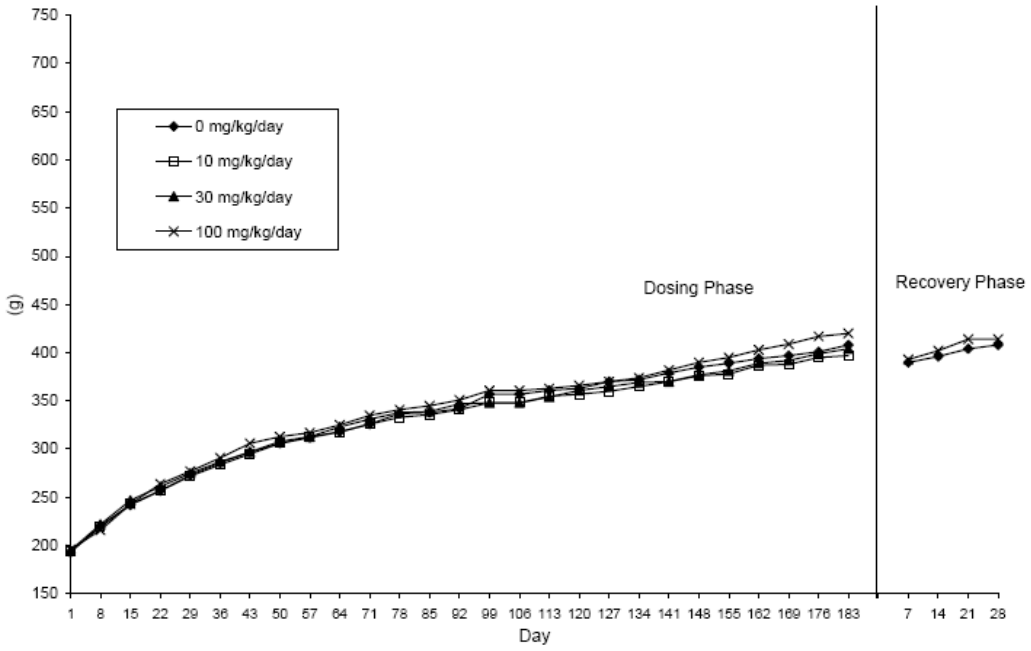


Figure 3: Mean Body Weight Data - Females

Feed Consumption: Mean food consumption was significantly lower during Week 1 in males and females given 100 mg/kg/day (decreased 26% and 13%, respectively). After Week 2, food consumption was generally similar between treated and control groups throughout the dosing and recovery phases. Small, statistically significant increases in food consumption were observed sporadically during the 26 week dosing phase and during the recovery phase for males and females given 100 mg/kg/day.

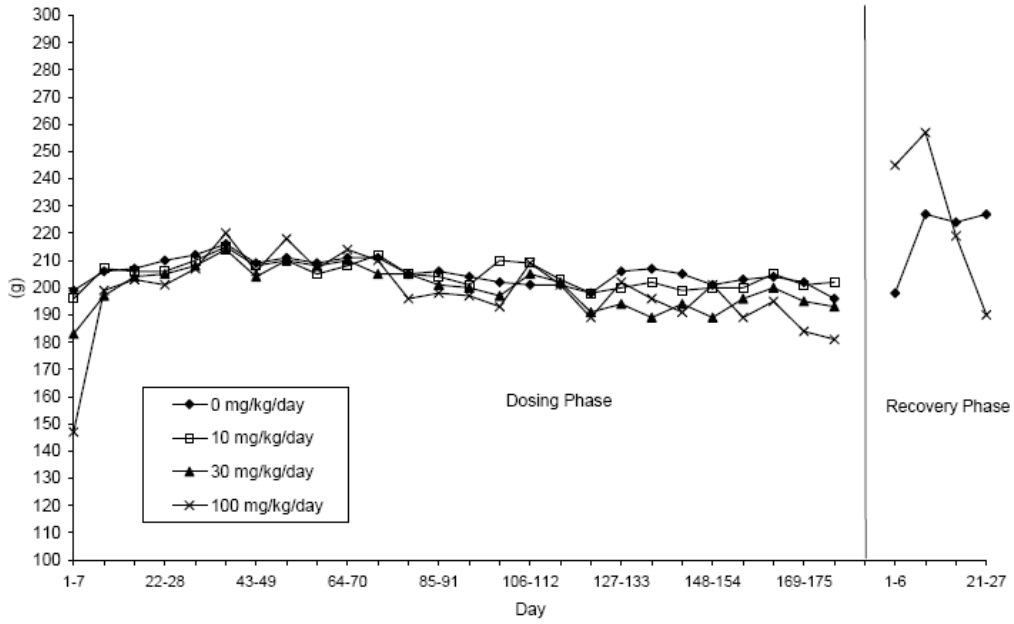


Figure 4: Mean Food Consumption Data - Males

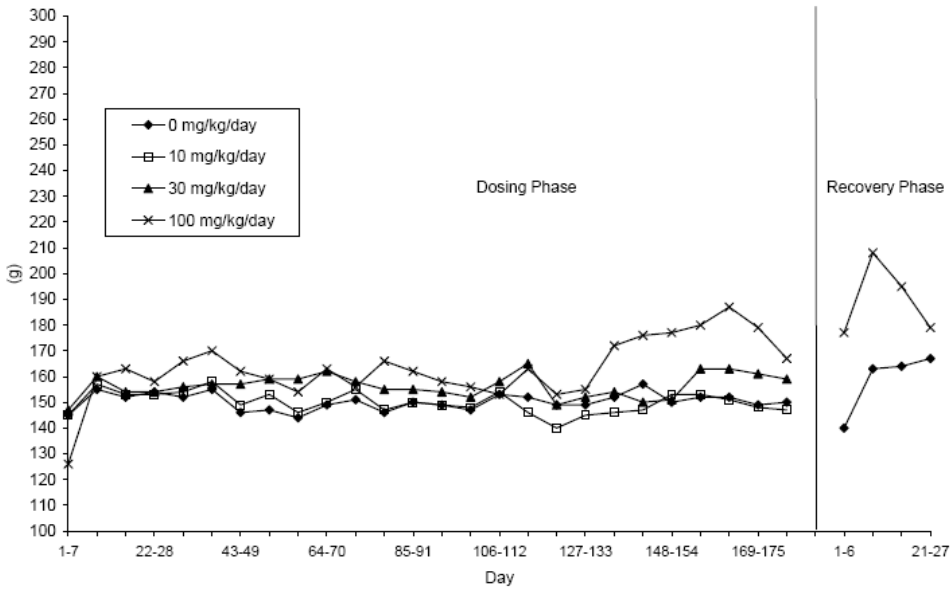


Figure 5: Mean Food Consumption Data - Females

Ophthalmoscopy: No abnormal findings were noted during ophthalmic examinations conducted pre-dose, or during Weeks 13 or 26.

Clinical Pathology: Hematology findings considered GS-5885-related affected only

animals given 100 mg/kg/day compared to controls and were limited to the following:
1. Mildly lower mean absolute reticulocyte count for males at Weeks 13 and 26 (30% and 25%, respectively).
2. Mildly higher mean absolute neutrophil count for females at Week 26 (1.67-fold control).
Lower absolute reticulocyte count for males given 100 mg/kg/day may have resulted from a normal negative feedback response because these males had slightly higher red cell mass (i.e., red blood cell count, hemoglobin, and hematocrit) than control males. Higher absolute neutrophil count for females given 100 mg/kg/day suggested a mild inflammatory response and was consistent with the findings of minimally higher globulin and lower albumin-to-globulin ratio for these females. However, no correlative anatomic pathology findings were observed.

Clinical chemistry findings considered GS-5885-related affected only animals given 100 mg/kg/day compared to controls and were limited to the following:

Males: minimally lower mean glucose at Week 26 (10%).
Females: minimally lower mean urea nitrogen at Weeks 13 and 26 (25% and 18%, respectively); minimally higher mean globulin at Week 26 (1.11-fold control); minimally lower mean albumin-to-globulin ratio at Weeks 13 and 26 (12% and 14%, respectively); minimally higher mean cholesterol at Week 26 (1.32-fold control); moderately higher mean triglycerides at Week 26 (2.20-fold control); minimally higher mean calcium at Week 26 (1.04-fold control).

Coagulation and Urinalysis: Test results were unaffected by GS-5885.

Gross Pathology:
Interim Sacrifice: In animals sacrificed after 13 weeks of dosing, no test article-related organ weight or macroscopic findings were noted.

Terminal Sacrifice:

Organ Weights: Statistically significant increases in absolute and/or relative (to body weight or brain weight) adrenal weights in males and females given 100 mg/kg/day (24 to 28% versus control) and in absolute and/or relative liver weights in females given 30 mg/kg/day (12% versus control) or 100 mg/kg/day (17 to 22% versus control) did not have microscopic correlates.

No test article-related macroscopic findings were noted in animals sacrificed after at least 26 weeks of dosing.

Histopathology: Test article-related histomorphologic alterations were observed.

Adequate Battery: yes
Peer Review: yes

Histological Findings: an increased incidence and severity of minimal to slight adrenal

cortical sinusoid dilatation/congestion in females given 100 mg/kg/day was observed. Minimal adrenal vacuolar degeneration in a single male and a single female given 100 mg/kg/day was seen. In the liver, random foci of hepatocyte necrosis, minimal or slight, were noted in males given 10, 30, or 100 mg/kg/day but not in controls. Minimal to slight bile duct hyperplasia was noted in males given 10, 30, or 100 mg/kg/day and in females given 30 or 100 mg/kg/day but not in controls.
Recovery Sacrifice: At the end of the 4-week recovery period, all of the test article-related organ weight findings exhibited evidence of reversibility, except increased relative (to body weight) adrenal weight in males. As there were no microscopic correlates, this finding is not considered toxicologically relevant. No test article-related macroscopic or microscopic findings were noted in recovery animals.

Toxicokinetics: Data are shown in Table below. Values for C_{max} and AUC_{0-24} increased from Day 1 to Week 26 (approximately 2-fold), indicating accumulation of GS-5885 after repeat dosing in rats. Exposure was only slightly (< 2-fold) higher at Week 26 compared to Week 13. Although C_{max} and AUC_{0-24} values in males were slightly higher than in females, sex differences in plasma exposure of GS-5885 were generally <2-fold.

Table 10: Toxicokinetic Parameters for GS-5885 in Rat Plasma - Day 1 and Weeks 13 and 26

Dose Level		AUC0-24 (ng·hr/mL)			Cmax (ng/mL)		
(mg/kg/day)	Sex	Day 1	Week 13	Week 26	Day 1	Week 13	Week 26
10	Male	4667	11699	16067	408	936	1005
	Female	2703	5256	7703	209	444	669
	Combined	3685	8477	11885	300	675	822
30	Male	12561	26866	36189	917	1720	2140
	Female	10361	16835	23563	765	1527	2083
	Combined	11461	21850	29876	841	1445	1965
100	Male	26807	51097	60842	1697	3900	3470
	Female	19340	43728	51175	1323	2737	2987
	Combined	23073	47413	56008	1448	3067	3228

Stability and Homogeneity: Solution stability was confirmed at concentrations of 0.0925 and 20.9 mg/mL for 15 days under room temperature and refrigerated conditions. Mean concentrations of the test article formulations ranged from 92.7 to 106.8% of the nominal concentration.

Note: The following study review was taken directly from the original IND-(b) (4) review by Dr. Pritam Verma.

Study title: 39-Week Oral Gavage Toxicity and Toxicokinetic Study of GS-5885 in Dogs with a 13-Week Interim Necropsy and a 4-Week Recovery Phase

Study no.: TX-256-2009
Study report location: electronic
Conducting laboratory and location: (b) (4)

Date of study initiation:	25 June 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-5885, 5885-XB-1 and 97.7%

Key Study Findings: GS-5885 given once daily by oral gavage to male and female Beagle dogs for 39 weeks was well-tolerated at dose levels up to 30 mg/kg/day. Therefore, the NOAEL for GS-5885 was 30 mg/kg/day (mean Week 39 C_{max} 4157 ng/mL; and AUC_{0-t} 62,563 ng·hr/mL; sexes combined).

Methods

Doses:	0, 3, 10 and 30 mg/kg/day
Frequency of dosing:	daily
Route of administration:	oral
Dose volume:	2 ml/kg
Formulation/Vehicle:	[45% propylene glycol and 15% Solutol HS-15 prepared in 40% pH-adjusted reverse osmosis water (pH 2.5 ± 0.1) (v/v/v)].
Species/Strain:	Beagle dogs
Number/Sex/Group:	7-9/sex/group; 3/sex/group for 13-week interim sacrifice, 4/sex/group for the terminal 39-week sacrifice, and 2/sex/control and high dose groups for recovery).
Age:	6 to 7 months old
Weight:	8.0 to 10.6 kg for males and 7.6 to 10.7 kg for females
Satellite groups:	none
Unique study design:	none
Deviation from study protocol:	none

Observations and Results

Mortality: One male (Animal No. H06413) given 30 mg/kg/day was sacrificed in moribund condition on Day 78 with clinical signs of general debilitation described as few and mucoid feces, hypoactivity, hunched posture, body weight loss (1.1 kg relative to the body weight on Day 71) and increased body temperature (40.2°C). This animal had a similar loss in body weight (0.9 kg) between Days 12 and 15, was subsequently put on a dosing holiday for 2 days and began receiving canned food supplementation. Dosing with GS-5885 resumed on Day 17 after the animal had regained body weight. Canned food supplementation was continued, and body weight continued to improve until the general debilitation was observed on Day 78. Hematology changes included minimally decreased red cell mass (decreased red blood cells, hematocrit, reticulocyte, and hemoglobin), a moderate increase in white blood cells, neutrophils, and monocyte counts, and mildly decreased eosinophil and basophil counts. These changes were suggestive of severe inflammation or bacterial infection and stress. Activated partial thromboplastin time was also mildly prolonged, suggesting consumption of non-enzymatic clotting factors (Factor V, Factor VIII, and/or fibrinogen), likely related to excessive coagulation.

Clinical chemistry changes were consistent with the moribund condition but also indicated possible systemic bacterial infection, especially involving the liver [i.e., decreased blood glucose concentration; increased globulin concentration; mild to moderate increases (4- to 7-fold) in AST, ALT, and gamma glutamyltransferase activities; marked increases (44-fold) in ALP; and marked increase (22-fold) in total bilirubin concentration compared with control values on Day 93].

Macroscopic findings in this animal included red discolored duodenum and ileum. Notable microscopic findings were observed in the liver, gallbladder, stomach, duodenum, ileum, heart, lung, and prostate gland. Changes in clinical pathology test results for this animal were consistent with a combination of microscopic findings, including hepatic inflammation and necrosis, bile duct hyperplasia, inflammation and vasculitis of the gallbladder, gastrointestinal inflammation, focal pulmonary inflammation, and necrotizing inflammation of a prostatic artery. Although bacteria were not visualized on a Brown and Brenn-stained section of the liver, the clinical sign of pyrexia (40.2°C), clinical pathology changes, and microscopic alterations are still Consistent with sepsis and not directly test article-related.

Clinical Signs: Over the course of the dosing phase, GS-5885-related clinical signs were limited to excessive salivation that was noted as early as Day 3 in treated animals. The number of animals with excessive salivation was higher for animals given 30 mg/kg/day and with higher incidence in females. The salivation was transient and often noted shortly before or after dosing.

Body Weights & Feed Consumption: Administration of GS-5885 had no statistically significant effects on absolute mean body weight in either sex. No GS-5885-related effect on mean food consumption was noted.

Ophthalmoscopy: No visible lesions were noted.

ECG: All the ECGs evaluated in this study were qualitatively and quantitatively interpreted and considered normal.

Blood Pressure: No GS-5885-related effects were noted on blood pressure parameters (systolic, diastolic, and mean arterial pressure) during the dosing or recovery phases.

Clinical Pathology: During Weeks 13, 26 and 39 of the dosing phase, no GS-5885-related changes were present in the hematology, coagulation, or clinical chemistry test results of animals given 3, 10 and 30 mg/kg/day.

Urinalysis: The mean urine volume was minimally decreased and specific gravity was minimally increased on Day 93 in animals given 30 mg/kg/day, but these changes were attributed to individual variation due to the small magnitude of change and lack of difference from control values.

Gross Pathology: No test article-related findings were noted at the interim, dosing, or recovery phase necropsies.

Organ Weights: No effects were noted.

Histopathology: Histomorphologic alterations were not observed.

Adequate Battery: yes
Peer Review: yes

Histological Findings: No test article-related findings were noted at the interim, dosing, or recovery phase necropsies.

Toxicokinetics: Data are shown in Sponsor Table below. The increases in mean C_{max} and AUC_{0-t} were, in general, approximately proportional between the 3 and 10 mg/kg/day dose levels and less than proportional between the 10 and 30 mg/kg/day dose levels. Mean C_{max} and AUC_{0-t} values were generally higher (generally less than 2-fold) during Weeks 13 and 39 than on Day 1, indicating slight accumulation of GS-5885 after repeat dosing in dogs. Sex differences were generally less than 2-fold in GS-5885 mean C_{max} and AUC_{0-t} values, although values for females were generally higher than males, especially during Week 39.

Table 11: Mean Toxicokinetic Parameters of GS-5885 in Dog Plasma: Day 1 and Weeks 13 and 39

Dose Level		AUC_{0-t} (ng·hr/mL)			C_{max} (ng/mL)		
(mg/kg/day)	Sex	Day 1	Week 13	Week 39	Day 1	Week 13	Week 39
3	M	4602	5239	5673	457	516	577
	F	4259	4455	8249	405	400	743
	Combined	4430	4847	6961	431	458	660
10	M	13080	15142	15897	1165	1328	1566
	F	16772	25323	32178	1293	2114	3190
	Combined	14926	20233	25200	1229	1721	2494
30	M	25178	35199	41318	2045	2945	2980
	F	19226	45685	80268	1823	3783	5138
	Combined	22202	40750	62563	1934	3389	4157

7 Genetic Toxicology

Note: The following study reviews were taken directly from the original IND- (b) (4) review by Dr. Pritam Verma.

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

Study title: Bacterial Reverse Mutation Assay with a Confirmatory Assay with GS-5885

Study no.:	TX-256-2005
Study report location:	4.2.3.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 23, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-5885, 3542-190-1 and 96.6%

Key Study Findings: These results indicate GS-5885 was negative in the Bacterial Reverse Mutation Assay.

Methods

Strains:	TA98, TA100, TA1535,and TA1537; WP2*uvr*A
Concentrations in definitive study:	6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3330, and 5000 µg/plate with and without S9 (one plate per dose).
Basis of concentration selection:	Dose range study
Negative control:	None
Positive control:	2-nitrofluorene, sodium azide, ICR-191, 4-nitroquinoline-N-oxide, Benzo[a]pyrene, 2-aminoanthracene
Formulation/Vehicle:	The vehicle control article was DMSO (dimethylsulfoxide)
Incubation & sampling time:	The plates were inverted and incubated for 52 ± 4 hours at 37 ± 2°C.

Study Validity: valid

Results: No positive increases in revertant colony counts were observed with any of the tester strains in the presence or absence of S9 mix. All positive and vehicle control values were within acceptable ranges, and all criteria for a valid study were met.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes with GS-5885

Study no.:	TX-256-2006
Study report location:	4.2.3.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 19, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-5885, 3542-190-1 and 96.6%

Key Study Findings: These results indicate that GS-5885 was considered negative for inducing chromosomal aberrations in cultured human lymphocytes with and without metabolic activation.

Methods

Cell line:	Human venous blood lymphocytes
Concentrations in definitive study:	2.71, 3.88, 5.54, 7.91, 11.3, 16.1, 23.1, 32.9, 47.1, 67.2, 96.0, 137, 196, 280, and 400 µg/mL were tested without and with metabolic activation.
Basis of concentration selection:	Dose range study
Negative control:	none
Positive control:	Mitomycin C (MMC) and Cyclophosphamide (CP)
Formulation/Vehicle:	Dimethylsulfoxide (DMSO)
Incubation & sampling time:	For the assay without metabolic activation, cells were incubated at 37 ± 2°C for ~22 hours. For the assay with metabolic activation, cultures were incubated at 37 ± 2°C for 3 hours.

Study Validity: valid

Results: No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed. The vehicle control cultures were within the historical control range for untreated cells with chromosomal aberrations and the positive control cultures had a significant increase in cells with chromosomal aberrations as compared with the vehicle control cultures.

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

Study title: *In Vivo* Rat Bone Marrow Micronucleus Assay with GS-5885

Study no:	TX-256-2007
Study report location:	4.2.3.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 23, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-5885, 3542-189-3 and 90.8%

Key Study Findings: Single oral administration of GS-5885 up to 450 mg/kg did not induce an increase of micronuclei in rat bone marrow under the conditions of this assay.

Methods

Doses in definitive study:	0, 112.5, 225 and 450 mg/kg
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10-20 ml/kg
Formulation/Vehicle:	45% propylene glycol, 15% Solutol HS-15 in 40% reverse osmosis water (v/v/v).
Species/Strain:	Young adult male and female CRL:CD(SD) rats
Number/Sex/Group:	5 animals/sex/group
Satellite groups:	none
Basis of dose selection:	Dose limit (high)
Negative control:	none
Positive control:	cyclophosphamide at 60 mg/kg

Study Validity: valid

Results: In male and female rats, there was no increase in exposure with increasing dose. The mean GS-5885 plasma concentrations at 4 hours post-dose ranged from 709-2550 ng/mL, thereby confirming exposure of the test article in the animals. GS-5885 did not induce statistically significant increases in micronucleated PCEs at any dose examined (112.5, 225, and 450 mg/kg). In addition, GS-5885 was not cytotoxic to the bone marrow (*i.e.*, no statistically significant decreases in the PCE:NCE ratios) at any dose. The positive control agent caused significant increases in the incidence of micronucleated immature erythrocytes confirming the sensitivity of the system.

7.4 Other Genetic Toxicity Studies

None

8 Carcinogenicity

Note: Rat and mouse studies with GS-5855 are currently in progress.

Study title: TWENTY-FOUR MONTH ORAL GLP CARCINOGENICITY STUDY OF PSI-7977 (GS-7977) IN MICE

Study no.:	TX-334-2002
Study report location:	4.2.3.4
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	November 19, 2010
GLP compliance:	Yes
QA statement:	Yes (final report)
Drug, lot #, and % purity:	PSI-7977 (GS-7977), 40410002 (used through Oct 21, 2011), 40410003 (used through Feb 2, 2012), 40410001 (used through April 14, 2012) and 40411001 (used through Oct 14, 2012) and 99.1-99.7%
CAC concurrence:	Yes

Key Study Findings: An evaluation of mortality, clinical signs, body weight, food consumption, hematology, gross pathology, histopathology (neoplastic and non-neoplastic lesions) and toxicokinetics was conducted in CD-1 mice administered vehicle (95% PEG 400, 5% Tween 80), water or GS-7977 at doses of 60, 200 and 600 mg/kg/day for ~92 weeks (females) or 20, 60 and 200 mg/kg/day for ~97 weeks (males) by oral gavage. All groups were sacrificed early since both male and female vehicle control groups reached the pre-specified minimal group survival criteria (20 animals) prior to 104 weeks. No significant GS-7977-related effects on survival were observed. In addition, no dose limiting toxicities were observed, with GS-7977-related findings limited to reduced body weight (from week 17 to 39 only) and a slight increase in the incidence and severity of papillary mineralization and/or necrosis in kidney in females administered 600 mg/kg/day (versus vehicle control).

Adequacy of Carcinogenicity Study: Despite the early sacrifice of all groups, the study appears to be adequate. The high dose levels were based on the MTD (>10% reduction in body weight gain in males and females in 3-month study), did not result in drug-related mortality and were consistent with CAC recommendations. GS-331007 $AUC_{0\text{-}24h}$ values at the high dose levels (on Day 178) are ≥4- and 17-fold higher in males and females, respectively, than clinical exposure levels.

Appropriateness of Test Models: GS-7977 is metabolized rapidly *in vivo* ultimately producing GS-331007, which accounts for the vast majority of total circulating drug-related material in all species (including humans). Therefore, the mouse is an appropriate model for evaluating the potential carcinogenicity of GS-7977.

Evaluation of Tumor Findings: Based on statistical criteria for rare and common tumors, no significant GS-7977-related tumor findings were noted in male or female mice.

Methods

Doses:	0 (vehicle), 0 (water), 20, 60, 200 mg/kg/day (males); 0 (vehicle), 0 (water), 60, 200, 600 mg/kg/day (females)-see Table below
Frequency of dosing:	Once daily
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	PSI-7977 in 95% PEG 400, 5% Tween 80
Basis of dose selection:	MTD (reduced BW gain) based on 3-month oral dose finding study (study #PSI-7851-09-0008). Note: ExecCAC concurred with the high dose levels selected. Note: Low/mid dose selection for males differs from execCAC recommendations (40 & 80 mg/kg). The sponsor apparently selected lower doses (20 & 60 mg/kg) in order to limit the study to 4 total GS-7977 formulations (as opposed to 5) to help prevent the potential for dosing errors.
Species/Strain:	Mouse/ICR (CD-1®)
Number/Sex/Group:	60 (main), 54 (TK), 25 (sentinel)
Age:	5 to 6 wks
Weight:	15 to 30 g
Animal housing:	Single housed
Paradigm for dietary restriction:	No restrictions-certified rodent diet (Harlan Teklad® 2016C) provided *ad libitum*
Dual control employed:	Yes
Interim sacrifice:	No
Satellite groups:	TK
Deviation from study protocol:	None that affected the integrity or conclusions of the study. Mice were treated for ~92-97 weeks (instead of 104 weeks as initially scheduled) prior to scheduled necropsy since vehicle treated controls reached the specified minimal group survival criteria of 20 animals on Study Day 647/648 (female) and 674 & 679 (male). Note: The sponsor's contingency plans regarding early euthanasia were consistent with execCAC recommendations.

Table 12: Summary of mouse 2-year carcinogenicity study design

Group (DCJ)	Males		Females	
	PSI-7977 Dosage (mg/kg)	PSI-7977 Conc. (mg/mL)	PSI-7977 Dosage (mg/kg)	PSI-7977 Conc. (mg/mL)
1	0 (vehicle)	0	0 (vehicle)	0
2	0 (water)	0	0 (water)	0
3	20	4	60	12
4	60	12	200	40
5	200	40	600	120

Observations and Results

Mortality: Not affected significantly by test-article administration (refer to Sponsor Figures and Tables below).

- Males: total number of early deaths [found dead (FD), sacrificed (moribund or in extremis) or died during dosing] in Group 1 through 5 was as follows: 41 (30 FD, 11 & 0), 37 (21 FD, 15 & 1), 44 (33 FD, 11 & 0), 40 (30 FD, 9 & 1) and 39 (24 FD, 15 & 0).
- Females: total number of early deaths [found dead (FD), sacrificed (moribund or in extremis) or died during dosing] in Group 1 through 5 was as follows: 40 (27 FD, 12 & 1), 40 (28 FD, 12 & 0), 35 (21 FD, 14 & 0), 37 (23 FD, 14 & 0) and 42 (32 FD, 10 & 0).

Note: Neoplasia was a fairly frequent cause of death in water control group only. This appeared to be due to the longer survival time of many mice in this group (*i.e.*, mice were older when they died versus mice in other groups who succumbed to early vehicle intolerance).

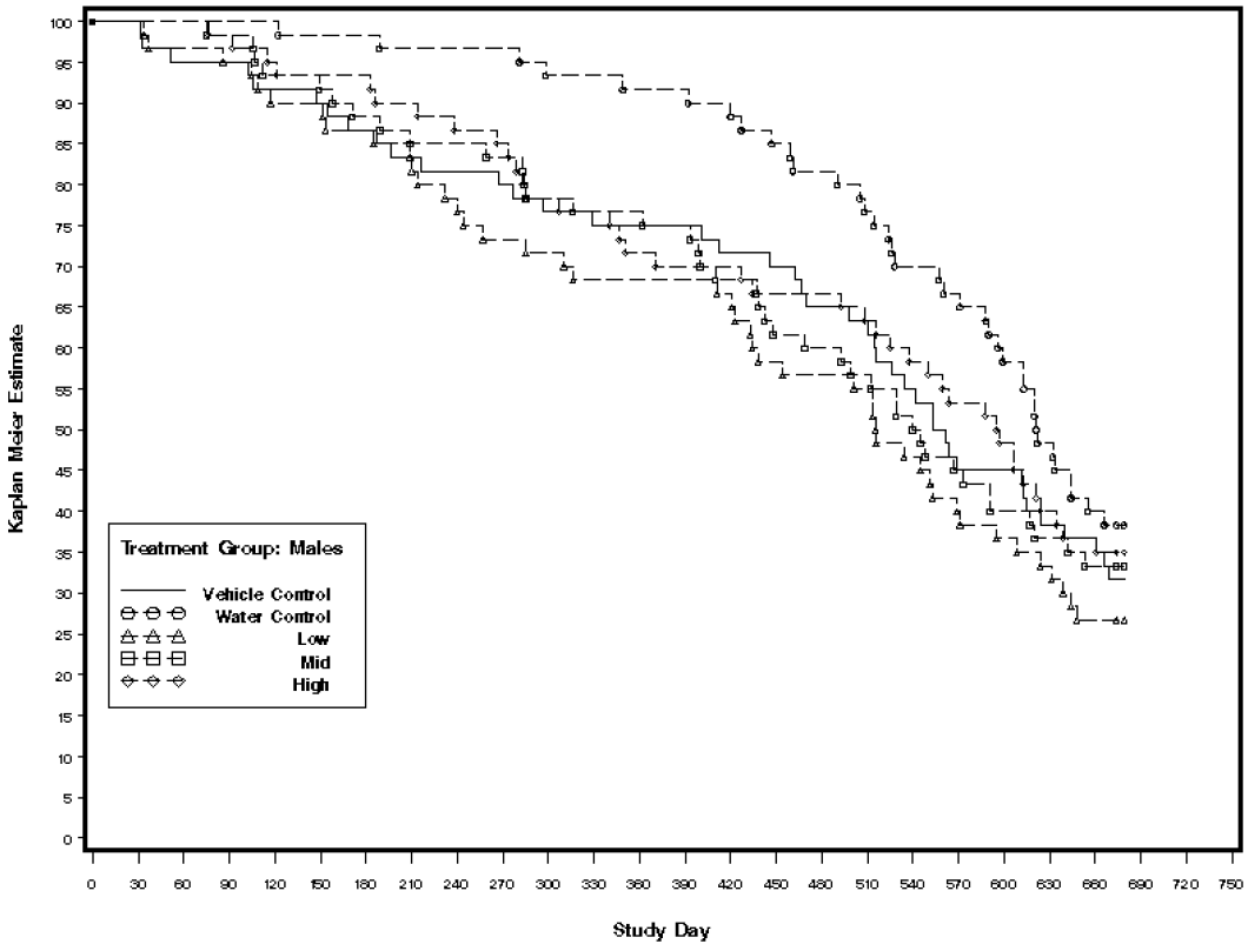


Figure 6: Kaplan-Meier survival estimates in male mice administered GS-7977, vehicle or water for ~97 weeks

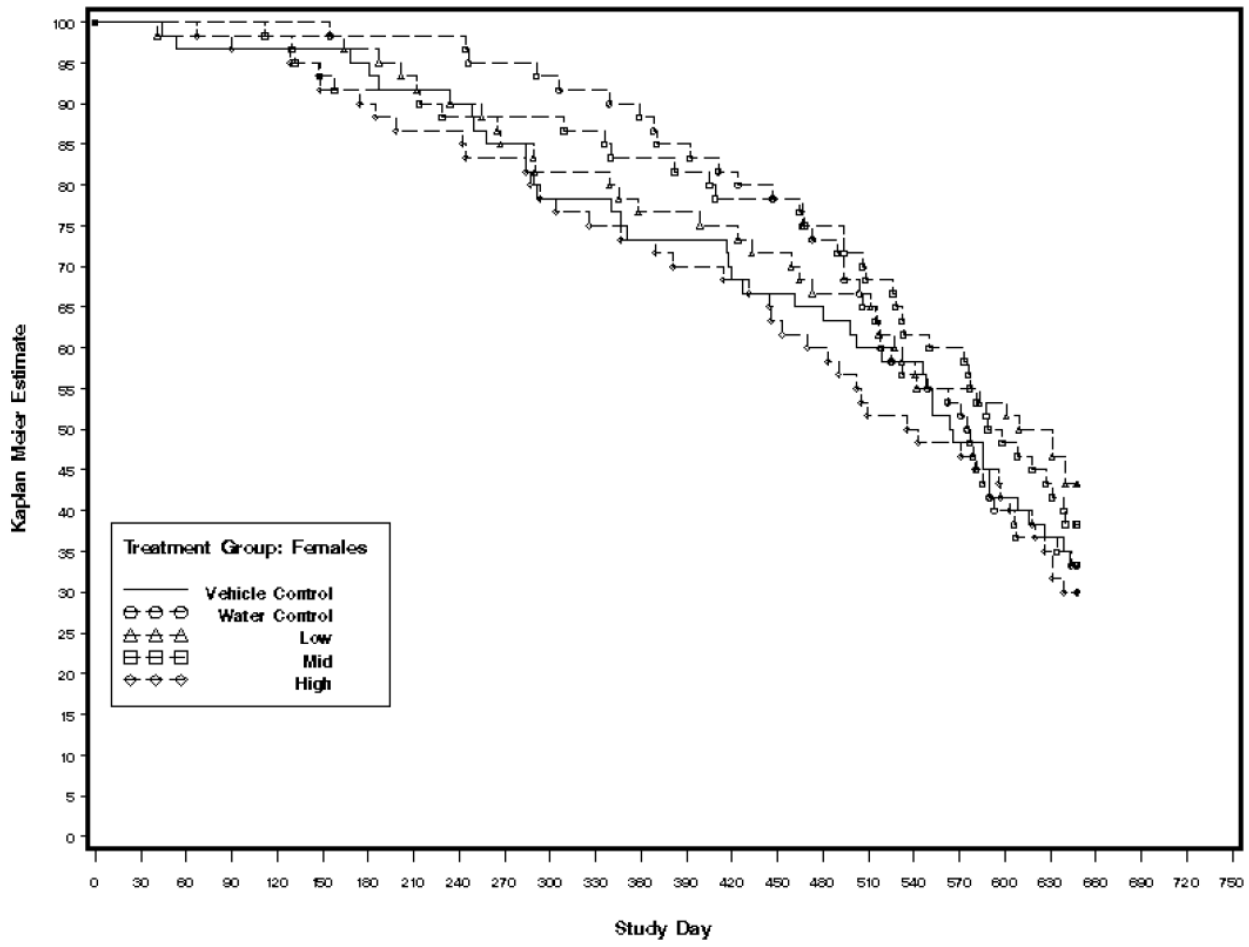


Figure 7: Kaplan-Meier survival estimates in female mice administered GS-7977, vehicle or water for ~92 weeks

Table 13: Summary of unscheduled mortality of CD-1 mice administered GS-7977, vehicle or water for ~92 (female) to 97 (male) weeks

Sex	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
No./Group	60	60	60	60	60	60	60	60	60	60
Number of early deaths	41	37	44	40	39	40	40	35	37	42
% Mortality	68	62	73	67	65	67	67	58	62	70
% Survival	32	38	27	33	35	33	33	42	38	30

Table 14: Cause of death summary of unscheduled mortalities in CD-1 mice administered GS-7977, vehicle or water ~92 (female) to 97 (male) weeks

Sex	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
Gastro-intestinal atony	6	-	13	6	7	2	-	2	2	5
Gavage – Related	7	2	3	6	7	5	3	5	2	9
Systemic Amyloidosis	10	14	13	8	11	8	11	10	8	11
Chronic Nephropathy	4	5	5	3	2	7	6	8	5	10
Neoplasia	2	14	5	5	6	8	14	11	8	6
Inflammation [a]	3	5	4	5	4	6	9	2	5	2
Cardiovascular [b]	-	2	-	1	1	2	3	2	2	2
Necrosis [c]	6	-	5	2	5	1	2	1	1	3
Urogenital dysfunction	6	3	2	3	1	-	-	-	-	-
Other [d]	-	-	-	-	-	-	-	1	1	-
Undetermined	7	2	5	5	3	6	1	1	8	3

[a] includes sepsis, ulceration
[b] includes hemorrhage, thrombosis, ascites, ovarian hematocytoma and uterine angiectasis, thrombosis and hemorrhage
[c] includes tissue necrosis, hepatic necrosis, infarcted/torsed liver
[d] gallbladder mineralization, endometrial cyst(s)

Clinical Signs: No clear test-article related changes noted.

- Gaseous distention of the abdomen observed in 17 & 10, 15 & 8, 31 & 15, 23 & 15 and 25 & 19 males & females in groups 1 through 5, respectively, was associated with gastrointestinal atony leading to severe bloating and death in some animals (refer to Sponsor Table above). Finding appears to be predominately vehicle-related indicating some level of vehicle intolerance, particularly in males.
- NutraGel (dietary supplement) administered to 154 animals (most of these with distended abdomens) to provide readily accessible water and nutrients.
- Respiratory crackles detected in 13, 0, 4, 8 and 19 males in groups 1 through 5. Finding appears primarily related to vehicle administration but the potential for a slight GS-7977 related effect (increased incidence in high dose animals) is possible.
- “Appears dehydrated” in 27, 23, 25, 26 and 39 females in groups 1 through 5.

Body Weights (1x/wk for 1st 13 weeks & then 1x/mos):

- Significant ($p<0.01$) reduction (5-7%) in BW observed in group 5 (versus group 1) females from week 17 to 39 only.
- Vehicle-related reduction in BW gain observed in males (BW increased 45% in group 1 versus 59% in group 2) and in females (BW increased 61% in group 1 versus 69% in group 2) at week 95 and 91, respectively, compared to pre-dose.

Feed Consumption (1x/wk for 1st 13 weeks & then 1x/wk-1x/mos): No test-article related changes noted.

Hematology: No test-article related changes noted.

Gross Pathology

- Gaseous distention of the GI tract observed (associated with GI atony leading to severe bloating and death in some animals-refer to Clinical Signs). Finding appears to be predominately vehicle-related.

Histopathology

Peer Review: Yes

Neoplastic: No significant test-article related changes noted.

- There was a statistically significant increase in the incidence of combined hepatocellular carcinoma/adenoma in the liver of water versus vehicle control groups in males only (refer to Sponsor Table below).
- In addition, malignant lymphoma was a common neoplasm in females with the highest incidence in the water control group.

Table 15: Summary of statistical analysis for selected tumor types in male mice administered GS-7977, vehicle or water for ~97 weeks

				Group					
Sex	Organ	R/C	Tumor		Vehicle Control	Water Control	Low	Mid	High	Dose Response
M	LIVER		TOTAL EXAMINED	(N)	60	60	59	60	59	
		C	HEPATOCELLULAR ADENOMA	(a)	3	14	1	5	2	
				(pVC)		0.0183	0.9186	0.2392	0.8321	0.4803
				(pWC)			0.9994	0.9865	0.9990	0.9992
		C	HEPATOCELLULAR CARCINOMA	(a)	2	12	4	4	5	
				(pVC)		0.0128	0.2590	0.3441	0.1078	0.1767
				(pWC)			0.8652	0.9476	0.9315	0.9560
		C	CARCINOMA/ADENOMA, HEPATOCELLULAR	(a)	5	23	5	8	6	
				(pVC)		0.0009*	0.3499	0.1862	0.3541	0.3402
				(pWC)			0.9973	0.9976	0.9995	0.9998
	SYSTEMIC NEOPLASMS		TOTAL EXAMINED	(N)	60	60	60	60	60	
		C	MALIGNANT LYMPHOMA	(a)	0	7	3	0	3	
				(pVC)		0.0186	0.0928	1.0000	0.1345	0.2177
				(pWC)			0.7650	1.0000	0.9118	0.9520

(N) Number of animals examined
(a) Number of animals with tumor
R/C Spontaneous tumor incidence rate < 1% (R=Rare tumor) or ≥ 1% (C=Common tumor)
(pVC,pWC) P-Values for peto analysis including control (vehicle control and water control, respectively):
Listed under water control: 2-sided pairwise comparison of vehicle control and water control
Listed under individual treatment group: 1-sided pairwise comparison of control with treatment group
Listed under 'Dose Response': 1-sided trend test including control and active treatment groups
Statistical Significance: Rare tumor - p≤0.025 (trend), p≤0.05 (pairwise); Common tumor - p≤0.005 (trend), p≤0.01 (pairwise)
* Statistically significant at the defined significance level

Non Neoplastic: No clear test-article related changes noted.

- Effects on nasal turbinates, consisting of variable amounts of serocellular exudate in nasal passages associated with inflammatory changes (dilated

mucosal glands, eosinophilic hyaline droplets, inflammation of submucosa) and hyperplasia of respiratory epithelium appear to be due primarily to mild irritation of the vehicle and/or gavage process. The incidence of these observations was increased in Group 5 females (refer to Sponsor Table below) so a GS-7977-related contribution to these findings in females cannot be ruled out.

- Gaseous distention of the GI tract, observed in animals found dead and sacrificed, correlated with gross pathology and clinical findings.
- Papillary mineralization in kidney observed in 3 of 60 Group 5 females (1 considered grade 3) versus 0, 1 and 1 of 60 Group 1, 3 and 4 females. Finding does not appear to be of significant concern given the low incidence.
- Papillary necrosis in kidney in 7 of 60 Group 5 females (6 considered grade 3) versus 2 of 60 Group 1 females (1 considered grade 3). Finding may be sporadic or a slight GS-7977-related effect.

Table 16: Incidence and mean severity of nasal turbinate histopathologic changes in CD-1 mice administered GS-7977, vehicle or water for ~92 (female) to 97 (male) weeks

Sex	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
NASAL TURBINATE I										
No. examined	60	60	59	60	60	59	60	59	60	60
Exudate, Serocellular	19 (1.8)	14 (1.4)	19 (1.6)	14 (1.4)	19 (1.7)	7 (1.6)	4 (1.3)	11 (1.5)	11 (1.4)	25 (1.8)
NASAL TURBINATE II										
No. examined	60	60	59	60	60	60	60	59	60	60
Exudate, Serocellular	18 (1.9)	10 (1.5)	20 (1.7)	24 (1.5)	23 (1.7)	17 (1.8)	10 (1.0)	17 (1.5)	14 (1.9)	29 (2.3)
NASAL TURBINATE III										
No. examined	59	60	59	60	60	60	60	60	60	60
Exudate, Serocellular	28 (2.4)	5 (1.2)	37 (2.3)	26 (2.4)	28. (2.6)	22 (2.0)	7 (1.9)	26 (2.3)	32 (2.2)	39 (2.4)
NASAL TURBINATE IV										
No. examined	59	60	60	60	60	60	60	60	60	60
Exudate, Serocellular	18 (2.1)	2 (1.5)	24 (2.5)	22 (2.3)	25 (2.6)	17 (1.8)	7 (1.7)	22 (2.1)	24 (2.2)	30 (2.3)

( ) = mean, sum of severities divided by number of animals affected

Toxicokinetics: Note: Blood samples collected pre-dose, 1, 2, 4, 8, 12, and 24 hours post-dose at Days 1 and 178 from 3/sex.

Table 17: Mean plasma TK parameters for the predominant GS-7977 (PSI-7977)-related metabolite, GS-331007 (PSI-6206), in mice following single and multiple (Day 178) GS-7977 administration

				Toxicokinetic Parameters							
Group	Dose (mg/kg/day)	Gender	Day	C_{max} (ng/mL)	t_{max} (h)	t_{last} (h)	AUC_{24h} (ng·h/mL)	$t_{1/2}$ (h)	C_{max}/Dose (ng/mL/ mg/kg/day)	AUC_{24h}/Dose (ng·h/mL/ mg/kg/day)	AUC_{24h} Ratio (Day 178/Day 1)
DCJ8	20	Males	1	1183	2	24	9154	5.3	59.2	457.7	NA
			178	1014	4	24	7905	4.5	50.7	395.3	0.9
	60	Females	1	4281	4	24	38739	3.3	71.3	645.7	NA
			178	4003	2	24	43452	4.0	66.7	724.2	1.1
DCJ9	60	Males	1	2608	2	24	15169	4.1	43.5	252.8	NA
			178	2190	1	24	19919	5.2	36.5	332.0	1.3
	200	Females	1	8031	2	24	68167	4.0	40.2	340.8	NA
			178	9277	2	24	100487	10.0	46.4	502.4	1.5
DCJ10	200	Males	1	5988	1	24	55355	2.3	29.9	276.8	NA
			178	5148	2	24	48028	4.8	25.7	240.1	0.9
	600	Females	1	15792	1	24	132266	2.9	26.3	220.4	NA
			178	30541	6	24	214194	ND	50.9	357.0	1.6

ND: Not Determined ($r^2 < 0.85$ or insufficient data)
NA: Not Applicable

Table from sponsor

Dosing Solution Analysis: Dosing formulations (0, 4, 12, 40 and 120 mg/ml) were analyzed for uniformity, concentration and/or stability. Duplicate "entrance" (from top, middle and bottom) and "exit" samples collected at week 1, while middle samples only were collected at months 3, 6, 9, 12, 15, 18 and 21. All mean assay results were within an acceptable concentration range (±15%), while the vehicle and the compound in this vehicle were shown to be stable under the conditions of this study.

Study title: TWENTY-FOUR MONTH ORAL GLP CARCINOGENICITY STUDY OF PSI-7977 (GS-7977) IN RATS

Study no.: TX-334-2001
Study report location: 4.2.3.4
Conducting laboratory and location: (b) (4)
Date of study initiation: October 27, 2010
GLP compliance: Yes
QA statement: Yes (final report)
Drug, lot #, and % purity: PSI-7977 and refer to Table below
CAC concurrence: Yes

Table 18: Characteristics of GS-7977 lots used for rat carcinogenicity study

Lot #/Expiration date	% purity	Dates used	Groups
40410002/6-29-12	99.7%	11-11-10 to 11-2-11	All
40410003/9-28-12	99.1%	11-3-11 to 1-11-12	All
40410001/5-6-12	99.2%	1-13-12 to 2-12-12	All

40410001/4-28-12	99.6%	2-13-12 to 3-19-12	All
40410003/9-28-12	99.1%	3-20-12 to 4-21-12	3, 4
40410001/4-28-12	99.6%	4-22-12 to 5-22-12	3
40410003/9-28-12	99.1%	5-23-12 to 6-1-12	3
40410001/4-28-12	99.6%	6-2-12 to 10-8-12	3
40410001/4-28-12	99.6%	4-22-12 to 10-3-12	4
40410001/5-6-12	99.2%	3-20-12 to 4-21-12	5
40410001/4-28-12	99.6%	4-22-12 to 7-30-12	5

Key Study Findings: An evaluation of mortality, clinical signs, body weight, food consumption, hematology, gross pathology, histopathology (neoplastic and non-neoplastic lesions) and toxicokinetics was conducted in Sprague Dawley rats administered vehicle (95% PEG 400, 5% Tween 80), water or GS-7977 at doses of 75, 250 and 750 mg/kg/day for ~20 months (all males and group 5 females) or for ~23 months (group 1-4 females) by oral gavage. GS-7977 administration was halted early in Group 5 females since only 20 animals remained. All groups were sacrificed early, since male and female control groups reached the pre-specified minimal group survival criteria (20 animals) prior to 104 weeks, with males and females sacrificed at ~20 and 23 months, respectively. Although not statistically significant (perhaps related to halting high dose administration ~3 months prior to sacrifice), a trend for a GS-7977-related decrease in survival was noted in females only. GS-7977-related findings were limited to a slightly higher incidence of various clinical signs, with no evidence of heart or skeletal muscle abnormalities noted.

Adequacy of Carcinogenicity Study: Despite the early sacrifice of all groups and a trend (not statistically significant) for drug-related mortality in females, the study appears to be adequate. The high dose level selected (based on ~1/3 lethal dose in 7 day study w/ GS-9851 since MTD >500 mg/kg/day in 3-month study w/ GS-7977) was consistent with CAC recommendations. GS-331007 $AUC_{0\text{-}24h}$ values at the high dose level (on Day 180) are ≥8- and 10-fold higher in males and females, respectively, than clinical exposure levels.

Appropriateness of Test Models: GS-7977 is metabolized rapidly *in vivo* ultimately producing GS-331007, which accounts for the vast majority of total circulating drug-related material in all species (including humans). Therefore, the rat is an appropriate model for evaluating the potential carcinogenicity of GS-7977.

Evaluation of Tumor Findings: Based on statistical criteria for rare and common tumors, no significant GS-7977-related tumor findings were noted in male or female rats.

Methods

Doses:	0 (vehicle), 0 (water), 75, 250, 750 mg/kg/day-designated groups 1 through 5, respectively
Frequency of dosing:	Once daily
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	PSI-7977 in 95% PEG 400, 5% Tween 80
Basis of dose selection:	High-dose selected (~1/3 lethal dose) based on a 90-Day oral toxicology study with PSI-7977 (GS-7977) (study #PSI-7977-09-0007) with MTD >500 mg/kg and a 7-Day oral toxicology study with PSI-7851 (GS-9851) (study # PSI-7851-08-001) with lethality observed at 2,000 mg/kg. Note: ExecCAC concurred with the dose levels selected.
Species/Strain:	Rat/Hsd: Sprague Dawley® SD®
Number/Sex/Group:	55 (main), 14 (TK), 25 (sentinel)
Age:	8 to 9 wks
Weight:	143 to 334 g
Animal housing:	Single housed
Paradigm for dietary restriction:	No restrictions-certified rodent diet (Harlan Teklad® 2016C) provided *ad libitum*
Dual control employed:	Yes
Interim sacrifice:	No
Satellite groups:	TK
Deviation from study protocol:	None that affected the integrity or conclusions of the study. Rats were treated for ~20 (all males and group 5 females) or 23 months (group 1 to 4 females) (instead of 24 months as initially scheduled) since control groups reached the specified minimal group survival criteria of 20 animals on Study Day 616 (group 2 males) and 694 (group 1 females). Test-article administration was halted on Day 628 in Group 5 females since only 20 animals remained. Males were sacrificed at 20 months, while all surviving females were sacrificed at 23 months. Note: The sponsor's contingency plans regarding early euthanasia were consistent with execCAC recommendations.

Observations and Results

Mortality: Although there was no statistically significant decrease in survival in GS-7977 treated groups (as compared to vehicle control), increased mortality in Group 5

females necessitated the halting of dose administration ~3 months prior to sacrifice. Thus, a positive trend on mortality was observed in females (but not in males) with slight increases in mortality occurring with increasing GS-7977 dose levels (refer to sponsor figures below).

- Males: total number of early deaths [found dead (FD), sacrificed (moribund or in extremis) or accidental/died during dosing] in Group 1 through 5 was as follows: (19 FD, 5 & 1), (21 FD, 13 & 1), (16 FD, 6 & 2), (30 FD, 3 & 1) and (17 FD, 6 & 1). Although the primary cause of death in males was due to nephropathy, a significant number died due undetermined causes.
- Females: total number of early deaths [found dead (FD), sacrificed (moribund or in extremis) or died during dosing] in Group 1 through 5 was as follows: (18 FD, 17 & 0), (9 FD, 24 & 0), (19 FD, 19 & 0), (19 FD, 20 & 1) and (17 FD, 22 & 0). The primary cause of death in females was due to moribund sacrifice resulting from the presence of benign mammary fibroadenomas, presenting as large palpable masses. Many of the remaining benign neoplasms causing early mortality were pituitary adenomas.

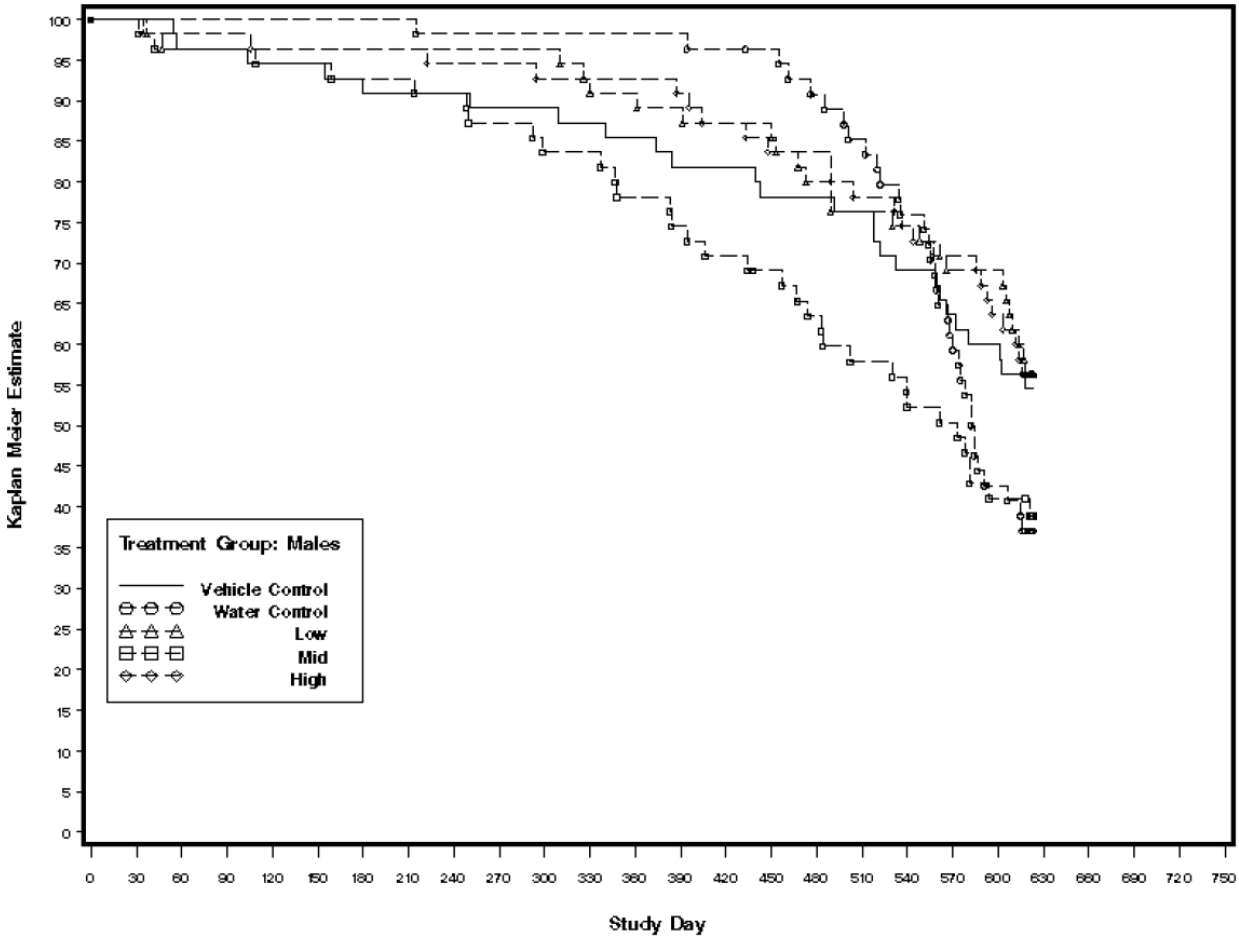


Figure 8: Kaplan-Meier survival estimates in male rats administered GS-7977, vehicle or water for ~20 months

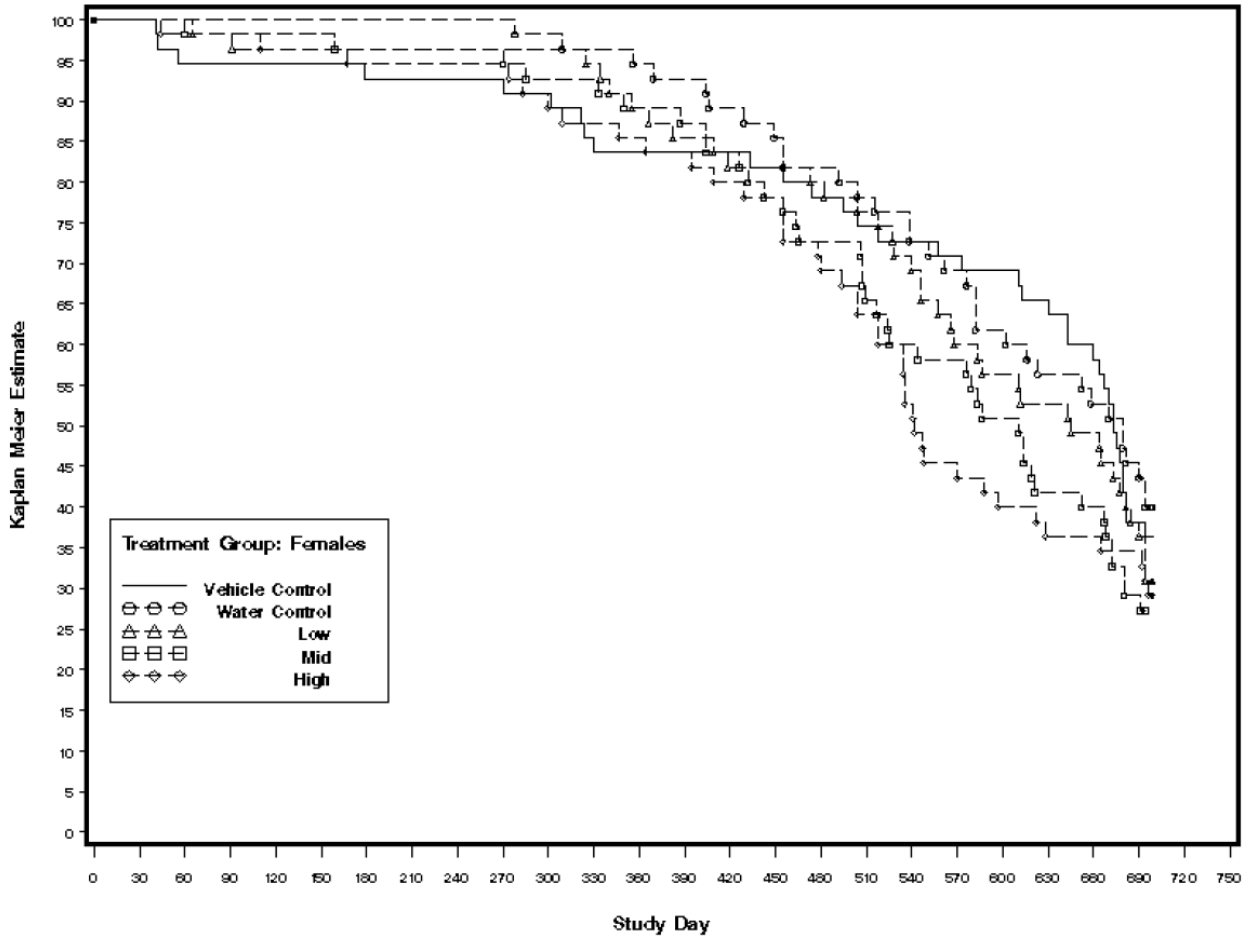


Figure 9: Kaplan-Meier survival estimates in female rats administered GS-7977, vehicle or water for up to ~20 to 23 months

Table 19: Mortality summary of rats administered GS-7977, vehicle or water for ~20 to 23 months

Sex	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
Termination Study Day	618-623					698-699			694	698-699
Number/Group	55	55	55	55	55	55	55	55	55	55
Number, One Year	8	1	6	12	4	9	3	6	6	9
% Mortality	15	2	11	22	7	16	5	11	11	16
% Survival	85	98	89	78	93	84	95	89	89	84
Number, Terminal Sacrifice	25	35	24	34	24	35	33	38	40	39
% Mortality	45	64	44	62	44	64	60	69	73	71
% Survival	55	36	56	38	56	36	40	31	27	29

Table from sponsor

Table 20: Cause of death summary of unscheduled mortalities in rats administered GS-7977, vehicle or water for ~20 to 23 months

Sex	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
Gavage-Related	3	-	2	6	3	5	-	1	2	2
Nephropathy	12	20	10	13	16	1	1	2	-	3
Cardiovascular[a]	-	1	-	-	-	1	-	1	1	-
Inflammatory[b]	-	-	1	-	-	1	1	-	1	-
Other[c]	-	-	-	-	-	-	-	-	-	1
Undetermined	9	10	9	11	3	6	5	8	14	9
Neoplasia										
Benign	-	3	-	1	-	16	21	23	15	18
Malignant	1	1	2	3	2	5	5	3	7	6

- = no findings
[a]Cardiovasular disesease (i.e, atrial thrombus, arteritis, and/or probable circulatory insufficiency)
[b]Abcess, ulcer
[c]Galactocele (mammary)

Table from sponsor

Clinical Signs

- Slightly higher incidence of material around eyes, mouth, nose and anogenital staining and respiratory crackles in group 5 animals. Signs were partially vehicle-related.
- Increased incidence of abnormal pinna (right, left or both) in group 5 ♂ [4 animals (7%) versus 0 in other groups combined]. No additional description/information (gross pathology, histopathology etc.) was provided in report. Finding appears unlikely to be a direct test-article related effect.
- Increased incidence of lame/limping in group 5 ♀ [3 animals (5.5%) versus 0 in other groups combined].
    - One animal had finding in the right hind-limb associated with fractured tibia and focal edema. Finding most likely due to accidental injury.
    - Second animal had finding described only as bilateral and generalized. No additional description/information (gross pathology, histopathology etc.) regarding this finding was provided in report. Uncertain whether finding is test-article related.
    - Third animal had finding in left fore-limb. No additional description/ information (gross pathology, histopathology etc.) regarding this finding was provided in report. Although no histopathological effects on skeletal muscle (biceps femoris) were noted, this animal had moderate myofiber degeneration with mild acute inflammation of the muscle in skin. This muscle finding appears unrelated to the fore-limb finding [although sample may have originated in fore-limb it is likely a smooth (not skeletal) muscle finding]. Uncertain whether finding is test-article related.
    - Given the low incidence of lameness/limping in group 5 ♀ (only 2 of 55 potentially test-article related) and the apparent lack of associated skeletal

muscle degeneration findings, this finding (and its relationship to a potential cardiac/skeletal muscle degeneration signal) appears to be of little or no significance.

- Males: total number of animals with masses or nodules in Group 1 through 5 was as follows: 16, 26, 24, 22 and 27.
- Females: total number of animals with masses or nodules in Group 1 through 5 was as follows: 37, 49, 47, 43 and 42.

Body Weights (1x/wk for 1st 13 weeks & then 1x/mos): No test-article related changes noted.

- Vehicle-related reduction in BW gain noted (Groups 1, 3, 4 & 5 versus Group 2).
  - BW gain ↓31% (from 1 to 87 weeks) in Group 1 versus Group 2 ♂.
  - BW gain ↓21% (from 1 to 99 weeks) in Group 1 versus Group 2 ♀.

Feed Consumption (1x/wk for 1st 13 weeks & then 1x/mos): No test-article related changes noted.

- Vehicle-related reduction in consumption noted (Groups 1, 3, 4 & 5 versus Group 2).

Hematology: No test-article related changes noted.

- Vehicle-related changes in some parameters noted in males (Groups 1 versus 2).

Gross Pathology: No test-article related changes noted.

Histopathology

Peer Review: Yes

Neoplastic: No significant test-article related changes noted.

- Minor trend for increased incidence of endometrial adenocarcinoma in the uterus with 0, 0, 0, 1 & 2 of 55 noted in groups 1 through 5, respectively.

Table 21: Summary of statistical analysis for selected tumor types in male rats administered GS-7977, vehicle or water for ~20 months

Sex	Organ	Tumor	R/C		Group: Vehicle Control	Water Control	Low	Mid	High	Dose Response
M	PROSTATE GLAND	TOTAL EXAMINED		(N)	55	55	55	55	55	
		ADENOMA	C	(a)	3	0	2	0	4	
				(pVC)		0.2706	0.7084	1.0000	0.3623	0.4280
				(pWC)			0.2140	1.0000	0.0836	0.0340

(N) Number of animals examined
(a) Number of animals with tumor
R/C Spontaneous tumor incidence rate < 1% (R=Rare tumor) or ≥ 1% (C=Common tumor)
(pVC,pWC) P-Values for peto analysis including control (vehicle control and water control, respectively):
Listed under water control: 2-sided pairwise comparison of vehicle control and water control
Listed under individual treatment group: 1-sided pairwise comparison of control with treatment group
Listed under 'Dose Response': 1-sided trend test including control and active treatment groups
Statistical Significance: Rare tumor - p≤0.025 (trend), p≤0.05 (pairwise); Common tumor - p≤0.005 (trend), p≤0.01 (pairwise)
* Statistically significant at the defined significance level

Table from sponsor

Table 22: Summary of statistical analysis for selected tumor types in female rats administered GS-7977, vehicle or water for ~20 to 23 months

Sex	Organ	Tumor	R/C		Group: Vehicle Control	Water Control	Low	Mid	High	Dose Response
F	ADRENAL GLANDS	TOTAL EXAMINED		(N)	55	55	55	54	55	
		PHEOCHROMOCYTOMA; BENIGN	C	(a)	6	1	6	2	0	
				(pVC)		0.1466	0.3996	0.8949	1.0000	0.9846
				(pWC)			0.0161	0.4719	1.0000	0.7286
		PHEOCHROMOCYTOMA; M/B/C	C	(a)	7	1	7	4	1	
				(pVC)		0.0679	0.3982	0.7276	0.9731	0.9579
				(pWC)			0.0085*	0.1149	0.7778	0.4667
	MAMMARY GLAND (REGION)	TOTAL EXAMINED		(N)	55	55	54	55	54	
		MAMMARYADENOCARCINOMA/CARCINOMA	C	(a)	5	12	4	10	6	
				(pVC)		0.0898	0.7225	0.0479	0.3440	0.0863
				(pWC)			0.9877	0.4462	0.8243	0.6456
	PITUITARY GLAND	TOTAL EXAMINED		(N)	54	53	53	54	54	
		ADENOMA	C	(a)	25	20	25	23	20	
				(pVC)		0.3526	0.1934	0.4189	0.3895	0.5297
				(pWC)			0.0406	0.0783	0.1411	0.1784
	THYROID GLAND (BOTH LOBES)	TOTAL EXAMINED		(N)	55	55	55	55	55	
		ADENOMA; C-CELL	C	(a)	9	10	15	15	8	
				(pVC)		1.0000	0.0522	0.0279	0.4374	0.3268
				(pWC)			0.0935	0.0452	0.4396	0.3798
		CARCINOMA/ADENOMA, C-CELL	C	(a)	9	13	15	15	8	
				(pVC)		0.4496	0.0522	0.0279	0.4374	0.3268
				(pWC)			0.2497	0.1310	0.6956	0.6189
	UTERUS	TOTAL EXAMINED		(N)	55	55	55	55	55	
		ADENOCARCINOMA; ENDOMETRIAL	R	(a)	0	0	0	1	2	
				(pVC)		1.0000	1.0000	0.4857	0.1803	0.0300
				(pWC)			1.0000	0.5313	0.1701	0.0307

(N) Number of animals examined
(a) Number of animals with tumor
R/C Spontaneous tumor incidence rate < 1% (R=Rare tumor) or ≥ 1% (C=Common tumor)
(pVC,pWC) P-Values for peto analysis including control (vehicle control and water control, respectively):
Listed under water control: 2-sided pairwise comparison of vehicle control and water control
Listed under individual treatment group: 1-sided pairwise comparison of control with treatment group
Listed under 'Dose Response': 1-sided trend test including control and active treatment groups
Statistical Significance: Rare tumor - p≤0.025 (trend), p≤0.05 (pairwise); Common tumor - p≤0.005 (trend), p≤0.01 (pairwise)
* Statistically significant at the defined significance level

Table from sponsor

Non Neoplastic: No clear test-article related changes noted.

- Effects on nasal turbinates, consisting of variable amounts of serocellular exudate in nasal passages associated with acute inflammatory changes appear to be due primarily to mild irritation of the vehicle and/or gavage process. The

incidence of these observations was increased in GS-7977 treated animals so a GS-7977-related contribution to these findings appears likely (refer to Sponsor Table below).

- Vacuolation of the renal tubular epithelium appears to be a vehicle-related effect.
- No evidence of heart or skeletal muscle abnormalities (degeneration etc.) was observed (refer to Sponsor Table below).

Table 23: Incidence and mean severity for GS-7977 related histopathologic changes in rats

Sex	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
Number	55	55	55	55	55	55	55	55	55	55
Kidney										
Vacuolation, Tubular Epithelium	9(1.7)	1(1.0)	6(3.0)	12(2.1)	10(2.1)	7(2.3)	-	8(1.8)	5(1.0)	9(1.7)
Nasal Turbinate I										
Exudate, Serocellular, Meatus	35(1.6)	21(1.1)	50(1.7)	43(1.5)	52(1.6)	18(1.5)	4(1.0)	16(1.6)	26(1.4)	31(1.6)
Inflammation, Acute, Nasal Epithelium	3(1.3)	-	3(1.0)	3(1.0)	7(1.6)	-	-	1(2.0)	-	3(1.7)
Nasal Turbinate II										
Exudate, Serocellular, Meatus	38(1.4)	14(1.2)	48(1.5)	43(1.5)	48(1.7)	20(1.7)	6(1.2)	20(1.3)	21(1.5)	27(1.7)
Inflammation, Acute, Nasal Epithelium	6(1.2)	2(1.5)	6(1.0)	4(1.0)	16(1.2)	-	-	-	-	5(1.2)
Nasal Turbinate III										
Exudate, Serocellular, Meatus	38(1.7)	16(1.2)	50(2.1)	46(2.2)	52(2.7)	19(2.0)	11(1.2)	25(1.8)	22(2.2)	33(2.4)
Inflammation, Acute, Nasal Epithelium	3(1.0)	3(1.0)	6(1.2)	2(1.0)	16(1.3)	-	-	2(1.0)	-	4(1.3)
Nasal Turbinate IV										
Exudate, Nasopharynx	-	1(4.0)	11(1.5)	7(1.7)	1(3.0)	4(1.5)	1(1.0)	7(1.7)	8(1.0)	4(1.8)
Exudate, Serocellular, Meatus	35(1.6)	18(1.2)	35(1.6)	40(1.9)	49(2.6)	17(1.5)	12(1.3)	20(1.7)	23(1.6)	30(2.2)
Inflammation, Acute, Nasal Epithelium	2(1.0)	4(1.0)	2(1.0)	2(1.0)	17(1.1)	-	-	1(1.0)	-	3(1.0)

- = no findings
( ) = mean, sum of severities divided by the number of animals affected

Table 24: Incidence and mean severity for histopathology findings in heart, skeletal muscle and tongue in rats

Sex	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
Heart / Number	55	55	55	55	55	55	55	55	55	55
Cardiomyopathy	29(1.2)	46(1.5)	40(1.1)	27(1.3)	39(1.4)	18(1.2)	18(1.2)	16(1.1)	18(1.0)	24(1.1)
Degeneration; Myofiber	-	-	1(1.0)	1(2.0)	1(2.0)	-	-	-	1(1.0)	-
Skeletal Muscle, Biceps Femoris / Number	55	55	54	54	55	55	55	55	55	55
Degeneration; Myofiber	1(2.0)	-	1(4.0)	1(2.0)	-	1(2.0)	-	-	-	-
Tongue / Number	55	55	55	55	55	55	55	55	55	55
Inflammation; Muscle	-	2(1.0)	1(1.0)	-	-	-	-	-	-	-
Regeneration; Myofibers	-	-	-	-	1(2.0)	-	-	-	-	-

- = no findings
( ) = mean, sum of severities divided by the number of animals affected

Toxicokinetics: Note: Blood samples collected pre-dose, 1, 2, 4, 6, 8, 12, and 24 hours post-dose on Day 1 and Day 180 from 3 rats/sex.

Table 25: Mean plasma TK parameters for the predominant GS-7977 (PSI-7977)-related metabolite, GS-331007 (PSI-6206), in rats following single and multiple (Day 180) GS-7977 administration

			Toxicokinetic Parameters							
Dose (mg/kg/day)	Gender	Day	C_{max} (ng/mL)	t_{max} (h)	t_{last} (h)	AUC_{24h} (ng·h/mL)	$t_{1/2}$ (h)	C_{max}/Dose (ng/mL/ mg/kg/day)	AUC_{24h}/Dose (ng·h/mL/ mg/kg/day)	AUC_{24h} Ratio (Day 180/Day 1)
75	Males	1	719	2	24	9555	9.5	9.6	127.4	NA
		180	978	4	24	12891	ND	13.0	171.9	1.3
	Females	1	563	2	24	5814	8.1	7.5	77.5	NA
		180	790	2	24	8270	11.5	10.5	110.3	1.4
250	Males	1	2558	6	24	34009	43.5	10.2	136.0	NA
		180	2536	4	24	38396	ND	10.1	153.6	1.1
	Females	1	1894	4	24	22729	ND	7.6	90.9	NA
		180	2627	4	24	27080	ND	10.5	108.3	1.2
750	Males	1	6458	8	24	65649	ND	8.6	87.5	NA
		180	7598	1	24	96641	17.2	10.1	128.9	1.5
	Females	1	6290	8	24	85168	ND	8.4	113.6	NA
		180	9317	2	24	125497	8.2	12.4	167.3	1.5

ND: Not Determined ($r^2 < 0.85$ or insufficient data)
NA: Not Applicable

Table from sponsor

Dosing Solution Analysis: Dosing formulations (0, 15, 50 and 150 mg/ml) were analyzed for uniformity, concentration and/or stability. Duplicate “entrance” (from top, middle and bottom) and “exit” samples collected at week 1, while middle samples only were collected at months 3, 6, 9, 12, 15, 18 and 21. All mean assay results were within an acceptable concentration range (±14%), while the vehicle and the compound in this vehicle were shown to be stable under the conditions of this study.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Note: The following study review was taken directly from the original IND-(b) (4) review by Dr. Pritam Verma.

Study title: Study of Fertility and Early Embryonic Development to Implantation of GS-5885 Administered by Oral (Gavage) in Rats

Study no.:	TX-256-2017
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	06 December 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-5885, 5885-03-A-1 and 99.5%

Key Study Findings: A significant reduction in mean body weight gain was observed transiently (first week only) in males given 100 mg/kg/day. In females given 100 mg/kg/day, there was a small but significant body weight loss during the first week of dosing. These body weight changes in males and females were associated with transient decreases in food consumption values in these groups. Since the body weight changes were small, transient and similar to or above control values at the end of dosing, they were not considered adverse. The average number of corpora lutea, implantations and viable embryos were slightly reduced in the 100 mg/kg/day dose group. No other GS-5885-related effects were noted on Caesarean sectioning or litter parameters (*i.e.*, pre-implantation or post-implantation loss).

As effects on body weight and food consumption in males were transient, the paternal NOAEL for GS-5885 was 100 mg/kg/day. There were no effects on mating and fertility of the male rats and the male reproductive NOAEL was 100 mg/kg/day. As effects on body weight and food consumption in females were transient, the maternal NOAEL for GS-5885 was 100 mg/kg/day. There were no effects on the mating of the female rats; however, the numbers of corpora lutea and implantation sites were reduced in females given 100 mg/kg/day; therefore, the female reproductive NOAEL was 30 mg/kg/day.

Methods

Doses:	0, 10, 30, or 100 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	5 ml/kg/day
Route of administration:	Oral gavage
Formulation/Vehicle:	45% propylene glycol and 15% Solutol HS-15 prepared in pH-adjusted deionized water
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	22 animals/sex/group
Satellite groups:	None
Study design:	Males were treated once daily beginning 28 days before cohabitation, during cohabitation (maximum of 21 days) and continuing through the day before euthanasia
Deviation from study protocol:	None

Observations: The following parameters and end points were evaluated: viability, clinical observations, body weights, body weight changes, food consumption, mating and fertility, ovarian and uterine examinations, male reproductive assessments (sperm motility, concentration, and morphology), organ weights and gross necropsy findings.

Mortality: There were no test article-related deaths. One male rat in each of the 0 (Vehicle) and 30 mg/kg/day dose groups was euthanized due to a gavage error.

Clinical Signs: Mild dehydration (based on skin turgor), and urine-stained abdominal fur occurred in 4 and 3 male rats, respectively, in the 100 mg/kg/day dose group. The incidences of these observations were statistically increased as compared to the vehicle control group values; however, because they occurred in a small number of rats over a limited number of days, they were not considered to be toxicologically important. Additionally, without clinical pathology data to confirm actual dehydration; the dehydration was not considered adverse.

Body Weight: Body weight gain was significantly reduced in males given 30 and 100 mg/kg/day during the first week of dosing (DSs 1 to 8); gains were 21% and 80% below the vehicle control group value, respectively. In males given 100 mg/kg/day, body weight gain was then significantly increased (1.5-fold) as compared to the control group for the second week of dosing (DSs 8 to 15), and for the remainder of the study body weight gain was similar to controls. Because body weight gain was comparable to or above control values after the first week, these initial reductions in body weight were not considered adverse. Body weights and body weight gains were unaffected in males given 10 mg/kg/day.

Feed Consumption: Absolute and relative food consumption values were significantly reduced in males given 30 and 100 mg/kg/day for the first week of the study (9% to 30% below control). Absolute and relative food consumption values were then significantly

increased in males given 100 mg/kg/day on DSs 15 to 22 and were similar to controls for the remainder of the study. Because food consumption was comparable to or above control values after the first week, these initial reductions in food consumption were not considered adverse. Absolute and relative food consumption values were unaffected in males given 10 mg/kg/day.

Toxicokinetics: Not reported

Dosing Solution Analysis: For all formulations analyzed, the mean sample concentration results were within or equal to 15% of the theoretical concentrations and ranged from 98.1% to 110.6% of target. Concentration verification specifications were met for all test article formulations.

Mating and Fertility: All mating and fertility parameters [numbers of days in cohabitation, rats that mated, the Fertility Index (number of pregnancies per number of rats that mated), rats with confirmed mating dates during the first week of cohabitation and number of pregnancies per number of rats in cohabitation] were unaffected by dosages of GS-5885 as high as 100 mg/kg/day. All values were comparable among the four dose groups and did not significantly differ.

Necropsy Observations: Included small epididymides, a soft tan mass in the epididymis and small and flaccid testes. However, all necropsy observations were considered unrelated to GS-5885 because: 1) the incidences were not dose dependent; 2) the observations occurred in only one rat.

Male rat #3850 (30 mg/kg/day) with small epididymides and small and flaccid testes mated but did not impregnate the cohort female rat, had a low sperm count and no motile sperm present in the motility sample. Male rat #3869 (100 mg/kg/day) with the mass on the right epididymis mated and impregnated the cohort female rat and all other sperm parameters were apparently normal. Because these were isolated incidences neither of these findings was considered test-article related.

Terminal Body Weights, Organ Weights and Ratios (%) of Organ Weights to Terminal Body Weights: Terminal body weights were comparable among the four dose groups and did not significantly differ. The weights of the epididymides, caudal epididymis, testes, seminal vesicles (with and without fluid) and prostate and the ratios of these organ weights to terminal body weight were unaffected by GS-5885.

Sperm Evaluation: All sperm parameters evaluated were unaffected by GS-5885. Values for number and percent motile sperm, number of non-motile sperm and total sperm count from the vas deferens and cauda epididymal sperm count and density were comparable among the four dose groups and did not significantly differ.

Sperm morphology data were unaffected by GS-5885. The small but statistically significant decrease (3% below control) in the number of normal sperm in males given 10 mg/kg/day, and the significant increases in the percentage of abnormal sperm and

the numbers of sperm with detached heads in males given 10 and 100 mg/kg/day were not considered to be test article related because these changes were not dose dependent.

F0 Generation Female Rats- Mortality and Clinical Observations: All female rats survived until scheduled euthanasia. Urine-stained abdominal fur occurred in an increased number of female rats (5) in the 100 mg/kg/day dose group during the gestation period.

Body Weight and Body Weight Changes: A small but statistically significant body weight loss occurred in females given 100 mg/kg/day during the first week of dose administration (DSs 1 to 8), as compared to females in the control group. Maternal body weight gain was also slightly reduced (-12.7% compared to control) in the 100 mg/kg/day dose group during the gestation dosing period [calculated as Days 0 to 8 of gestation (DGs 0 to 8)]. After the completion of dosing on DGs 8 to 10, body weight gains were significantly increased above the vehicle control group value in females given 100 mg/kg/day. Since the body weight changes were small, transient and similar to or above control values at the end of dosing, they were not considered adverse. Body weights and body weight gains during the pre-cohabitation and gestation periods were unaffected in females given dosages of GS-5885 up to 30 mg/kg/day.

Absolute (g/day) and Relative (g/kg/day) Food Consumption Values: Absolute and relative food consumption values were reduced or significantly reduced (12% to 20% below control) in females given 100 mg/kg/day for the first two weeks of the study (DSs 1 to 8, 8 to 15 and 1 to 15). Absolute and relative food consumption values during the pre-cohabitation period were unaffected by dosages of GS-5885 as high as 30 mg/kg/day. Absolute and relative food consumption values during the gestation period were generally comparable to control values during the gestation dosing period (DGs 0 to 8), and were significantly increased above control group values during the post-dose period (DGs 8 to 13), reflecting rebounds in body weight gain during this period.

Estrous Cycling, Mating and Fertility: The number of estrous stages per 14 days was comparable among the four dose groups before the start of administration and during the pre-cohabitation period. All mating and fertility parameters [numbers of days in cohabitation, rats that mated, the Fertility Index (number of pregnancies per number of rats that mated), rats with confirmed mating dates during the first or second week of cohabitation and number of pregnancies per number of rats in cohabitation] were unaffected by dosages of GS-5885 as high as 100 mg/kg/day. All values were comparable among the four dose groups and did not significantly differ.

Necropsy Observations: There were no test article-related necropsy observations in the female rats. The only necropsy observation was the presence of pup tissues in the stomach of one control group rat that delivered a litter.

Terminal Body Weights, Organ Weights and Ratios (%) of Organ Weights to Terminal Body Weights: Terminal body weights were comparable among the four

dose groups and did not significantly differ. Weights of the ovaries and the ratios of the ovary weight to the terminal body weight were generally comparable among the four dose groups. A statistically significant reduction in the weight of the left ovary and the ratio of the left ovary to the terminal body weight in the 30 mg/kg/day dose group was not considered to be adverse or test article related because it was not dose dependent and a comparable change did not occur for the right ovary.

Caesarean-Sectioning and Litter Observations: Pregnancy occurred in 22 (100%), 22 (100%), 20 (90.9%) and 22 (100%) rats in the four GS-5885 dose groups, 0, 10, 30 and 100 mg/kg/day, respectively. One rat in the control group without a confirmed mating date delivered a litter. As a result of this delivery, Caesarean-sectioning and litter observations were based on 21, 22, 20 and 22 pregnant rats in Groups 1 through 4, respectively.

The average number of corpora lutea and implantations were significantly reduced (10.3% and 9.9% below control, respectively) in females given 100 mg/kg/day. Although not statistically significant, the average number of viable embryos was also reduced (13% below control) in this group. The reduction in corpora lutea and implantations was considered adverse and potentially test-article related.

No other Caesarean-sectioning or litter parameters were affected by dosages of GS-5885 as high as 100 mg/kg/day. The litter averages for pre-implantation loss, nonviable embryos and post-implantation loss were comparable among the four dose groups and did not significantly differ. No dam had a litter consisting of only nonviable embryos. All placentae appeared normal.

9.2 Embryonic Fetal Development

Note: The following study reviews were taken directly from the original IND-(b) (4) review by Dr. Pritam Verma.

Study title: Oral Gavage Study for Effects on Embryo-Fetal Development with GS-5885 in Rats

Key study findings: GS-5885-related effects on mean maternal body weight, body weight gain, and food consumption were noted at 100 mg/kg/day. During the dosing phase, a small decreases (96.5 to 97.8% of control) occurred from GD 10 through 18. The cesarean section data were unremarkable and indicated that GS-5885 had no effects on embryo/fetal viability. There were no GS-5885-related effects on mean fetal weights or external or soft tissue variations or malformations. There were no GS-5885-related skeletal malformations. A small statistically significant increase occurred in the total fetal and litter incidences of common skeletal variations affecting the vertebra, sternebrae, and ribs in animals given 100 mg/kg/day. In the absence of other evidence of fetal toxicity or associated malformations, these common skeletal variations are likely secondary to general maternal toxicity (decreased food consumption and body weight gain) rather than a direct developmental effect of GS-5885.

The NOAEL for maternal toxicity of GS-5885 when given once daily by oral gavage to pregnant rats during the period of organogenesis was 30 mg/kg/day based on significantly decreased maternal body weight gain and food consumption at 100 mg/kg/day. There were no GS-5885-related effects on embryo/fetal viability and no fetal visceral or skeletal anomalies; therefore, the NOAEL for developmental toxicity is 100 mg/kg/day.

Study no.: TX-256-2012

Volume # and page #: electronic

Conducting laboratory and location: (b) (4)

Date of study completion: 26 May 2011

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: GS-5885, 5885-XB-1 and 100%

Methods

Doses: 0, 10, 30 and 100 mg/kg/day (feasible doses)

Basis of dose selection: The choice for the high dose level was based on the results of a dose-range finding developmental toxicity study (TX-256-2011). In the developmental range-finding study, GS-5885 was administered at 10, 30 and 100 mg/kg/day. The NOAEL for maternal toxicity was 30 mg/kg/day based on decreased body weight gain observed at the 100 mg/kg/day dose, and the NOAEL for fetal toxicity was 100 mg/kg/day. Therefore, the highest dose selected for this study was 100 mg/kg, which was within the tolerated range of dosing volume and formulation concentration. The mid and low doses of 30 and 10 mg/kg GS-5885, respectively, were chosen to provide a range of systemic exposures.

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

Number/sex/group: 25 rats/group

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

Study design: Administered by oral gavage daily on GD 6 through 17 at a dose volume of 5 mL/kg.

Vehicle: The vehicle control article was 45% propylene glycol and 15% Solutol® HS-15 prepared in pH-adjusted reverse osmosis water [(pH 2.5 ± 0.1 adjusted with dilute hydrochloric acid).

Parameters and endpoints evaluated: Abnormalities of the placenta or amniotic sac were described, if applicable. 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. Each fetus (live or dead) was sexed, weighed, and examined for external abnormalities. Live fetuses were sacrificed via an intracisternal injection of sodium pentobarbital followed by thoracic penetration.

Results

Mortality & Clinical signs (dams): All animals survived to scheduled necropsy, and there were no GS-5885-related clinical signs of toxicity.

Body weight (dams): GS-5885-related effects on mean maternal body weight and body weight gain occurred at 100 mg/kg/day. During the dosing phase (GD 6 to 18), there were no significant decreases in mean maternal body weight, although mean body weight gain from GD 6 to 8 was significantly decreased (33% of control). The effects on mean body weight gain reversed during the post-dose phase (GD 18 to 21), where the mean body weight gain was 1.5 fold the control mean. There were no effects on body weight at 10 and 30 mg/kg/day.

Food consumption (dams): Mean food consumption of animals given 100 mg/kg/day decreased significantly from GD 6 to 8, GD 8 to 10, GD 10 to 12, GD 12 to 14, and GD 6 to 18 (78.9 to 89.4% of control). The effects on mean food consumption reversed during the post-dose phase (GD 18 to 21). There were no effects on food consumption at 10 and 30 mg/kg/day.

Toxicokinetics: Exposure to GS-5885 increased with the increase in dose level from 10 to 100 mg/kg/day (refer to Sponsor Table below). The increases in C_{max} and AUC_{0-24} were dose proportional between 10 and 30 mg/kg/day, and slightly less than proportional between 30 and 100 mg/kg/day. Approximately 2-fold accumulation of GS-5885 was observed after multiple dosing of GS-5885 in pregnant rats.

Table 26: Toxicokinetic Parameters for GS-5885 in Rat Plasma.

Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)	T_{last} (hr)	C_{last} (ng/mL)
		GD 6				
10	F	251	4.00	2720	24.0	13.3
30	F	673	4.00	7535	24.0	52.6
100	F	1507	4.00	18325	24.0	266
		GD 17				
10	F	368	4.00	4881	24.0	51.4
30	F	1400	6.00	18218	24.0	244
100	F	2580	4.00	39159	24.0	1087

Terminal Procedures:

Gross Pathology: There were no remarkable observations attributed to GS-5885 at the parental necropsy.

Uterine Findings: There was no effect of GS-5885 on uterine weights, corrected terminal body weight, or net corrected body weight changes. Means were similar between control and GS-5885 treated groups.

The cesarean section data were unremarkable and indicated that GS-5885 had no effects on embryo/fetal viability.

Fetal External, Soft Tissue, and Skeletal Evaluations:

Fetal Weight: There were no GS-5885-related effects on mean fetal weights.

There were no GS-5885-related fetal external abnormalities. The fetal and litter incidences of fetal external variations were similar to control.

Soft Tissue Evaluations:

There were no GS-5885-related soft tissue variations or malformations. Dilated ureter(s) showed a positive trend in fetal incidence at 100 mg/kg/day; however, this trend was not statistically significant and the percent fetal and litter incidences were within the historical control range for this common variation. The fetal and litter incidences of the remaining soft tissue variations and malformations were similar between the control and GS-5885 groups.

Skeletal Evaluations:

There were no GS-5885-related skeletal malformations. A small increase was noted in the fetal and litter incidences of common variations affecting the vertebra, sternebrae, and ribs in animals given 100 mg/kg/day.

Commonly noted findings of bipartite vertebral centrum(a), 5th/6th sternebra(e) incomplete ossification, 5th sternebra(e) unossified showed a positive trend in fetal and litter incidences, and the presence of a 7th cervical rib had a positive trend in fetal incidence. Although the incidence of some of these common variations were outside the historical control range, none were statistically significant compared to control. The presence of 14th rudimentary rib(s) showed a positive trend in fetal incidence. The fetal incidence of this common variation was outside of the historical control range and statistically significant ($p<0.05$) only at 30 mg/kg/day. In the absence of other evidence of fetal toxicity, such as fetal weight effects, alterations in fetal viability, or associated malformations, these common skeletal variations are deemed unrelated to GS-5885.

There was a positive trend identified in the fetal and litter incidences of total skeletal variations with statistical significance at 100 mg/kg/day compared to control. This likely reflects the effects of lower maternal weight gain and significantly decreased food consumption in a majority of the dams in this group during the dosing phase. These variations are considered related to general maternal toxicity (decreased food consumption and body weight gain) rather than a direct developmental insult from GS-5885.

Conclusion: The NOAEL for maternal toxicity of GS-5885 when given once daily by oral gavage to pregnant rats during the period of organogenesis was 30 mg/kg/day based on significantly decreased maternal body weight gain and food consumption at 100 mg/kg/day. There were no GS-5885-related effects on embryo/fetal viability and no fetal visceral or skeletal anomalies; therefore, the NOAEL for developmental toxicity is 100 mg/kg/day.

Study title: Oral Gavage Study for Effects on Embryo-Fetal Development with GS-5885 in Rabbits

Key study findings: There were no test article-related deaths. Seven early deaths were distributed similarly in control and GS-5885-treated groups (2, 0, 2 and 3 deaths at 0, 30, 60 and 180 mg/kg/day, respectively). Deaths were associated with severe weight loss, inappetence, possible dosing errors, or poor pregnancy outcome (early delivery/abortion) that also showed no dose-related incidence. Fecal changes (few, none, and soft) were noted in all groups but with a higher incidence in the 180 mg/kg/day dose group. There were no remarkable maternal necropsy findings. The cesarean section data were unremarkable and indicated that GS-5885 had no effects on embryo-fetal viability. The NOAEL for maternal toxicity was 180 mg/kg/day. The

NOAEL for developmental toxicity was 180 mg/kg/day (mean GD 20 C_{max} 1,399 ng/mL and AUC_{0-24} 20,831 ng·hr/mL), the highest dose tested in this study.

Study no.: TX-256-2013

Volume # and page #: electronic

Conducting laboratory and location: (b) (4)

Date of study completion: 22 June 201

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: GS-5885, 5885-XB-1 and 98.3%

Methods

Doses: 0, 30, 60 and 180 mg/kg/day

Basis of dose selection: The choice of the high dose level was based on all available data including those obtained from an oral gavage dose range-finding developmental toxicity study in rabbits ((b) (4) 8231-635, Gilead Sciences TX-256-2010).

Vehicle: The vehicle control article was 75% propylene glycol/25% Solutol® HS-15.

Species/strain: Female pathogen free New Zealand White (PF NZW) rabbits

Number/sex/group: 20 rabbits/group

Study design: Animals were identified using an implantable microchip identification device and/or cage card/tag and assigned to study groups as follows:

Table 27: Study design summary of rabbit EFD study

Group[a]	No. of Females	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)	Dosing Schedule Days of Gestation
Main-Study Animals				
1 (Vehicle Control)	20	0	0	7-20
2 (Low GS-5885)	20	30	10	7-20
3 (Mid GS-5885)	20	60	20	7-20
4 (High GS-5885)	20	180	60	7-20
Toxicokinetic Animals				
5 (High GS-5885)	3	180	60	7-20

[a] Group 1 received vehicle control article only.

Route, formulation, volume, and infusion rate: oral gavage; 3 ml/kg/day

Toxicokinetics: Toxicokinetic evaluation at the 180 mg/kg/day level was conducted by (b) (4) Toxicokinetic evaluation at dose levels of 30, 60 and 100 mg/kg/day was conducted during the rabbit dose range finding study (study#TX-256-2010).

Parameters and endpoints evaluated: On GD 29, the remaining rabbits were sacrificed by Beuthanasia® followed by exsanguination. Uterine contents were examined. Each fetus (live or dead) was weighed and examined for external abnormalities.

Results

Mortality (dams): Seven animals died or were euthanized as described in Sponsor Table below. These deaths were generally associated with severe weight loss, inappetence, possible dosing errors, or poor pregnancy outcomes (early delivery/abortion) that also showed no dose-related incidence.

Table 28: Summary of Unscheduled Deaths in Rabbit EFD Study

Dose Level (mg/kg)	Animal Number	Status	Day of Death (GD)	Comments
0	F73225	Died	9	Inappetence, no remarkable necropsy observations
	F73238	Sacrificed	27	Severe weight loss (18% from the highest body weight), inappetence, thin appearance, no or few feces, late resorptions
60	F73262	Sacrificed	20	Early weight loss, inappetence, few, no feces, hypoactivity, irregular respiration. Died just before necropsy. Lungs failure to collapse.
	F73266	Died	10	Early weight loss, inappetence, no remarkable clinical signs. Thoracic cavity red fluid, all lobes of lungs dark. Possible dosing error.
180	F73289	Aborted	25	Weight loss, inappetence, few or no feces, no remarkable necropsy observations. Aborted therefore euthanized per protocol.
	F73290	Died (not pregnant)	11	No remarkable clinical observations. Lungs dark red, failure to collapse possible dosing error.
	F73296	Sacrificed	12	Inappetence. Hypoactivity, irregular respiration. Few or no feces, liquid and mucoid feces, heart enlarged.

Clinical signs (dams): Fecal abnormalities (few or none, liquid and/or mucoid feces) were observed in all groups, with a slightly higher incidence at 180 mg/kg/day relative to the control group.

Body weight (dams): There were no statistically significant differences in mean maternal body weight or body weight gain between the GS-5885 groups and control. Animals given 180 mg/kg/day had decreased mean body weight gain during intervals GD 9 to 11 and 18 to 21. However, mean body weight gain of this group during the full dosing period (GD 7 to 21) was similar to (1.2-fold above) the control group.

Food consumption (dams): During the GD 8 to GD 15 interval, mean food consumption in animals given 180 mg/kg/day was 14% lower than controls. All animals that ate less than 25 grams of food were provided dietary supplements (additional timothy hay and

vegetables) to stimulate appetite. Daily food consumption from GD 7 to 21 (dosing period) decreased slightly (8% less than control) in animals given 180 mg/kg/day but this difference was not statistically significant. Observation of the entire study interval (GD 4 to 29) showed no difference in food consumption between the control and treated groups.

Toxicokinetics: The mean toxicokinetic parameters of GS-5885 (180 mg/kg/day) in pregnant rabbit plasma are presented in Sponsor Table below. Exposure to GS-5885 was observed out to 24 hours post-dose. Values for mean C_{max} and AUC_{0-24} were 1,102 ng/mL and 13,079 ng·hr/mL, respectively, on GD 7 and 1,399 ng/mL and 20,831 ng·hr/mL, respectively, on GD 20. Possible accumulation of GS-5885 was observed after repeat dosing in pregnant rabbits.

Table 29: Summary of the Mean Toxicokinetic Parameters for GS-5885 in Pregnant Rabbit

Interval	Dose Level (mg/kg/day)		C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)	T_{last} (hr)	C_{last} (ng/mL)	$T_{1/2}$ (hr)
GD 7	180	Mean	1102	4.00	13079	24.0	208	7.48
		SD	234	0	2752	0	42	NA
		N	3	3	3	3	3	2
GD 20	180	Mean	1399	10.7	20831	24.0	706	5.53
		SD	674	11.5	10704	0	777	NA
		N	3	3	3	3	3	1

Dose range study (TX-256-2010): Exposure to GS-5885 increased with the increase in dose level from 30 to 300 mg/kg/day (refer to Sponsor Table below). The increase in exposure was proportional with dose levels from 30 to 60 mg/kg/day, but was less than proportional with dose levels between 60 to 300 mg/kg/day. Approximately 2-fold accumulation of GS-5885 was observed after repeat dosing of GS-5885 in pregnant rabbits, which was consistent with the observed half-lives of GS-5885 in this study.

Table 30: Summary of the Mean Toxicokinetic Parameters for GS-5885 in the Plasma of Pregnant

Dose Group	Dose Level (mg/kg/day)	Sex		C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24} (ng•hr/mL)	T_{last} (hr)	C_{last} (ng/mL)	$t_{1/2}$ (hr)
					GD 7				
8	30	F	Mean	444	3.33	3377	24.0	40.4	8.40
			SD	85	1.15	326	0	14.6	0.72
			N	3	3	3	3	3	2
9	60	F	Mean	889	4.00	8092	24.0	117	8.69
			SD	367	0	4968	0	109	0.74
			N	3	3	3	3	3	3
10	100	F	Mean	790	4.00	7742	24.0	112	8.70
			SD	108	0	1295	0	39	2.00
			N	3	3	3	3	3	3
11	300	F	Mean	1124	10.7	19868	24.0	927	NA
			SD	396	11.5	7713	0	581	NA
			N	3	3	3	3	3	0
					GD 20				
8	30	F	Mean	704	2.67	6847	24.0	112	8.47
			SD	276	1.15	2661	0	104	NA
			N	3	3	3	3	3	2
9	60	F	Mean	1490	4.00	19354	24.0	399	9.03
			SD	439	0	8609	0	255	NA
			N	3	3	3	3	3	1
10	100	F	Mean	1743	6.67	24891	24.0	790	8.57
			SD	206	4.62	16613	0	1002	NA
			N	3	3	3	3	3	2
11	300	F	Mean	2870	16.2	57323	24.0	2593	NA
			SD	930	13.6	20520	0	1038	NA
			N	3	3	3	3	3	0

Terminal Procedures: There were no remarkable GS-5885-related observations at scheduled maternal necropsy.

Gravid Uterine Weights, Corrected Terminal Weights, and Net Body Weight Changes

Gravid uterine weights, corrected terminal body weights, and net body weight changes were similar between the vehicle control and GS-5885-treated groups.

Cesarean Section: There was no effect of GS-5885 on ovarian, uterine, and fetal litter data. Pregnancy rates were 85, 100, 95 and 90% in the 0, 30, 60 and 180 mg/kg/day groups, respectively, due to animals found not pregnant at cesarean section. There were no early deliveries. The numbers of corpora lutea and implantation sites were similar to control values. The number and mean percent of early, late and total

resorptions and mean post-implantation loss for animals evaluated at scheduled cesarean section were similar between animals dosed with GS-5885 and controls. There were no significant differences in mean fetal weights.

Fetal External, Soft Tissue, and Skeletal Evaluations

External Evaluations: There were no remarkable external observations among the groups. At 180 mg/kg/day two fetuses in one litter had flexed front paws but this incidence was low and not accompanied by other malformations and therefore this finding was considered unrelated to GS-5885 treatment.

Soft Tissue Evaluations: The fetal soft tissue variations observed were generally similar across the groups in both fetal and litter incidences with no apparent effect of GS-5885. Across dose groups, only one fetal soft tissue malformation (cardiomegaly) was observed in the 30 mg/kg/day dose group.

Skeletal Evaluations: Common variations such as angulated hyoid wings, 5th/6th sternebra(e) incomplete ossification, 13th rudimentary rib(s) and 13th unilateral full rib were observed with similar incidence across groups.

Conclusion: GS-5885 given once daily by oral gavage to pregnant rabbits during organogenesis had no adverse effect on maternal or embryo/fetal viability and growth, or on the incidence of fetal anomalies. Based on the transient decrease in maternal body weight gain and food consumption the NOAEL for maternal toxicity was 180 mg/kg/day. The NOAEL for developmental toxicity was 180 mg/kg/day (mean GD 20 C_{max} 1,399 ng/mL and AUC_{0-24} 20,831 ng·hr/mL), the highest dose tested in this study.

9.3 Prenatal and Postnatal Development

Study title: An Oral (Gavage) Study of the Effects of GS-5885 on Pre- and Postnatal Development, Including Maternal Function in Rats

Study no.:	TX-256-2020 ((b) (4) -604030)
Study report location:	4.2.3.5.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 4, 2013
GLP compliance:	Yes (final report)
QA statement:	Yes
Drug, lot #, and % purity:	GS-5885, 5885-03-AC-3P and 99.3%

Key Study Findings: Potential GS-5885 related effects on pre- and post-natal development (PPND) were evaluated in pregnant rats (25/sex/group) administered oral doses of 10, 30, 100 mg/kg or vehicle (45% propylene glycol and 15% Solutol) control from GD6 to lactation day 20 (LD20). The NOEL for PPND was estimated to be 100 mg/kg/day [maternal: AUC_{0-t}~22.2 & 37.6 μg.h/ml for GS-5885 at GD6 & LD10,

respectively; F_1 rat pups: AUC_{0-t}~9.8 μg.h/ml for GS-5885 at PND10 (LD10)], while the NOAEL for maternal toxicity was estimated conservatively to be 30 mg/kg/day [maternal: AUC_{0-t}~8.6 & 11.4 μg.h/ml for GS-5885 at GD6 & LD10, respectively; F_1 rat pups: AUC_{0-t}~2.6 μg.h/ml for GS-5885 at PND10 (LD10)], given adverse maternal findings at 100 mg/kg/day. A trend for reduced body weight in the F_1 generation that was not statistically significant was observed, which appears to be attributed to maternal toxicity and not a direct LDV-related effect. At 100 mg/kg/day, one gravid female was euthanized *in extremis* on GD 18 following body weight loss, low food consumption and clinical findings. In addition, mean maternal body weight was decreased at GD 20, correlating with lower mean food consumption, but was not significantly different than the control group by LD 7. No other significant PPND or maternal effects were observed.

Methods

Doses:	0, 10, 30, 100 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	Oral gavage
Route of administration:	5 ml/kg
Formulation/Vehicle:	GS-5885 in 45% propylene glycol & 15% Solutol HS-15 (pH=2.5)
Species/Strain:	Rat/Crl:CD(SD) Sprague Dawley
Number/Sex/Group:	25 ♀ (main), 9 (TK), 2 pups (F_1)/sex/litter (25 pups/sex/group for both subsets A & B)
Satellite groups:	TK
Study design:	• GS-5885 administered to pregnant females from gestation day (GD) 6 to lactation day (LD) 20 and sacrificed on LD21. F_1 pups not selected for F_1 generation sacrificed on PND21. • F_1 generation split into subsets A or B (see Sponsor table below). F_1 pups not selected for breeding phase sacrificed after attainment of developmental landmarks. • Laparohysterectomies performed on GD 20 (F_2 generation morphological assessment) for F_1 animals selected for breeding phase (reproductive assessment). F_1 males sacrificed.
Deviation from study protocol:	None that affected the integrity or conclusions of the study.

Table 31: Offspring allocation of F1 generation

No. Selected	Age	Evaluation
1/pup/sex/litter (A)[a]	PND 20 and 60	Auditory startle
1/pup/sex/litter (A)[a]	PND 21 and 61	Locomotor Activity
1/pup/sex/litter (B)	PND 22	Learning and Memory [b]
1/pup/sex/litter (A)[a]	PND 62	Learning and Memory [b]
1/pup/sex/litter (A)[a]	Minimum of 85 days	Breeding

[a] = The same pup subset was used for auditory startle, locomotor activity, PND 62 learning and memory, and breeding.
[b] = Different pups were evaluated on PND 22 and 62.

Note: Biel maze swimming trial conducted to assess learning and memory (measured escape time and errors).

Observations and Results

F_0 Dams

Survival:	In the 100 mg/kg/day group, one gravid female (had 6 late resorptions and 1 early resorption) was euthanized *in extremis* on GD 18 following body weight loss of 7% from GD 15-18 and low food consumption (↓60% versus other HD ♀). Clinical findings of pale eyes and red material around the nose and urogenital area were noted on the day of euthanasia. Macroscopic findings at necropsy included dark red contents in the uterus and vagina and red skin matting on the urogenital area. Findings considered GS-5885-related but of low incidence (1 of 25). In the 30 mg/kg/day group, one female was euthanized due to total litter loss on LD 10. All other females in the vehicle control, 10, 30, and 100 mg/kg/day groups survived to the scheduled necropsies.
Clinical signs:	Signs of maternal toxicity at 100 mg/kg/day included slightly higher incidence of slight/moderate forelimb hair loss.
Body weight:	~10% decrease of mean body weight gain (and transient body weight loss from GD 6-9) was noted in 100 mg/kg/day group animals (versus vehicle control) from GD 6-20, resulting in ~6% lower mean body weights at GD 20. Mean body weight gain was lower in the 30 mg/kg/day group (versus vehicle control) from GD 6-9 only. Significantly higher mean body weight gain was noted at 100 mg/kg/day (versus vehicle control) from LD 1-7 and 10-21, attributed to a slight developmental delay of the F_1 pups (by conducting lab), resulting in mean body weights that were not significantly different than the vehicle control group by LD 7.
Feed consumption:	Significantly lower mean food consumption was noted in the 100 mg/kg/day group (versus vehicle control) throughout the entire gestation period (GD 6-20). In the 30 mg/kg/day group, significantly lower mean food consumption was noted (versus vehicle control) during GD 6-9 only.
Uterine content:	No test-article related findings noted. Gestation length was 22.0, 21.9 and 22.0 days in the 10, 30 and 100 mg/kg groups versus 22.0 days in the vehicle control. Refer to Table below.
Necropsy observation:	No test-article related findings noted.
Toxicokinetics:	Note: Samples collected pre-dose, 0.5, 1, 2, 4, 8, 12 & 24 hours post-dose from 3 ♀/group/timepoint on

	GD 6 & LD10 and 1 pup/sex/litter/group @ PND 10 (LD 10). See Sponsor Table below.
Dosing Solution Analysis	All dosing formulations (0, 2, 6 and 20 mg/ml) were analyzed for concentration. Assay results were within an acceptable concentration range (±10%), while the vehicle and the compound in this vehicle were shown to be stable under the conditions of this study.
Other:	None

Table 32: Summary of uterine content in F_0 dams administered GS-5855

	0 (vehicle)	10 mg/kg	30 mg/kg	100 mg/kg
# of dams that delivered:	25	25	24	24
Implantation sites/litter:	16.3	16.4	15.9	16.0
# pups born/litter:	15.2	15.8	14.4	14.8
Live litter size (PND 0)/litter:	15.1	15.2	14.1	14.5
% male:	48.8	50.7	47.5	48.1

Table 33: Mean plasma GS-5885 TK parameters in pregnant and lactating F_0 rats and F_1 offspring following maternal GS-5885 administration

Interval	Group	Dose Level (mg/kg/day)	Generation	Sex	C_{max} (ng/mL)	AUC_{0-t} (ng•hr/mL)	M:O Ratio AUC_{0-t}
GD 6	2	10	F_0	F	299	3030	NA
	3	30		F	658	8580	NA
	4	100		F	1530	22200	NA
LD 10	2	10	F_0	F	267	2620	NA
	3	30		F	846	11400	NA
	4	100		F	2610	37600	NA
LD 10	2	10	F_1	M	34.2	597	4.39
				F	29.2	593	4.41
				MF	31.3	598	4.38
	3	30	F_1	M	163	2670	4.28
				F	119	2500	4.56
				MF	122	2590	4.42
	4	100	F_1	M	483	10100	3.71
				F	514	9420	3.99
				MF	498	9770	3.85

NA = Not applicable
M:O Ratio = Maternal (F_0) to offspring (F_1) AUC_{0-t} ratio
Note: Combined (MF) data is based on the analysis of the combined mean concentration data for both F_1 sexes.

F_1 Generation

Survival:	No clear test-article related findings noted. Trend for increased pups found dead/euthanized/missing in GS-5885 dose groups that was not statistically significant.
Clinical signs:	No test-article related findings noted.
Body weight:	No clear biologically significant test-article related findings noted. However, a trend for a slightly reduced BW and BW gain in 100 mg/kg/day dose group (that was generally not statistically significant) was noted (refer to Table below). For example, BW was 4.1 & 5.8% less in males & females respectively at PND 1 and ~6.5% less in males & females at PND 21 (the result of decreased BW gain during PND 4-7 & 17-21).
Feed consumption:	Not evaluated
Physical development:	No test-article related findings noted.
Neurological assessment:	No test-article related changes on startle response (PND 20 or 60), motor activity (PND 21 or 61) or learning and memory (Biel maze at PND 22 or 62) noted. Differences observed either did not reach statistical significance or were consistent with historical control values.
Reproduction:	Refer to Table below.
Other:	No test-article related effects noted on gestation length, parturition or gross pathology.

Table 34: Summary of survival, body weight, clinical and selected developmental parameters in the F1 generation

	0 (vehicle)	**10 mg/kg**	**30 mg/kg**	**100 mg/kg**
# of pups examined (clinical signs):	374	376	331	338
# of pups found dead:	9	15	17	19
# of pups euthanized *in extremis*:	0	2	7	0
# of pups missing (cannibalized):	4	9	6	14
Mean pup weight on PND 1 (♂):	7.3 g	7.2 g	7.7 g	7.0 g
Mean pup weight on PND 1 (♀):	6.9 g	6.8 g	7.1 g	6.5 g
Mean pup weight on PND 21 (♂):	53.5 g	50.6 g	54.8 g	50.1 g
Mean pup weight on PND 21 (♀):	51.0 g	47.3 g	52.0 g	47.7 g
Mean body weight on PND 126 (♂):	624 g	625 g	633 g	600 g
Mean body weight on PND 84 (♀):	290 g	281 g	291 g	277 g
PN survival (%)/litter (birth to PND 4):	97.5	94.3	95.2	92.9
PN survival (%)/litter (PND 4 to 21):	98.5	98.5	93.8	95.5
Balanopreputial separation (PND):	43.5	44.2	43.2	43.6
Mean BW at attainment age (♂):	247.8 g	247.9 g	249.6 g	239.0 g

Vaginal patency (PND):	32.2	32.0	31.8	32.1
Mean BW at attainment age (♀):	119.8 g	112.4 g*	119.4 g	112.4 g*

PND=post-natal day; BW=body weight; *denotes statistical significance

Table 35: Summary of F_1 reproductive performance

Parameter	Dosage Level (mg/kg/day) 0	10	30	100	WIL HC[a] Mean (Range)
Male Mating Index (%)[b]	92.0 (23)	96.0 (24)	95.7 (22)	87.5 (21)	95.8 (84.0-100.0)
Female Mating Index (%)[b]	96.0 (24)	100.0 (25)	95.7 (22)	95.8 (23)	98.2 (92.0-100.0)
Male Fertility Index (%)[b]	92.0 (23)	96.0 (24)	82.6 (19)	75.0 (18)	90.0 (60.0-100.0)
Female Fertility Index (%)[b]	96.0 (24)	96.0 (24)	82.6 (19)	83.3 (20)	92.9 (60.0-100.0)
Male Copulation Index (%)[b]	100.0 (23)	100.0 (24)	86.4 (19)	85.7 (18)	93.2 (71.4-100.0)
Female Conception Index (%)[b]	100.0 (24)	96.0 (24)	86.4 (19)	87.0 (20)	92.9 (65.2-100.0)
Estrous Cycle Length (days) (n)	4.3 (25)	4.3 (24)	4.4 (20)	4.2 (24)	4.3 (4.0-5.0)
Pre-Coital Interval (days) (n)	3.5 (24)	3.2 (25)	2.5 (22)	3.9 (23)	3.3 (2.4-4.8)

[a] = (b) (4) historical control data

[b] = Presented as percentage confirmed, with number of animals confirmed in parentheses.

Table 36: Summary of uterine content in F_1 dams administered GS-5855

	0 (vehicle)	10 mg/kg	30 mg/kg	100 mg/kg
# of gravid females:	24	23	18	20
Corpora lutea/litter:	17.4	17.2	16.9	17.3
Implantation sites/litter:	16.6	15.3	15.9	16.2
# pups born/litter:	15.1	14.3	14.7	15.4
Live litter size (PND 0)/litter:	14.8	14.0	14.3	14.6
% male:	52.8	49.7	42.9	47.9

F_2 Generation

Survival: No test-article related findings noted.
Body weight: No test-article related findings noted.
External evaluation: No test-article related findings noted.
Male/Female ratio: No test-article related findings noted.
Other: Not applicable

Table 37: Summary of fetal data for F2 generation

	0 (vehicle)	10 mg/kg	30 mg/kg	100 mg/kg
# of litters:	24	23	18	20
# of pups found dead:	23	7	8	18
# of pups missing (cannibalized):	1	10	2	2
Mean fetal weight (sexes combined):	6.9	6.9	7.0	6.9

10 Special Toxicology Studies

Study title: 2-Week Oral Gavage Qualification Toxicity and Toxicokinetic Study with GS-5885 in Male Rats

Study no.:	TX-256-2035
Study report location:	4.2.3.7.6
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 3, 2013
GLP compliance:	Yes
QA statement:	Yes (final report)
Drug, lot #, and % purity:	GS-5885, 5885-03-AC-3P and 99.3%; GS-5885, 5763-30-25 and 98.3%

Key study findings: A 14-day oral toxicity study was conducted in male SD rats (10/group) by administering daily GS-5885 doses of up to 100 mg/kg/day or vehicle (45% propylene glycol and 15% Kolliphor HS-15). Standard toxicity endpoints were evaluated including clinical signs, body weight, food consumption, hematology, coagulation, clinical chemistry, urinalysis and gross and histopathology. Administration of both lots of GS-5885 was well-tolerated at up to 100 mg/kg/day, with only transient, non-adverse reductions in mean body weight gain and food consumption as well as mild increases in cholesterol and triglycerides noted. The NOAEL was 100 mg/kg/day for both lots (for 5885-03-AC-3P: C_{max} =2,610 ng/mL, AUC_{0-t} =47,700 ng·hr/mL; for 5763-30-25: C_{max} =2,230 ng/mL, AUC_{0-t} =39,400 ng·hr/mL at 14 days). Overall the toxicity findings with these two lots were similar to those described previously.

Study title: 2-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-5885 and GS-5885-02 in Rats

Study no.:	TX-256-2014
Study report location:	4.2.3.7.7
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 16, 2010
GLP compliance:	Yes
QA statement:	Yes (final report)
Drug, lot #, and % purity:	GS-5885 (free base), 5885-XB-1 and 100%; GS-5885-02 (b) (4) 5885-02-B-1 and 99.2%

Key study findings: A 14-day oral toxicity study was conducted in SD rats (10/sex/group) by administering daily GS-5885 or GS-5885-02 doses of up to 100 mg/kg/day or vehicle 1 (45% propylene glycol and 15% Kolliphor HS-15) or vehicle 2

[0.2% (w/v) hydroxypropylmethylcellulose (HPMC) E4M, 0.2% (v/v) Tween 20 and 0.9% (v/v) benzyl alcohol]. Standard toxicity endpoints were evaluated including clinical signs, body weight, food consumption, ophthalmic examinations, hematology, coagulation, clinical chemistry, urinalysis and gross and histopathology. Administration of GS-5885 and GS-5885-02 was well-tolerated at up to 100 mg/kg/day, with only transient, non-adverse reductions in mean body weight gain and food consumption noted in males only. One TK group male given 100 mg/kg/day of GS-5885 was sacrificed on Day 8 due to general debilitation (clinical signs of hunched/thin, low fecal production) and was considered a sporadic event. Thus, the NOAEL was 100 mg/kg/day (for GS-5885: C_{max} =2,072 ng/mL, AUC_{0-t} =34,622 ng·hr/mL; for GS-5885-02: C_{max} =903 ng/mL, AUC_{0-t} =10,992 ng·hr/mL at 14 days). Overall the toxicity findings with GS-5885 and GS-5885-02 were similar to those described previously.

Study title: Phototoxicity Evaluation of GS-5885-02 Administered as a Single Oral (Gavage) Dose in Hairless Mice

Study no.:	TX-256-2015
Study report location:	4.2.3.6
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Not provided (around March 1, 2011)
GLP compliance:	Yes
QA statement:	Yes (final report)
Drug, lot #, and % purity:	GS-5885-02 (b) (4) 5885-02-B-1 and 100.4%

Key study findings: A single dose oral phototoxicity study was conducted in albino female Crl:SKH1-*hr* hairless mice (6/group) by administering GS-5885-02 doses of 22.3, 74.5, 224 or 300 mg/kg/day, a vehicle control [0.2% (w/v) hydroxypropylmethylcellulose (HPMC) E4M, 0.2% (v/v) Tween 20 and 0.9% (v/v) benzyl alcohol] or the positive control 8-Methoxypsoralen (8-MOP). Toxicity endpoints evaluated including clinical signs, body weight and skin observations. Mice were exposed to UVR [for 30 ± 5 min at an exposure dose equivalent to 0.5 minimal erythema dose (MED)] either 4 hrs ± 15 min (approximate T_{max}) or 1 hr ± 10 min after administration of GS-5885-02 or 8-MOP, respectively. One mouse administered 224 mg/kg of GS-5885-02 was found dead after sham UVR exposure, which was considered a likely procedure-related event (due to restraint and anesthesia/sedation). No skin reactions indicative of phototoxicity occurred in albino female hairless mice administered single oral doses of GS-5885-02 up to 300 mg/kg followed by a single exposure to solar-simulated UVR. Thus, the NOEL for dermal phototoxicity was 300 mg/kg/day (mean C=2,960 ng/mL @ 4hrs post-dose).

Study title: A Multiple Dose Phototoxicity Study to Determine the Effects of Oral (Gavage) Administration of GS-5885 and GS-5885 + (b) (4) on Eyes in Pigmented Rats

Study no.:	TX-256-2038
Study report location:	4.2.3.7
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Not provided (protocol signed May 2, 2014)
GLP compliance:	Yes
QA statement:	Yes (draft report)
Drug, lot #, and % purity:	GS-5885 (0.1% (b) (4) 5885-03-AC-4P and 99.6%; GS-5885 ((b) (4) % (b) (4) MK-5916-066 and 85.6%

Key Study Findings: A 3-day multiple dose oral (gavage) ocular phototoxicity study was conducted in pigmented male Long Evans (LE) rats (5/group) administered GS-5885 or GS-5885 (spiked w/ (b) (4) % (b) (4) once a day at doses of up to 100 mg/kg/day, a vehicle control [45% propylene glycol and 15% Kolliphor® HS-15 (pH 2.5)] or the positive control 8-Methoxypsoralen (8-MOP) (refer to Sponsor Table below). Toxicity endpoints evaluated including clinical signs, body weight, ophthalmology (pre-dose and on Day 6), gross necropsy (unscheduled deaths only) and ocular histopathology (cornea, lens, bulbar conjunctiva, vitreous & aqueous chambers, optic nerve, retina, sclera, iris, ciliary body and choroid evaluated in both eyes on Day 6). Rat eyes were exposed to a Xenon lamp (emits light at wavelengths throughout the UVB, UVA, visible and into the near IR spectrum), producing ultraviolet radiation (UVR) (single ~40 min exposure of ~10 J/cm^2 of UVA & ~145 mJ/cm^2 of UVB), either 4 hrs ± 15 min or 1 hr ± 10 min after administration of GS-5885 with or without (b) (4) or 8-MOP, respectively. Ophthalmological observations, ocular histopathological changes and/or clinical observations characteristic of phototoxicity were noted in animals administered 8-MOP including swollen soft eyelids, diffuse corneal stromal edema and neutrophil infiltration, loss of corneal endothelium, intercellular edema and/or vacuolation of corneal epithelium and lenticular degeneration (hyperplasia of central lenticular epithelium or swelling and necrosis of lens fibers in nuclear bow region) consistent with early cataract development in lens, while inferior focal retinopathy (degenerative injury) was observed following UVR exposure in control and GS-5885 treated groups. Two rats (group 1 & 3) found dead after UVR exposure were considered procedure-related events (due to restraint and sedation with ketamine and xylazine). Slight body weight loss was observed during dosing phase at 100 mg/kg/day (groups 3-5), while these groups gained weight during Days 4 to 6. No eye reactions indicative of phototoxicity occurred in pigmented male rats administered oral doses of GS-5885 with or without (b) (4) up to 100 mg/kg for 3 days followed by a single exposure to solar-simulated UVR. An increased incidence of minimal/mild corneal dystrophy was observed in GS-5885 treated groups (60, 90, 63 & 80% of group 2, 3, 4 & 5 eyes, respectively, affected versus 25% in control). Although possibly GS-5885-related, these minor changes appear to be due primarily to corneal desiccation as a result of

procedure-related manipulation. Thus, the NOEL for ocular phototoxicity was 100 mg/kg/day for LDV (C_{max} = 3,120 ng/mL, AUC_{0-24} = 61,500 ng·hr/mL at 3 days) and ~86 and 13 mg/kg/day for LDV and (b) (4) respectively (LDV: C_{max} =3,540 ng/mL, AUC_{0-24} = 58,400 ng·hr/mL at 3 days).

Table 38: Experimental design summary of ocular phototoxicity study in male Long Evans rats

Group	Number of Rats	Descriptor	Dose Level (mg/kg/day)	Concentration (mg/mL)	Dose Volume (mL/kg)	UVA Dose, J/cm^2	Interval Between Dose and UVR Exposure[a] (Hours)
1	5	Vehicle	0	N/A	10	10 ± 1	4 hours ± 15 min.
2	5	GS-5885 + GS-459666	30	3	10	10 ± 1	4 hours ± 15 min.
3	5	GS-5885 + GS-459666	100	10	10	10 ± 1	4 hours ± 15 min.
4	5	GS-5885	100	10	10	10 ± 1	4 hours ± 15 min.
5	5	GS-5885	100	10	10	0	N/A[b]
6	3	8-MOP	(50[c])	(5[c])	10	10 ± 1	1 ± 10 minutes

N/A = Not applicable

[a] Interval based on the Tmax of the test articles. The actual exposure time of each group was based on the average dosing time of the group.

[b] Sham exposure; rats were sedated and restrained but not exposed to UVR.

[c] Group 6 rats were administered 50 mg/kg 8-MOP once at a concentration of 5 mg/mL.

Table 39: Mean plasma GS-5885 TK parameters in male Long Evans rats following administration of GS-5885 with or without (b) (4)

Group / Descriptor	GS-5885 Dose Level (mg/kg/day)	T_{max} (hour)	C_{max} (ng/mL)	AUC_{0-24} (ng.h/mL)
GS-5885 + GS-459666	30	8	1410	24000
GS-5885 + GS-459666	100	8	3540	58400
GS-5885	100	12	3121	61500

Table from sponsor

11 Integrated Summary and Safety Evaluation

Harvoni™, a once daily fixed-dose combination (FDC) tablet containing 90 mg of ledipasvir and 400 mg of sofosbuvir, is intended to be indicated for treatment of chronic hepatitis C virus (HCV) genotype 1 infection in adult patients. Ledipasvir (LDV, GS-5885) 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 (as a component of a combination antiviral treatment regimen) was approved for marketing in the U.S. in December 2013 (refer to NDA-204,671). With the exception of the rodent 2-year carcinogenicity studies, nonclinical safety studies for SOF to support the FDC were reviewed previously. Refer to the Pharmacology/ Toxicology review for

NDA-204,671 for the detailed summary of SOF nonclinical data as well as Table 41 for updated SOF clinical exposure multiples.

Pharmacokinetics:

The oral bioavailability of LDV was estimated to be ~33 to 53% in rats, monkeys and dogs, following administration by gavage, while oral T_{max} values for LDV in these species ranged from ~4 to 5 hours. LDV has low intrinsic aqueous solubility and low solubility in simulated intestinal fluid. Despite this low solubility and a saturation of absorption, absorption of LDV from the GI tract was sufficient to achieve adequate circulating LDV exposure levels in the toxicology studies. Increases in SOF (and its main circulating metabolite, GS-331007) exposure were observed clinically when administered as a FDC w/ LDV, which appears to be the result of increased intestinal absorption of SOF due to LDV inhibition of intestinal transporters.

The steady state volume of distribution (V_{ss}) ranged from ~1.2 to 2.7 L/kg in rats, dogs and monkeys, values greater than total body water volume, with mean residence times (MRT) from ~6 to 13 hours. In addition, LDV was bound highly to plasma protein in all species (>99.8% bound). These data with LDV appear reasonable given its hydrophobic structure and low intrinsic aqueous solubility. Tissue distribution of LDV and related metabolites was examined using quantitative whole body autoradiography in male mice (CD-1) and rats [Long-Evans (LE) and Sprague-Dawley (SD)]. In mice, the highest concentrations of radioactivity were observed in the GI tract, gall bladder, liver, harderian gland and kidney, in the absence of significant penetration of the blood-testes and blood-brain barriers. In SD rats, concentration of LDV and related metabolites in liver were 23 times that in plasma 4 hours post-dose. In addition to liver, the highest concentrations were measured in the GI tract, bile, adrenal, salivary, thyroid, pituitary and harderian glands, pancreas, adipose tissue (brown) and kidney.

The systemic clearance of LDV was low in all species tested with terminal half-lives of ~5 to 10 hours in rats, dogs and monkeys. Unchanged parent drug was the major circulating component, representing ~87 to 98% of total plasma AUC following oral administration of ^{14}C-LDV to mice, rats, dogs and healthy human subjects. Unchanged parent drug was not only the major component in feces (accounting for greater than 80% of the total) in these species, but also accounted for ~44% and 80% of the radioactivity recovered in bile from rats and dogs, respectively. Several minor metabolites were identified in plasma, feces, bile and/or urine, primarily formed via oxidation and *N*-demethylcarboxylation. Approximately 93 to 96% of the administered LDV-derived radioactivity was excreted in feces from mice, rats and dogs. In addition, it was estimated that ~86% and 98% of the absorbed dose was eliminated via biliary excretion in rats and dogs, respectively, so biliary excretion of unchanged parent compound was a major route of elimination for LDV.

Safety Pharmacology:

No significant effects on neurologic (modified Irwin test) or respiratory parameters (respiratory rate, tidal or minute volumes) were observed in male rats following single oral doses of LDV up to 100 mg/kg (estimated C_{max}~1.63 μg/ml for GS-5885), providing an ~5-fold rat to human exposure multiple at the recommended LDV dose. This C_{max} value (Day 1) was obtained from the 2-week toxicology study in rats (study #TX-256-2003). In addition, no significant cardiovascular effects on

hemodynamic (HR and BP) or electrocardiographic parameters were noted for up to 25 hours post-dose in telemetry-monitored male dogs given single oral doses of LDV up to 30 mg/kg ($C_{4.5hr}$=1.93 to 2.96 μg/ml; estimated C_{max}~5.86 μg/ml for GS-5855), providing ~6- to 9- (at $C_{4.5hr}$) & ~18-fold (at estimated C_{max}) dog to human exposure multiples at the recommended LDV dose. This (estimated) C_{max} (Day 1) value was obtained from the 2-week toxicology study in dogs (study #TX-256-2004). GS-5885 did not significantly inhibit hERG current *in vitro* at the maximal feasible concentration [445(0.5) ng/mL(μM)], based on solubility limitations. No significant hemodynamic or electrocardiographic heart effects have been observed in clinical trials with LDV.

Repeat-Dose Toxicology Studies:

In mice, increases in mean body weight gain (associated with increased mean food consumption and increased body weight in males) were observed in both males and females administered an oral LDV dose of 300 mg/kg/day (AUC_{0-24hr}~164 & 271 μg.h/ml for GS-5885 in females and males, respectively). In addition, slight increases in ALP and/or ALT were observed in males and females at 300 mg/kg/day that were also observed to some extent at other dose levels, and so these elevations appeared LDV-related but were not clearly dose dependent. It is possible that the ALP and ALT elevations and a slight increase in liver/gall bladder weight (in males administered 300 mg/kg/day only) are indicative of mild hepatobiliary toxicity. However, in the absence of correlating histopathology changes in the liver, these findings were not considered to be adverse. Additional organ weight changes in males administered 300 mg/kg/day included decreased seminal vesicle weight that was not considered to be toxicologically significant, since there were no correlating histopathology findings. These non-adverse body weight and hepatobiliary findings were observed at GS-5885 AUC exposure ~30-fold higher than that in humans at the recommended LDV dose.

In rats, decreased body weight gain and food consumption, increases in cholesterol (up to 32% in 26-week study) and triglycerides (up to 2.2-fold in 26-week study) were noted at 100 mg/kg/day (AUC_{0-24hr}~56 μg.h/ml for GS-5885) in both the 14-day and 26-week studies. In addition, increased incidence of minimal to slight adrenal cortical sinusoid dilatation/congestion in females with minimal adrenal vacuolar degeneration in a single male and female given 100 mg/kg/day were observed. In the liver, minimal to slight random foci of hepatocyte necrosis in males and bile duct hyperplasia in males and females were noted at all or most LDV dose levels following 26-weeks of LDV administration, and so these findings were not clearly dose dependent. Two (of 15) females administered 100 mg/kg/day in the 26-week study were sacrificed at around 8 and 19 weeks, respectively, due to general debilitation including few feces and 13-15% body weight loss. Clinical signs in these animals included red hair coat (front legs/facial and/or perineal regions), which was also observed at higher incidence in males and other females administered ≥30 and 100 mg/kg/day, respectively. Red hair coat was attributed, at least in part, to struggling during dosing that occurred for the first few weeks, particularly in females administered 100 mg/kg/day. The only noteworthy findings in sacrificed animals were increased absolute neutrophil count in one female and a slight ulceration/erosion and epithelial hyperplasia of the non-glandular stomach in the other female, which are common findings in debilitated rats and so were not likely a direct LDV effect. Thus, the cause of death was

undetermined for both animals and of uncertain relationship to treatment. These findings, which were considered either non-adverse or not clearly LDV-related, were observed at GS-5885 AUC exposure ~8-fold higher than that in humans at the recommended LDV dose.

In dogs, LDV-related adverse effects, including body weight loss and lower food consumption during the first week of dosing, were observed in several animals administered 30 mg/kg/day in the 2-week toxicology study. However, this dose level was well-tolerated for 2-weeks when nutritional supplementation was provided. Large cerebral ventricles (described microscopically as minimal dilatation of ventricles) were noted in one female administered 30 mg/kg/day for 14 days. Although the significance is uncertain, this finding was observed in only a single animal and was not seen in longer duration studies and so appears to be sporadic and therefore not LDV-related. The only clear LDV-related finding in the 39-week study at 30 mg/kg/day ($AUC_{0\text{-}24hr}$~41.3 & 80.3 μg.h/ml for GS-5885 in males and females, respectively) was transient post-dose salivation. In addition, one (of 9) male administered 30 mg/kg/day was sacrificed in moribund condition at around 11 weeks with clinical signs of general debilitation (few and mucoid feces, hypoactivity, hunched posture, body weight loss and increased body temperature). This animal also lost body weight during the first two weeks of dosing and was subsequently put on a brief dosing holiday and began receiving food supplementation. Although the reason for the potential intolerability in this animal is not totally clear, the clinical sign of pyrexia, clinical pathology changes, and microscopic alterations in this animal were consistent with sepsis, and therefore not directly LDV-related. These findings, which were considered either non-adverse or not clearly LDV-related, were observed at GS-5885 AUC exposure ~9-fold higher than that in humans at the recommended LDV dose.

Phototoxicity:

Tissue distribution of LDV was examined in male pigmented [Long-Evans(LE)] and non-pigmented (Sprague-Dawley) rats following a single 10 mg/kg dose of ^{14}C-LDV. Differences between pigmented and non-pigmented rats were observed, most notably in the eye uveal tract. Concentration of LDV and related metabolites in the eye uveal tract in LE rats peaked at 8 hours post-dose (at ~4 times that in plasma) and was still detectable at 168 hours post-dose. The half-life of LDV in the uveal tract of the eye was slightly less than seven days following a single dose, occurring at LDV AUC exposure similar to that in humans at the recommended LDV dose. Although a slight increase in LDV distribution to pigmented versus non-pigmented skin was observed in LE rats, direct binding of LDV to melanin appears unlikely.

Since LDV (and related metabolites/impurities/degradants) absorbs ultraviolet (UV) light, accumulates in the uveal tract of the eye in pigmented rats and includes an impurity/photodegradant of potential phototoxic concern ([(b) (4)] based on structure (LDV converted to a potentially more reactive molecule by visible light), a 3-day multiple dose ocular phototoxicity study was conducted in male LE rats administered LDV or LDV (spiked w/[(b) (4)]% [(b) (4)] This study was undertaken not only for general hazard identification and risk assessment purposes but also to qualify the [(b) (4)] impurity/photodegradant, given the specific cause for concern identified. No eye reactions indicative of phototoxicity occurred in male pigmented rats administered oral gavage doses of LDV with or without [(b) (4)] up to 100 mg/kg

once a day for 3 days followed by a single exposure to solar-simulated UVR. The NOEL for ocular phototoxicity was 100 mg/kg/day for LDV (C_{max} = 3,120 ng/mL, AUC_{0-24} = 61,500 ng·hr/mL at 3 days) and ~86 and 13 mg/kg/day for LDV and (b) (4) respectively (LDV: C_{max} =3,540 ng/mL, AUC_{0-24} = 58,400 ng·hr/mL at 3 days), resulting in LDV AUC exposure ~8-fold higher than that in humans at the recommended LDV dose. Additionally, human equivalent dose multiples (based on body surface area conversion) for (b) (4) are estimated to be greater than 1-fold (based on (b) (4) as a photodegradant, assuming the worst case scenario of 100% photodegradation of LDV) and 190-fold (based on (b) (4) as an impurity, assuming highest exposure allowed under the current LDV drug substance specification) higher than that in humans at the recommended LDV dose. No ocular toxicity was noted in (pigmented) dogs administered up to 30 mg/kg/day for 39 weeks, at GS-5885 AUC exposure ~9-fold higher than that in humans at the recommended LDV dose. In addition, no skin reactions indicative of phototoxicity occurred in albino female hairless mice administered single oral doses of LDV of up to 300 mg/kg followed by a single exposure to solar-simulated UVR.

Reproductive and Developmental Toxicology:

The NOEL for male fertility is 100 mg/kg/day (estimated AUC_{0-24hr}~34.5 μg.h/ml for GS-5885), the highest dose tested, while the NOEL for female fertility and early embryonic development is considered to be 30 mg/kg/day (estimated AUC_{0-24hr}~12.1 μg.h/ml for GS-5885) in rats administered oral doses of LDV. In females at 100 mg/kg (estimated AUC_{0-24hr}~25.1 μg.h/ml for GS-5885), the average number of corpora lutea, implantations and viable embryos were reduced slightly and were associated with non-adverse maternal toxicity findings consisting of slight body weight loss and reduced food consumption during first one and two weeks of dosing, respectively. The rat to human GS-5885 AUC exposure multiples at the NOEL are estimated to be ~2- and 5, for females and males respectively, at the recommended LDV dose, while the exposure multiple in females at 100 mg/kg is estimated to be ~3.4. Since TK were not conducted in this study, exposures at the 30 and 100 mg/kg/day dose levels were estimated based on data obtained at these dose levels following 2-weeks of LDV administration (study #TX-256-2003). In the absence of 28-day GS-5885 exposure data in males, using this 2-week exposure data to estimate exposure multiples seem appropriate, since females and males were treated for 15 and 28 days, respectively, prior to mating, with females administered test-article through gestational day (GD) 7.

The NOEL for embryo-fetal development (EFD) toxicity is considered to be 100 mg/kg/day (AUC_{0-24hr}~18.3 & 39.2 μg.h/ml for GS-5885 at GD6 & 18, respectively), the highest dose tested, in pregnant rats administered oral doses of LDV. A small increase in total fetal and litter incidence of common skeletal variations (affecting vertebra, sternebrae and ribs) were observed at 100 mg/kg/day, which appear to be attributed to maternal toxicity (indirect effect). The NOAEL for maternal toxicity was 30 mg/kg/day, based on significantly decreased maternal body weight gain and food consumption at 100 mg/kg/day. Thus, no clear EFD or teratogenicity findings were observed in rats at GS-5885 AUC exposure ~4-fold higher than that in humans at the recommended LDV dose.

The NOAEL for maternal toxicity and NOEL for EFD toxicity is 180 mg/kg/day ($AUC_{0\text{-}24hr}$~13.1 & 20.8 μg.h/ml for GS-5885 at GD7 & 20, respectively), the highest dose tested, in pregnant rabbits administered oral doses of LDV. Thus, no EFD or teratogenicity findings were observed in rabbits at GS-5885 AUC exposure ~2.3-fold higher than that in humans at the recommended LDV dose. Maternal findings at this dose level were limited to mild stool abnormalities (few/none, liquid and/or mucoid feces). This 180 mg/kg dose level was selected based on results of a pilot study in which early death and significantly decreased body weight gain and food consumption were observed at 300 mg/kg/day, with the NOAEL for maternal toxicity determined to be 100 mg/kg/day.

The NOEL for PPND toxicity was estimated to be 100 mg/kg/day [maternal: $AUC_{0\text{-}t}$~22.2 & 37.6 μg.h/ml for GS-5885 at GD6 & LD10, respectively; F_1 rat pups: $AUC_{0\text{-}t}$~9.8 μg.h/ml for GS-5885 at PND10 (LD10)], while the NOAEL for maternal toxicity was estimated conservatively to be 30 mg/kg/day [maternal: $AUC_{0\text{-}t}$~8.6 & 11.4 μg.h/ml for GS-5885 at GD6 & LD10, respectively; F_1 rat pups: $AUC_{0\text{-}t}$~2.6 μg.h/ml for GS-5885 at PND10 (LD10)], given adverse maternal findings at 100 mg/kg/day. A trend (not statistically significant) for reduced body weight in the F_1 generation was observed, which appears to be attributed to maternal toxicity (indirect effect). At 100 mg/kg/day, one gravid female was euthanized *in extremis* on GD 18 following body weight loss, low food consumption and clinical findings. In addition, mean maternal body weight was decreased at GD 20, correlating with lower mean food consumption, but was not significantly different than the control group by LD 7. At 30 mg/kg/day, GS-5885 AUC exposure is ~1.6-fold higher than that in humans at the recommended LDV dose. No other significant LDV-related effects on pregnancy, parturition and lactation of maternal animals (F0) or on the growth, viability, development or reproductive performance of the F1 generation, or survivability of the F2 generation were noted at 100 mg/kg/day. At 100 mg/kg/day, GS-5885 AUC exposure is ~5-fold higher than that in humans at the recommended LDV dose. In addition, neonatal rats were exposed to significant amounts of LDV and related metabolites via milk consumption, with LDV AUC exposure ~25% of that observed maternally.

Genetic Toxicology and Carcinogenicity:

Ledipasvir (LDV) was not mutagenic or clastogenic as tested in the Ames assay, the *in vitro* chromosomal aberration assay in human peripheral blood lymphocytes and the *in vivo* rat micronucleus assay. In addition, mouse and rat carcinogenicity studies with LDV are in progress.

Sofosbuvir (SOF) was not mutagenic or clastogenic as tested with GS-9851 in the Ames assay, the *in vitro* chromosomal aberration assay in human peripheral blood lymphocytes and the *in vivo* mouse micronucleus assay. In addition, no significant SOF-related tumor findings were noted in male or female CD-1 mice administered SOF doses of up to 200 mg/kg/day (for ~97 weeks in males) or 600 mg/kg/day (for ~92 weeks in females) by oral gavage ($AUC_{0\text{-}24hr}$~48 & 214 μg.h/ml for GS-331007 in males and females, respectively, on Day 178), the highest doses tested, resulting in GS-331007 AUC exposure ~4- to 18-fold higher than those in humans at the recommended SOF dose. In rodents, SOF is metabolized rapidly *in vivo* ultimately producing GS-331007, which accounts for the vast majority of total circulating drug-related material in

all species (including humans). The high dose levels in this study were selected based on the MTD (>10% reduction in body weight gain in 3-month study) and did not affect survival in this study. All groups were sacrificed early since vehicle control groups reached the pre-specified minimal group survival criteria prior to 104 weeks. SOF-related findings were limited to transient reductions in body weight and a slight increase in the incidence and severity of papillary mineralization and/or necrosis in kidney in females administered 600 mg/kg/day.

No significant SOF-related tumor findings were noted in male or female Sprague Dawley rats administered SOF doses of up to 750 mg/kg/day for ~20 months (all males and high-dose females) or for ~23 months (all other female groups) by oral gavage ($AUC_{0\text{-}24hr}$~97 & 125 μg.h/ml for GS-331007 in males and females, respectively, on Day 180), the highest dose tested, resulting in GS-331007 AUC exposure ~8- to 10-fold higher than those in humans at the recommended SOF dose. The high dose level was selected based on ~1/3 lethal dose in the 7 day toxicology study w/ GS-9851 (since MTD >500 mg/kg/day in 3-month study w/ SOF). SOF administration was halted early in females administered 750 mg/kg/day due to a low number of surviving animals. All groups were sacrificed early, since control groups reached the pre-specified minimal group survival criteria prior to 104 weeks, with males and females sacrificed at ~20 and 23 months, respectively. Although not statistically significant, a trend for a SOF-related decrease in survival was noted in females only. SOF-related findings were limited to a slightly higher incidence of various clinical signs, with no evidence of heart or skeletal muscle abnormalities noted.

Table 40: Summary of Systemic Exposure Multiples for Ledipasvir Toxicology Studies

Species	Study Type/ Duration/Toxicity	Dose (mg/kg)	Exposure Multiple Based on GS-5885 AUC*
Mouse	1-month RD	300 (NOAEL)	~30 [~37(♂); ~22(♀)]
Rat	RD Ocular Phototoxicity	100 (♂ NOEL)	~8
	6-month RD	100 (NOAEL)	~8
	↓ corpora lutea/ implantations/viable embryos Fertility & EED	100 (♀) 100 (♂ NOEL) 30 (♀ NOEL)	~3.4 ~4.7 ~1.7
Rat (pregnant)	EFD	100 (NOEL)	~4 (~2.5 @ GD6 & ~5.4 @ GD18)
	↓ F_1 body weight PPND	100 30 (NOEL)	~5 ~1.6
Rabbit (pregnant)	EFD	180 (NOEL)	~2.3 (~1.8 @ GD6 & ~2.9 @ GD18)
Dog	9-month RD	30 (NOAEL)	~9 [~6(♂); ~11(♀)]

*AUC in HCV-infected human subjects given FDC (LDV & SOF): 7,290 ng.hr/ml at 90 mg QD; RD=repeat-dose toxicology study; EFD=embryo-fetal development study; PPND=pre- and post-natal development study; EED=early embryonic development; GD=gestational day; NOEL=no effect level; NOAEL=no adverse effect level

Table 41: Summary of Systemic Exposure Multiples for Sofosbuvir Toxicology Studies (modified from original Table included in NDA-204,671)

Species	Study Type/ Duration/Toxicity	Dose (mg/kg)	Exposure Multiple Based on GS-331007 AUC*	Exposure Multiple Based on GS-7977 AUC^
Mouse	Exceeded MTD 3-month RD	1000 (>MTD) 300 (>♂ MTD) 100	~24 ~7 ~2 (♂); ~7(♀)	NA
	24-Month Carcinogenicity	600 (♀ NOEL) 200 (♂ NOEL)	~18 ~4	NA

Rat	Lethality & heart toxicity 7-day RD	2000 GS-9851 (1000 GS-7977) 250 (NOAEL)	~17 (~9) 2.6	NA
	6-month RD	500 (NOAEL)	5.5	NA
	Fertility & EED	500 (NOEL)	~5	NA
	24-Month Carcinogenicity	750 (NOEL)	~8 (♂) ~10 (♀)	NA
Rat (pregnant)	EFD	500 (NOEL)	2.8 @ GD6 & 6 @ GD18	NA
	PPND	500 (NOEL)	3.3 @ GD6 & ~7 @ LD10	NA
Rabbit (pregnant)	EFD	300 (NOEL)	~7 @ GD6 & ~17 @ GD19	~2 @ GD6 & 6.6 @ GD19
Dog	GI toxicity 3-month RD	500 100 (NOAEL)	~23 ~7	~76 ~17
	GI toxicity 9-month RD	500 100 (NOAEL)	~17 ~7	NA

*AUC in HCV-infected human subjects given FDC (LDV & SOF): 12,000 ng.hr/ml at 400 mg QD; ^AUC in HCV-infected human subjects given FDC (LDV & SOF): 1,320 ng.hr/ml at 400 mg QD; NA= not applicable (either not measured or below the level of detection); RD=repeat-dose toxicology study; EFD=embryo-fetal development study; PPND=pre- and post-natal development study; EED=early embryonic development; GD=gestational day; MTD=maximal tolerated dose; NOEL=no effect level; NOAEL=no adverse effect level

12 Appendix/Attachments

12.1 Histopathology inventory (for SOF carcinogenicity studies)

Study	TX-334-2002	TX-334-2001
Species	mouse	rat
Adrenals	X	X
Aorta	X	X
Bone Marrow smear	X	X
Bone (femur)	X	X
Brain	X	X
Cecum	X	X
Cervix	X	X
Colon	X	X
Duodenum	X	X

Epididymis	X	X
Esophagus	X	X
Eye	X	X
Fallopian tube		
Gall bladder		
Gross lesions	X	X
Harderian gland		
Heart	X	X
Ileum	X	X
Injection site		
Jejunum	X	X
Kidneys	X	X
Lachrymal gland	X	X
Larynx	X	X
Liver	X	X
Lungs	X	X
Lymph nodes, cervical	X	X
Lymph nodes mandibular		
Lymph nodes, mesenteric	X	X
Lymph nodes, popliteal		
Lymph nodes, unilateral		
Mammary Gland	X	X
Nasal turbinates	X	X
Optic nerves	X	X
Ovaries	X	X
Pancreas	X	X
Parathyroid	X	X
Peripheral nerve	X	X
Peyer’s patches	X	X
Pharynx		
Pituitary	X	X
Prostate	X	X
Rectum	X	X
Salivary gland	X	X
Sciatic nerve	X	X
Seminal vesicles	X	X
Skeletal muscle	X	X
Skin	X	X
Spinal cord*	X	X

Spleen	X	X
Sternum	X	X
Stomach	X	X
Testes	X	X
Thymus	X	X
Thyroid	X	X
Tongue	X	X
Trachea	X	X
Urinary bladder	X	X
Uterus	X	X
Vagina	X	X
Zymbal gland		

X, histopathology performed; *cervical and thoracic

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/s/

CHRISTOPHER E ELLIS
07/10/2014

HANAN N GHANTOUS
07/10/2014

I concur with Dr. Ellis's conclusion, the sponsor provided sufficient nonclinical safety information on ledipasvir in support of approval for marketing in the U.S.

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: 205-834

Supporting document/s:

Supporting Document	Sponsor Submission Date	CDER Received Date
0	2/8/14	2/10/14
22	6/26/14	6/26/14

Product: Sofosbuvir/Ledipasvir Fixed-Dose Combination

Indication: treatment of chronic hepatitis C genotype 1 infection in adults

Applicant: Gilead Sciences Inc.

Review Division: DAVP

Reviewer: Mark W. Powley, Ph.D.

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

Division Director: Debra B. Birnkrant, M.D

Project Manager: Linda C. Onaga, M.P.H.

Disclaimer

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1 Executive Summary

Gilead Sciences Inc. has submitted an NDA to support the fixed dose combination therapy of ledipasvir (HCV NS5A inhibitor) and sofosbuvir (approved HCV N25B polymerase inhibitor) to treat chronic hepatitis C genotype 1 infection in adults. The proposed clinical dose regimen includes 90 mg/day ledipasvir and 400 mg/day sofosbuvir [redacted]

This review focuses on qualification of ledipasvir impurities, residual solvents, metals, and degradants. Proposed sofosbuvir specifications are not covered as these are consistent with the previously reviewed NDA (NDA#204-671). Regulatory decision making utilizes information presented in ICH guidelines M7 (draft) "Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk", Q3C(R5) "Impurities: Guideline for Residual Solvents", and Q3D (draft) "Guideline for Elemental Impurities".

Overall, the proposed ledipasvir specifications summarized below are considered acceptable from a pharmacology/toxicology perspective (tables taken or constructed from the Sponsor submission).

Table 1. Proposed ledipasvir drug substance impurity specifications

NMT	[redacted] of total impurities, with
NMT	[redacted] % of [redacted]
NMT	[redacted] % of the sum of [redacted]
NMT	[redacted] % each of [redacted]
NMT	[redacted] % each of [redacted] and
NMT	[redacted] % each of any individual unspecified impurity

Table 2. Proposed ledipasvir drug substance residual solvent specifications

NMT	[redacted] % of [redacted]
NMT	[redacted] % of [redacted]
NMT	[redacted] % of [redacted]
NMT	[redacted] % each of [redacted]
NMT	[redacted] % of [redacted] and
NMT	[redacted] % or the PDE (per ICH Q3C) of any unspecified residual solvent, whichever is lower

Table 3. Proposed ledipasvir drug substance metal specifications

NMT	[redacted] ppm [redacted] and
NMT	[redacted] ppm each of [redacted] and
NMT	[redacted] ppm [redacted]

Table 4. Proposed ledipasvir drug product degradant specifications

At Release:	A total of NMT (b) (4)% of ledipasvir-related degradation products with NMT (b) (4)% of (b) (4) and NMT [redacted]% each of any ledipasvir-related unspecified degradation product.
During Shelf Life:	A total of NMT (b) (4)% ledipasvir-related degradation products with NMT (b) (4)% of (b) (4) and NMT [redacted]% each of any ledipasvir-related unspecified degradation product

2 Qualification of Ledipasvir Drug Substance

2.1 Impurities

2.1.1 Specified Impurities

The qualification of specified impurities in the drug substance is based on results from general toxicology studies, assessment of potential mutagenicity using (quantitative) structure-activity relationship [(Q)SAR] models, and/or phototoxicity evaluation.

General Toxicology – Specified impurities were present in the drug lots tested in 2-week studies in male rats (TX-256-2014 and TX-256-2035: see detailed pharmacology/toxicology review by Dr. Chris Ellis). Using the NOAELs established in these general toxicology studies, qualified levels of the impurities summarized in Table 5 (table taken from the Sponsor submission) are deemed adequate to support the proposed specifications.

Table 5. Summary of ledipasvir specified impurity general toxicology qualification

Impurity	Maximum Observed in Toxicological Studies (%)	NOAEL from Study (mg/kg/day)	Qualified Level (%)	Batch Number	Toxicology Study Number
(b) (4	(b) (4)	100	(b) (4)	5885-02-B-1	TX-256-2014
		100		5763-30-25	TX-256-2035
		100		5763-30-25	TX-256-2035
		100		5763-30-25	TX-256-2035
		100		5763-30-25	TX-256-2035
		100		5763-30-25	TX-256-2035
		100		5763-30-25	TX-256-2035
		100		5763-30-25	TX-256-2035

TX-256-2014 and TX-256-2035 were conducted in rats

(b) (4)

Note - qualified level = (% impurity x NOAEL) / (body surface area conversion factor x maximum clinical dose)

Mutagenicity – The specified ledipasvir stereoisomers (i.e., (b) (4)) are qualified by the Ames negative API. The remaining specified impurities were evaluated for potential mutagenicity using (Q)SAR. The Sponsor's (Q)SAR assessments rely on predictions from Derek Nexus (DX) and Leadscope Model Applier (LMA). Additional assessments by the CDER Computational Toxicology Consultation Service include predictions from DX, LMA, and CASE Ultra (CU). These assessments are consistent with regulatory recommendations provided in the ICH M7 draft guideline. Overall, the impurities are deemed to lack mutagenic potential. Results from the (Q)SAR evaluations are provided in the Appendix.

Phototoxicity – Ledipasvir spiked with (b) (4) ((b) (4) was tested in a phototoxicity study in pigmented rats (TX-256-2038: see detailed pharmacology/toxicology review by Dr. Chris Ellis). Based on an NOAEL of 100 mg/kg/day of spiked ledipasvir and the maximum clinical dose of 90 mg, (b) (4) is qualified for phototoxicity to a level of (b) (4) %. The proposed specification is, therefore, acceptable.

2.1.2 Unspecified Impurities

(Q)SAR was also used to evaluate the mutagenic potential of impurities unspecified in the drug substance. The Sponsor describes the structures selected for evaluation as "… all starting materials, intermediates, likely process byproducts, and impurities specified in the starting materials, intermediates, or ledipasvir. The compounds screened were chosen to provide coverage of all major structural features present in the intermediates and specified impurities."

Although the Sponsor's (Q)SAR analysis utilizes 2 methodologies, LMA predictions are not provided for several impurities as the structures fell outside of the model's domain of applicability. In these cases, the CDER analysis is used to support a final conclusion. Results from the (Q)SAR evaluations are provided in the Appendix.

The (Q)SAR analysis identified structural alerts for (b) (4) The known mutagenic and/or carcinogenic chemicals (b) (4) are also reported by the Sponsor. In addition, the presence of a structural alert in (b) (4) is mentioned but is not described in the Sponsor's (Q)SAR evaluation. Each of these potentially or known reactive impurities is effectively purged (see detailed CMC review by Dr. George Lunn) and does not pose a substantial risk.

2.2 Residual Solvents

Appropriate limits for the residual solvents are summarized in Table 6. With the exception of (b) (4) (b) (4), recommended limits for all specified residual solvents are provided in ICH Q3C(R5). (b) (4)

Table 6. Summary of ledipasvir residual solvent qualification

Solvent	ICH Q3C(R5) [redacted]	Qualified Level	Proposed Specification
[redacted]	5000 ppm	[redacted]	[redacted]
	5000 ppm		
	5000 ppm		
	880 ppm		
	600 ppm		
	410 ppm		
	N/A		

[a] *risk assessment provided below*

According to the ICH Q3C(R5) [redacted] *are acceptable.* [redacted] *The Sponsor submission describes efforts to reduce the levels of these solvent; therefore, the proposed specifications are acceptable.*

[redacted] – *The Sponsor calculated a permissible daily exposure (PDE) of* [redacted] *using the LD_{50} data for rats. A more appropriate source of data comes from an oral developmental toxicity study in rats* [redacted] *In this study, female Wistar rats were administered oral doses of 100, 200, 600, and 900 mg/kg/day of* [redacted] *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 =* [redacted] *mg/kg/day. Additional published data suggests the chemical is not mutagenic in TA98 or TA100* [redacted].

The NOAEL supports a PDE = [redacted] *mg/day (calculation below) or* [redacted] *% of the maximum recommended clinical dose of ledipasvir. Based on both the LD_{50} and developmental toxicity studies, the proposed specification for* [redacted] *is acceptable.*

PDE = NOAEL x [redacted] kg (body weight) / (F1 x F2 x F3 x F4 x F5)

NOAEL = [redacted] mg/kg/day
F1 = 5 (extrapolation from rats to humans)
F2 = 10 (account for variability between individuals)
F3 = 1 (entire period of organogenesis)
F4 = 10 (severe toxicity)
F5 = 1 (NOAEL used)

= [redacted] / (5 x 10 x 1 x 10 x 1)
= [redacted] mg/day

References

(b) (4)

2.3 Metals

Appropriate limits for the metals are summarized in Table 7. Based on comparison with recommended limits described in the draft ICH Q3D guideline, all proposed specifications are considered acceptable.

Table 7. Summary of ledipasvir metal qualification

Metal	ICH Q3D Limit	Qualified Level[a]	Proposed Specification
(b) (4)	100 µg/day 5 µg/day 5 µg/day 40 µg/day 15 µg/day		(b) (4)

calculation based on maximum recommended ledipasvir dose of 90 mg

3 Qualification of Ledipasvir Drug Product

The qualification of (b) (4) *is based on results from a general toxicology study, (Q)SAR evaluation for mutagenicity, and phototoxicity. Detailed results of these analyses are provided in section 2 above. Overall, the data support the proposed specification.*

4 Qualification of Sofosbuvir Drug Substance and Product

Proposed specifications for sofosbuvir impurities, residual solvents, metals, and degradants are consistent with those described in the approved NDA.

5 Qualification of (b) (4)

There is the potential for exposure to (b) (4) *of* (b) (4) *if ledipasvir is completely metabolized. Because of this possibility, pharmacology/toxicology was asked to provide a risk assessment.*

According to the Agency for Toxic Substances and Disease Registry (ATSDR, 2003), there is extensive human data derived from exposure to (b) (4)*. The endpoint most commonly evaluated in these studies is hip fractures. The data used to support the ATSDR risk assessment comes from a study reported by* (b) (4)*). These authors evaluated the incidence of hip fractures in Chinese adults (i.e., ages ≥ 50) exposed to a range of* (b) (4) *concentrations in* (b) (4)*. The NOAEL established in this study was* (b) (4)*. By including a 3x safety factor to account for human variability, the ATSDR calculated a chronic-duration oral minimal risk level (MRL; estimate of human exposure likely to be without appreciable risk of*

adverse non-cancer effects) of (b) (4) *Note that while the potential exposure to* (b) (4) *from ledipasvir slightly exceeds the ATSDR chronic limit, the maximum duration of ledipasvir administration in the clinic is 12 weeks. Based on the totality of information,* (b) (4) *potentially liberated from ledipasvir does not pose a substantial risk at the levels and dosing duration described.*

Reference

(b) (4)

Appendix

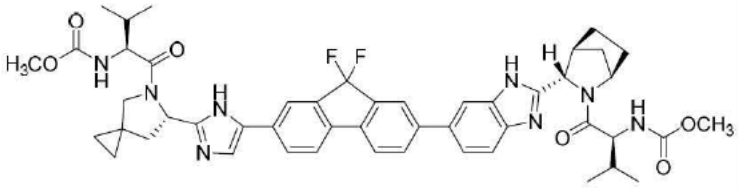
(Q)SAR Evaluation

Evaluation of mutagenic potential was performed by both Gilead and the CDER Computational Toxicology Consultation Service. The Sponsor provided predictions using Derek Nexus (DX; v3.0.1) and Leadscope Model Applier (LMA; v.1.7.4). The CDER analysis included predictions from DX (v.4.0.5), LMA (v.1.8.3-1), and CASE Ultra (CU; v1.4.6.6). Structures evaluated by CDER were limited to 1) specified impurities not evaluated by the Sponsor and 2) impurities reported by the Sponsor as "not in domain" for LMA. The overall (Q)SAR assessment is consistent with recommendations in the draft ICH M7 guideline (e.g., 2 prediction methodologies).

The Sponsor's (Q)SAR analysis identified structural alerts for (b) (4) *No additional potentially mutagenic impurities were identified by either the Sponsor or CDER evaluation.*

Detailed results of the evaluations are included in the following table (table taken from the Sponsor submission) and report for the CDER evaluation.

Table 8. Summary of (Q)SAR data

Material Name	Structure	Derek Screening Result	Leadscope Screening Prediction
Ledipasvir (GS-5885)		No Structural Alerts	Negative
[redacted] (b) (4)		No Structural Alerts	Not in Domain[a]
		No Structural Alerts	Not in Domain[a]
		No Structural Alerts	Negative
		Derek Structural Alert	Not in Domain[a]
		No Structural Alerts	Not in Domain[a]
		No Structural Alerts	Not in Domain[a]

a The compound was not within the domain of applicability for the model and no prediction could be calculated.

Material Name	Structure	Derek Screening Result	Leadscope Screening Prediction
(b) (4)		No Structural Alerts	Not in Domain[a]
		Derek Structural Alert	Positive
		No Structural Alerts	Not in Domain[a]
		No Structural Alerts	Not in Domain[a]
		Derek Structural Alert	Positive

a The compound was not within the domain of applicability for the model and no prediction could be calculated.

Material Name	Structure	Derek Screening Result	Leadscope Screening Prediction
(b) (4)		No Structural Alerts	Negative
		No Structural Alerts	Negative
		No Structural Alerts	Not in Domain[a]
		No Structural Alerts	Not in Domain[a]
		No Structural Alerts	Negative
		No Structural Alerts	Not in Domain[a]

a The compound was not within the domain of applicability for the model and no prediction could be calculated.

To: **Mark Powley**
cc: **Hanan Ghantous**
From: **CDER/OTS/OCP/DARS: The Chemical Informatics Group**
Re: **NDA 205834**
Date: **June 18, 2014**

Ledipasvir and 12 impurities have been evaluated by CDER/OTS/OCP/DARS for bacterial mutagenicity using (quantitative) structure-activity relationship [(Q)SAR] models. Three software programs were used: *Derek Nexus* 4.0.5 (*DX*), *Leadscope Model Applier* 1.8.3-1 (*LMA*), and *CASE Ultra* 1.4.6.6 (*CU*). To maximize sensitivity and negative predictivity, a positive prediction from any one software program was used to justify a positive overall software prediction.

The (Q)SAR assessment of mutagenic potential for the impurities is consistent with recommendations described in the draft ICH M7 guideline (e.g., prediction of bacterial mutagenicity using multiple complementary methodologies). The API, **Ledipasvir,** is included in the report for comparison purposes.

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1. Ledipasvir (GS-5885)

Bacterial Mutagenicity[1]

Software	*Salmonella* Mutagenicity	*E. coli* Mutagenicity
Derek Nexus	–	–
Leadscope Model Applier	–	–
CASE Ultra	Eqv	–
Overall Software Prediction	–	–
Overall Expert Prediction	–	–

Ledipasvir is known to be negative for bacterial mutagenicity (i.e., both *Salmonella* and *E. coli* mutagenicity). The structure is predicted to be negative for *Salmonella* and *E. coli* mutagenicity by both *DX* and *LMA*. (b) (4)

(b) (4)

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[1] + = positive; – = negative; Eqv = equivocal; NC = test chemical features are not adequately represented in the model training data set, leading to a no call.

2. Impurities (b) (4)

Bacterial Mutagenicity[1]

Software	*Salmonella* Mutagenicity	*E. coli* Mutagenicity
Derek Nexus	–	–
Leadscope Model Applier	–	–
CASE Ultra	Eqv	–
Overall Software Prediction	–	–
Overall Expert Prediction	–	–

Impurities (b) (4) are predicted to be negative for *Salmonella* and *E. coli* mutagenicity by *DX* and *LMA*. (b) (4)

(b) (4)

3. Impurities (b) (4)

Bacterial Mutagenicity[1]

Software	*Salmonella* Mutagenicity	*E. coli* Mutagenicity
Derek Nexus	–	–
Leadscope Model Applier	NC	NC
CASE Ultra	Eqv	–
Overall Software Prediction	–	–
Overall Expert Prediction	–	–

Impurities (b) (4) are predicted to be negative for *Salmonella* mutagenicity by *DX* and negative for *E. coli* mutagenicity by both *DX* and *CU*. *LMA* did not provide predictions due to a lack of coverage. (b) (4)

(b) (4)

4. Impurities (b) (4)

Bacterial Mutagenicity[1]

Software	*Salmonella* Mutagenicity	*E. coli* Mutagenicity
Derek Nexus	–	–
Leadscope Model Applier	NC	NC
CASE Ultra	+	–
Overall Software Prediction	+	–
Overall Expert Prediction	–	–

Impurities (b) (4) are predicted to be negative for *Salmonella* mutagenicity by *DX* and negative for *E. coli* mutagenicity by both *DX* and *CU*. *LMA* did not provide predictions due to a lack of coverage.

(b) (4)

(b) (4)

5. Impurities (b) (4)

Bacterial Mutagenicity[1]

Software	*Salmonella* Mutagenicity	*E. coli* Mutagenicity
Derek Nexus	–	–
Leadscope Model Applier	NC	NC
CASE Ultra	Eqv	–
Overall Software Prediction	–	–
Overall Expert Prediction	–	–

Impurities (b) (4) are predicted to be negative for *Salmonella* mutagenicity by *DX* and negative for *E. coli* mutagenicity by both *DX* and *CU*. *LMA* did not provide predictions due to a lack of coverage. (b) (4)

(b) (4)

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6. Impurity (b) (4)

Bacterial Mutagenicity[1]

Software	*Salmonella* Mutagenicity	*E. coli* Mutagenicity
Derek Nexus	–	–
Leadscope Model Applier	NC	NC
CASE Ultra	Eqv	+
Overall Software Prediction	–	+
Overall Expert Prediction	–	–

Impurity (b) (4) is predicted to be negative for *Salmonella* and *E. coli* mutagenicity by *DX*. *LMA* did not provide predictions due to a lack of coverage. (b) (4)

(b) (4)

(b) (4)

7. **Impurity** (b) (4)

Bacterial Mutagenicity[1]

Software	*Salmonella* Mutagenicity	*E. coli* Mutagenicity
Derek Nexus	–	–
Leadscope Model Applier	NC	NC
CASE Ultra	–	–
Overall Software Prediction	–	–
Overall Expert Prediction	–	–

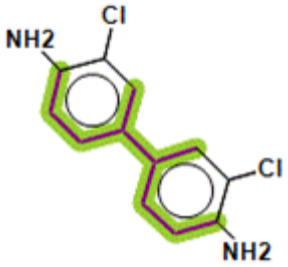
Impurity (b) (4) is predicted to be negative for *Salmonella* and *E. coli* mutagenicity by DX and CU. *LMA* did not provide predictions due to a lack of coverage.

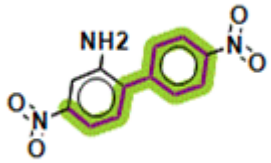
(b) (4)

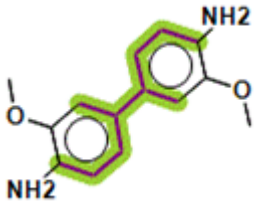
This report has been reviewed and approved by CDER/OTS/OCP/DARS.

Appendix

Ames positive training set structures for *E. coli* alert 152.







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/s/

MARK W POWLEY
07/04/2014

HANAN N GHANTOUS
07/07/2014

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 205,834 **Applicant:** Gilead Sciences **Stamp Date:** February 10, 2014

Drug Name: Ledipasvir (LDV, GS-5885) & Sofosbuvir (SOF, GS-7977) **NDA Type:** Original

On **initial** overview of the NDA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		In eCTD format
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		Carcinogenicity studies for SOF in rats and mice were submitted (also submitted as PMRs to NDA-204,671). Carcinogenicity studies for LDV in rats and mice are in progress. As agreed upon by the Agency, the Sponsor plans to submit study results post-approval.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		Further nonclinical assessment of ocular phototoxicity risk was requested in an IR letter sent on March 7th.

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

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		Appropriateness of content will be determined upon review and discussed at labeling meetings.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		Whether sponsor has addressed issues adequately is a review issue.
11	Has the applicant addressed any abuse potential issues in the submission?		X	Not applicable
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?		X	Not applicable

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ___Yes___

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Not applicable

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Not applicable

Christopher Ellis, Ph.D.	March 11, 2014
Reviewing Pharmacologist	Date
Hanan Ghantous, Ph.D. DABT	March 11, 2014
Team Leader/Supervisor	Date

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

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/s/

CHRISTOPHER E ELLIS
03/12/2014

HANAN N GHANTOUS
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