

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

205834Orig1s000

**CLINICAL PHARMACOLOGY AND
BIOPHARMACEUTICS REVIEW(S)**

OFFICE OF CLINICAL PHARMACOLOGY REVIEW

NDA: 205834	Submission Date(s): February 10, 2014
Brand Name	To be Determined
Generic Name	Ledipasvir/Sofosbuvir
Clinical Pharmacology Reviewers	Jenny H. Zheng, Ph.D. (primary), Leslie Chinn, Ph.D.
Secondary Reviewer	Shirley K. Seo, Ph.D.
PM Reviewer	Jeffry Florian, Ph.D.
Secondary PM Reviewer	Yaning Wang, Ph.D.
OCP Division	Division 4
OND division	DAVP
Applicant	Gilead Sciences
Relevant IND(s) and NDA(s)	INDs 106739, 112681, (b) (4) 115268 and NDA 204671
Submission Type	Priority
Formulation; Strength(s)	Fixed dose combination tablets; 90 mg/400 mg
Indication	Treatment of chronic hepatitis C (CHC) genotype 1 infection in adults

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LIST OF ABBREVIATIONS and NOMENCLATURES

[¹⁴ C]	radiolabeled carbon 14
3TC	lamivudine
ABC	abacavir
ADME	absorption, distribution, metabolism, and excretion
AE	adverse event
ARV	antiretroviral
ATR	Atripla® (efavirenz/emtricitabine/tenofovir disoproxil fumarate, coformulated)
ATV	atazanavir
AUC _{inf}	area under the plasma/serum/PBMC concentration versus time curve from time zero to infinity
AUC _{tau}	area under the plasma/serum/PBMC concentration versus time curve over the dosing interval
BA	bioavailability
BCRP	breast cancer resistance protein
BCS	Biopharmaceutics Classification System
BID	twice daily
BMI	body mass index
BOC	boceprevir
BSEP	bile salt export pump

CatA	cathepsin A
CES1	carboxyl esterase 1
CI	confidence interval
CL/F	apparent oral clearance
CLcr	creatinine clearance
C _{max}	maximum observed plasma/serum/PBMC concentration of drug
COBI	cobicistat
CPA	Complera® (emtricitabine/rilpivirine/tenofovir disoproxil fumarate, coformulated)
CPT	Child-Pugh-Turcotte classification
CsA	cyclosporine (cyclosporin A)
C _{tau}	observed drug concentration at the end of the dosing interval
CV	coefficient of variation
CYP	cytochrome P450 enzyme(s)
d4T	stavudine
DAA	direct-acting antiviral
DCV	daclatasvir
DDI	drug-drug interaction
DRV	darunavir
EC _{50/90}	half-maximal/90% effective concentration
ECG	electrocardiogram
EE	ethinyl estradiol
EFV	efavirenz
eGFR	estimated glomerular filtration rate
E _{max}	maximum effect
ESRD	end-stage renal disease
EU	European Union
EVG	elvitegravir
FDC	fixed-dose combination
FMO	flavin monooxygenase
FTC	emtricitabine
GLSM	geometric least-squares mean
GMR	geometric mean ratio
GT	genotype
H2RA	H ₂ -receptor antagonist
HCV	hepatitis C virus
HINT1	histidine triad nucleotide binding protein 1
HIV, HIV-1	human immunodeficiency virus, type 1
IC ₅₀	half-maximal inhibitory concentration
IFN	interferon
IL28	interleukin 28
IL28B	interleukin 28B gene
LC/MS/MS	liquid chromatography/tandem mass spectrometry
LDV	ledipasvir
LLOQ	lower limit of quantitation
LOD	limit of detection
MATE1	multidrug and toxin extrusion protein 1
mRNA	messenger ribonucleic acid
MRP2	multidrug resistance-associate protein 2
N or n	number of subjects in a population (N) or subset (n)
NA	not applicable
ND	not determined
NG	norgestrel
NGMN	norelgestromin
NI	nucleoside inhibitor

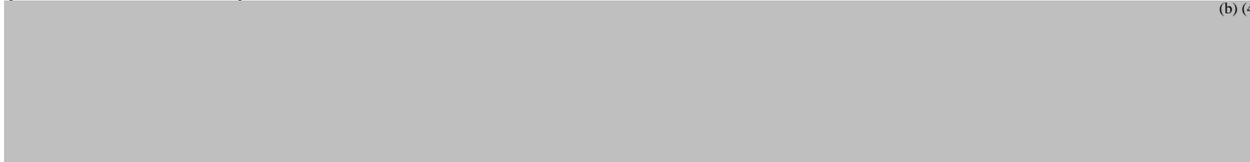
NNI	nonnucleoside inhibitor
NOAEL	no observed adverse effect level
NOEL	no observed effect level
OAT	organic anion transporter
OATP	organic anion transporting polypeptide
OC	Ortho Tri-Cyclen Lo®
OCT	organic cation transporter
PD	pharmacodynamic(s)
Peg-IFN	pegylated interferon
P-gp	p-glycoprotein
PI	protease inhibitor
PK	pharmacokinetic(s)
PPI	proton pump inhibitor
Q1, Q2, Q3, Q4, Q12	first quartile, second quartile, third quartile, fourth quartile, first 2 quartiles
QD	once daily
QT	electrocardiographic interval between the beginning of the Q wave and termination of the T wave, representing the time for both ventricular depolarization and repolarization to occur
$\Delta\Delta\text{QTc}$	time-matched, baseline-adjusted, placebo-corrected QTc
QTc	QT interval corrected for heart rate
QTcB	QT interval corrected for heart rate using the Bazett formula
QTcF	QT interval corrected for heart rate using the Fridericia formula
QTcl	QT interval corrected for heart rate using subject-specific correction factor
QTcN	QT interval corrected for heart rate using population-specific correction factor
RAL	raltegravir
/r	boosted with ritonavir
RAV	resistance-associated variant
RBV	ribavirin
RGT	response-guided therapy
RIF	rifampin
RNA	ribonucleic acid
RPV	rilpivirine
RTV	ritonavir
SAE	serious adverse event
SD	standard deviation
(b) (4)	(b) (4)
SMV	simeprevir
SOF	sofosbuvir
SVR	sustained virologic response
SVRXX	sustained virologic response "XX" weeks following completion of all treatment
TAC	tacrolimus
TDF	tenofovir disoproxil fumarate
TE	treatment experienced
TFV	tenofovir
TGV	tegobuvir
TN	treatment naive
TVD	Truvada® (emtricitabine/tenofovir disoproxil fumarate, coformulated)
TVR	telaprevir
UGT	uridine diphosphate glucuronosyltransferase
ULN	upper limit of the normal range
UMP-CMP	uridine monophosphate-cytidine monophosphate
VDV	vedoprevir
vRVR	very rapid virologic response
ZDV	zidovudine

1. EXECUTIVE SUMMARY

Gilead Sciences is seeking approval of ledipasvir (LDV, GS-5885) and sofosbuvir (SOF, GS-7977) together as an oral fixed-dose combination (FDC) tablet (LDV/SOF 90 mg/400 mg) for the treatment of chronic genotype 1 hepatitis C virus (HCV) infection (for both genotypes 1a and 1b as efficacy was similar between the subgenotypes). LDV/SOF was granted Breakthrough Therapy Designation on July 22, 2013.

LDV is a novel HCV NS5A inhibitor that has demonstrated potent anti-HCV activity against genotype 1a and 1b HCV infection. SOF is a novel nucleotide NS5B polymerase inhibitor that inhibits HCV RNA replication and has been approved for use in combination with other agents for the treatment of chronic HCV infection in adults (tradename Sovaldi®; NDA 204671).

The proposed LDV/SOF dosage regimen is one 90 mg/400 mg tablet, taken orally, once daily with or without food. The following treatment durations are proposed by the applicant, based on prior treatment experience and cirrhosis status:



The consideration for approval of this NDA is based on efficacy data from 3 pivotal LDV/SOF Phase 3 trials in a total of 1518 subjects with genotype 1 chronic hepatitis C (CHC): 0108 (ION-3, HCV treatment-naïve adults without cirrhosis), 0102 (ION-1, treatment-naïve adults with or without cirrhosis), and 0109 (ION-2, adults who failed prior therapy with or without cirrhosis); as well as pooled safety data from these three Phase 3 clinical trials and other trials which evaluated use of LDV/SOF or LDV/SOF + ribavirin (RBV) for 8, 12, and 24 weeks. The final treatment duration recommendation for each patient population remains under review at this time.

1.1 Recommendation

The Office of Clinical Pharmacology has determined that there is sufficient clinical pharmacology information provided in the NDA to support a recommendation of approval of LDV/SOF.

1.2 Phase IV Commitments

None.

1.3 Summary of Important Clinical Pharmacology Findings

A comprehensive program of Phase 1 clinical studies characterized the PK of SOF and LDV when administered either as single agents or as the FDC. Additionally, both intensive and sparse plasma concentration data from 391 healthy subjects and 2147 HCV-infected subjects who received LDV/SOF FDC, SOF and LDV administered together as single agents, or LDV as a single agent from 14 clinical studies (9 Phase 1, 2 Phase 2, and 3 Phase 3 studies) were used for population PK evaluation of SOF, its predominant circulating metabolite GS-331007, and LDV.

Key PK characteristics for LDV as a single agent are summarized below:

- A high-fat meal reduced LDV AUC and C_{max} by approximately 45%.
- LDV is >99.8% bound to human plasma proteins.
- The half-life of LDV is approximately 47 hours
- Following a single 90 mg oral dose of [¹⁴C]-LDV, mean total recovery of the [¹⁴C]-radioactivity in feces and urine was approximately 87%, with most of the radioactive dose recovered from feces (approximately 86%). Unchanged LDV excreted in feces accounted for a mean of 70% of the administered dose and the oxidative metabolite M19 accounted for 2.2% of the dose. The data indicate that biliary excretion of unchanged LDV is a major route of elimination with renal excretion being a minor pathway.
- LDV exhibits dose linearity for AUC_{inf} and C_{max} over the 3- to 100-mg range.
- LDV PK is not affected by race (as determined by both popPK analysis of phase 3 data as well as one dedicated phase 1 study in Japanese subjects) or age (18-80 years)
- Relative to healthy subjects, LDV AUC₀₋₂₄ and C_{max} were 24% lower and 32% lower, respectively, in HCV patients.
- AUC and C_{max} of LDV were 77% and 58% higher, respectively, in females than males. After correcting for body weight differences between genders, females still have approximately 40% higher exposure as compared to males. This observation has no clinical relevance because neither response rate nor rate or severity of adverse events was significantly different between genders.
- Renal impairment has no clinically significant effect on LDV PK and no dose adjustment of LDV is needed. The effect of hemodialysis on LDV PK was not evaluated; however, due to the high protein binding of LDV, hemodialysis is unlikely to have a significant impact.
- Hepatic impairment (including cirrhosis) has no clinically significant effect on LDV PK, and no dose adjustment of LDV is needed for any degree of hepatic impairment.
- LDV solubility decreases as pH increases. Drugs that increase gastric pH are expected to decrease systemic concentrations of LDV.
 - A substantial decrease in LDV plasma exposure (~ 42% to 48% lower AUC and C_{max}) was observed upon administration of the proton pump inhibitor (PPI) omeprazole (20 mg) 2 hours prior to LDV administration.
 - LDV absorption was unaffected upon simultaneous or staggered (12 hours) administration of the H₂-receptor antagonist (H₂RA) famotidine (20 mg).
- LDV is a substrate of the drug transporters P-gp and BCRP.
- LDV is an inhibitor of P-gp and BCRP.
- LDV is not expected to inhibit OATP1B1, OATP1B3 and BSEP at concentrations achieved in vivo at the recommended dose
- LDV 30 mg once daily increased SMV C_{max} and AUC by 161% and 169%, respectively, due to P-gp inhibition, which is a similar magnitude of the effect of DRV/RTV on simeprevir (SMV) and DRV/RTV is not recommended to be coadministered with simeprevir. Thus, SMV is not recommended to be coadministered with LDV/SOF.
- At the supratherapeutic dose of 120 mg twice daily, LDV does not prolong QTc to a clinically relevant extent.

SOF is a nucleotide prodrug that undergoes intracellular metabolism to form the pharmacologically active uridine analog triphosphate (GS-461203). GS-461203 is not measurable in plasma. The PK characteristics for SOF and its major metabolite GS-331007 following oral administration of SOF as a single agent are summarized below:

- When SOF was administered as a single agent, a high-fat meal slowed the rate of absorption of SOF but did not substantially affect the extent of absorption as compared to

fasting conditions. The exposure of GS-331007 was not altered in the presence of a high-fat meal. Protein binding is about 65% for SOF, minimal for GS-331007.

- The half-life is approximately 0.4 hr for SOF, and 27 hrs for GS-331007.
- Following a single 400 mg oral dose of [¹⁴C]-sofosbuvir, mean total recovery of the dose was greater than 92%, consisting of approximately 80%, 14%, and 2.5% recovered in urine, feces, and expired air, respectively. The majority of the sofosbuvir dose recovered in urine was GS-331007 (78%) while 3.5% was recovered as sofosbuvir. These data indicate that Renal clearance is the major elimination pathway for GS-331007.
- Sofosbuvir and GS-331007 AUCs are near dose proportional over the dose range of 200 mg to 1200 mg.
- SOF and GS-331007 PK are not affected by gender, race, disease state, or age.
- For renal impairment, the sofosbuvir AUC_{0-inf} was 61%, 107% and 171% higher in mild, moderate and severe renal impairment, while the GS-331007 AUC_{0-inf} was 55%, 88% and 451% higher, respectively. No dose adjustment is required for patients with mild or moderate renal impairment. Use of SOF is currently not recommended for use in patients with severe renal impairment or ESRD.
- For hepatic impairment (including cirrhosis): no clinically significant effect was observed. Therefore, no dose adjustment of SOF is recommended for any degree of hepatic impairment.
- SOF is a substrate of drug transporters P-gp and BCRP while GS-331007 is not.
- Drugs that are intestinal P-gp inducers (e.g., rifampin (RIF), St. John's Wort) may alter the concentrations of SOF and thus should not be used with SOF.
- The interaction between SOF and the following drugs was evaluated in clinical trials and no dose adjustment is needed for either drug: cyclosporine (CsA), darunavir/ritonavir (DRV/RTV), efavirenz (EFV), emtricitabine (FTC), methadone, raltegravir (RAL), rilpivirine (RPV), tacrolimus (TAC), tenofovir disoproxil fumarate (TDF), or oral contraceptive agents containing ethinyl estradiol (EE) and norgestimate.
- At a dose three times the maximum recommended dose, sofosbuvir does not prolong QTc to a clinically relevant extent
- LDV causes a 2.3- and 2.2-fold increase in SOF AUC_{inf} and C_{max} respectively, due to P-gp and BCRP inhibition by LDV. Therefore, the results of SOF PK as a single agent should be carefully interpreted in the context of the increased SOF concentrations in LDV/SOF FDC as compared to SOF as a single dose.

Key PK characteristics for SOF, GS-331007 and LDV following oral administration of LDV/SOF FDC are summarized below:

- When administered as the LDV/SOF FDC, a high-fat meal caused a similar magnitude of effect on SOF and GS-331007 as compared to the single agent and also did not affect LDV AUC and C_{max} to a clinically relevant extent. Thus, LDV/SOF FDC can be administered without regard to food (as instructed in phase 3 studies).
- LDV/SOF FDC tablets have been studied with: abacavir/lamivudine (ABC/3TC), Atripla® (ATR), Complera® (CPA), atazanavir/ritonavir (ATV/RTV), DRV/RTV, elvitegravir+cobicistat (EVG+COBI), and acid-reducing agents. The following are the results from these studies:
 - No clinically significant effects on SOF and GS-331007 exposures were observed with any of the above agents, which is similar to what was observed for SOF as a single agent
 - The effects of these drugs on LDV AUC or C_{max} is in the range of 34%↓ by ATR to ~100%↑ by ATV/RTV
 - Famotidine (40 mg single dose) was administered either with simultaneous administration or 12 hours apart with LDV/SOF, while omeprazole (20 mg) was

studied only with simultaneous administration with LDV/SOF. Since the onset of the antisecretory effect of H2RAs occurs within 1 hour, while the onset of antisecretory effect of PPIs may be delayed and prolonged, staggered administration of LDV/SOF with H2RAs or PPIs may result in lower LDV concentrations. As shown in Study GS-US-256-0110, a substantial decrease in LDV plasma exposure (~42% to 48% lower AUC and Cmax) was observed upon administration of omeprazole 2 hours earlier than LDV as a single agent. Therefore, H2RAs should only be administered simultaneously or 12 hours apart with LDV/SOF, and PPIs should only be administered simultaneously with LDV/SOF at doses comparable to omeprazole 20 mg once daily (or lower).

2. QUESTION BASED REVIEW

2.1 General Attributes

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

SOF is a nucleotide prodrug of 2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate that is converted to the active uridine triphosphate form (GS-461203) within the hepatocyte and is a HCV NS5B-directed inhibitor that has displayed potent inhibition of HCV replicon RNA replication in vitro. Please refer to Clinical Pharmacology Review for NDA 204671 for additional details on the physical-chemical properties of the SOF drug substance.

Ledipasvir (b) (4) is defined as the drug substance. (b) (4)

(b) (4) The following shows physical-chemical properties of the drug substance (b) (4)

Chemical Name: (b) (4)

Empirical Formula: (b) (4)

Molecular Weight: (b) (4) (889 for LDV)

Chemical Structure:

(b) (4)

pKa: (b) (4)

Partition Coefficient: log P = (b) (4)

Solubility: [REDACTED] (b) (4)

LDV/SOF fixed-dose combination tablets contain 400 mg of sofosbuvir and 90 mg of LDV. The tablet formulation utilizes LDV [REDACTED] (b) (4) The quantitative composition of LDV/SOF tablets is listed in Table 1.

Table 1: Quantitative Composition of Ledipasvir/Sofosbuvir Tablets

Component	Composition (% w/w)	Unit Formula (mg/tablet)	Quality Standard	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)	(b) (4)	JSP, Ph. Eur.	(b) (4)
(b) (4)	(b) (4)	(b) (4)	JSP, Ph. Eur.	(b) (4)
Lactose Monohydrate	(b) (4)	(b) (4)	NF, Ph. Eur.	(b) (4)
Microcrystalline Cellulose	(b) (4)	(b) (4)	NF, Ph. Eur.	(b) (4)
Croscarmellose Sodium	(b) (4)	(b) (4)	NF, Ph. Eur.	(b) (4)
Colloidal Silicon Dioxide	(b) (4)	(b) (4)	NF, Ph. Eur.	(b) (4)
Magnesium Stearate	(b) (4)	(b) (4)	NF, Ph. Eur.	(b) (4)
(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)	NF, Ph. Eur.	(b) (4)
(b) (4)	(b) (4)	(b) (4)	NF, Ph. Eur.	(b) (4)
(b) (4)	(b) (4)	(b) (4)	NF, Ph. Eur.	(b) (4)
(b) (4)	(b) (4)	(b) (4)	-	(b) (4)
(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)	In-house	(b) (4)
(b) (4)	(b) (4)	(b) (4)	JSP, Ph. Eur.	(b) (4)

(b) (4)

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

LDV is an HCV inhibitor targeting the HCV NS5A protein, which is essential for both RNA replication and the assembly of HCV virions. Biochemical confirmation of NS5A inhibition of LDV is not currently possible as NS5A has no enzymatic function. In vitro resistance selection and cross-resistance studies indicate LDV targets NS5A as its mode of action.

SOF is a pan-genotypic inhibitor of the HCV NS5B RNA-dependent RNA polymerase, which is essential for viral replication. SOF is a nucleotide prodrug that undergoes intracellular metabolism to form the pharmacologically active uridine analog triphosphate (GS-461203), which can be incorporated by HCV NS5B and acts as a chain terminator. In a biochemical assay, GS-461203 inhibited the polymerase activity of the recombinant NS5B from HCV genotypes 1b, 2a, 3a and 4a with IC50 values ranging from 0.7 to 2.6 μ M. GS-461203 is not an inhibitor of human DNA and RNA polymerases or an inhibitor of mitochondrial RNA polymerase.

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

The proposed dose of LDV /SOF is 90 mg/400 mg, taken orally, once daily with or without food. The proposed duration of treatment is based on treatment experience and cirrhosis status.

(b) (4)

The recommended duration of treatment is still under review. Although the applicant has proposed

(b) (4)
DAVP is under active negotiations with the applicant for extending the treatment durations.

2.2 General Clinical Pharmacology

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

A comprehensive range of clinical studies was conducted to characterize the pharmacokinetics (PK) of SOF, its predominant circulating metabolite GS-331007, and LDV. This submission includes 18 SOF single-agent studies, 19 LDV single-agent studies, and 10 LDV/SOF FDC studies containing biopharmaceutic and clinical pharmacology data that were conducted as part of the LDV/SOF clinical development program. In vitro studies for SOF, GS-331007 and LDV were also conducted to assess the potential for drug-drug interactions (DDI). Most of the clinical pharmacology studies for SOF as a single agent have been reviewed in NDA 204671, with the exception of the DDI study with oral contraceptives.

Dose Selection: The dose of LDV 90 mg was selected based on the results from the Phase 1, 3-day proof-of-concept dose-ranging monotherapy study (GS-US-256-0102, LDV dose range: 1 mg to 90 mg) and the data from the Phase 2 study in genotype 1 HCV-infected subjects (GS-US-248-0120). Although the maximum effect (Emax) modeling indicated that exposures achieved following administration of LDV \geq 30 mg provide >95% of maximal antiviral response in genotype 1a HCV-infected subjects, and LDV dosing beyond 90 mg was unlikely to cause further meaningful reductions in HCV RNA, study GS-US-248-0120 showed that the incidence of virologic breakthrough in the LDV 90 mg-containing group was approximately half of that observed in the LDV 30 mg-containing group (10.6% versus 19.6%). Treatment with LDV 90 mg

plus DAAs for 12 or 24 weeks resulted in numerically higher, though not statistically different, SVR24 rates as compared with LDV 30 mg plus DAAs for 24 weeks. These findings supported further evaluation of the 90-mg dose of LDV in the clinical development program for LDV/SOF.

SOF 400 mg is approved for use in combination with other agents for the treatment of chronic HCV infection in adults. No dose adjustment for either agent was required on co-administration (Study GS-US-334-0101) because the increase in the systemic exposure of SOF by LDV was not considered clinically significant. Therefore, LDV/SOF (90 mg/400 mg) FDC tablets were used to support initiation of Phase 3.

Phase 2 and 3 Clinical Efficacy and Safety Development Program:

The efficacy and safety of LDV/SOF FDC were evaluated in 3 pivotal Phase 3 studies: GS-US-337-0102 (ION-1), GS-US-337-0109 (ION-2), and GS-US-337-0108 (ION-3); as well as 3 Phase 2 clinical studies: P7977-0523 (ELECTRON) Part 4 (Groups 12 and 13) and Part 6 (Groups 16 to 18, 20, and 21), GS-US-337-0118 (LONESTAR), and GS-US-337-0122 (ELECTRON-2 [Cohort 2, Groups 3 and 4]).

The Phase 3 studies were all conducted in subjects with genotype 1 HCV infection.

- Study GS-US-337-0102 evaluated 12 or 24 weeks of LDV/SOF±RBV in treatment-naïve subjects;
- Study GS-US-337-0109 evaluated 12 or 24 weeks of LDV/SOF±RBV in treatment-experienced subjects; and
- Study GS-US-337-0108 evaluated LDV/SOF+RBV for 8 weeks and LDV/SOF (without RBV) for 8 and 12 weeks in treatment-naïve subjects.

Studies GS-US-337-0102 and GS-US-337-0109 each included a subset of subjects ($\leq 20\%$) with compensated cirrhosis. Each of the Phase 3 studies have reached post-treatment Week 12 and achieved their primary endpoints (SVR12), which form the basis for submitting the application at this point in the development program.

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

The goal of treatment of chronic HCV infection is long-lasting viral eradication, generally defined as SVR (i.e., undetectable virus [LLOQ or limit of detection for assay] 12 [SVR12] or 24 [SVR24] weeks after the completion of therapy). Previously, achieving SVR24 has been proven as a reliable predictor of long-term clearance of HCV RNA for PEG+RBV treatment and is generally accepted as a cure of infection. Recently, analyses of large datasets demonstrated a high concordance between SVR12 and SVR24. SVR12 was the primary endpoint used for approval of Sovaldi®. Therefore, SVR12 was selected as the primary endpoint for LDV/SOF Phase 2/3 clinical trials for LDV/SOF FDC.

2.2.3 Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure-response relationships?

Plasma concentrations for LDV, SOF, and GS-331007 were used to assess PK parameters and exposure-response relationships.

LDV is an HCV inhibitor targeting the HCV NS5A protein, and thus is an active moiety. Following a single 90 mg oral dose of [¹⁴C]-LDV, mean total recovery of the [¹⁴C]-radioactivity in feces and urine was approximately 87%, with most of the radioactive dose recovered from feces (approximately 86%). Unchanged LDV excreted in feces accounted for a mean of 70% of the administered dose and the oxidative metabolite M19 accounted for 2.2% of the dose. Therefore, LDV plasma concentrations are appropriate to evaluate LDV PK/PD relationships.

SOF is a nucleotide prodrug that undergoes intracellular metabolism to form the pharmacologically active uridine analog triphosphate (GS-461203). Nonclinical characterization of the disposition of SOF across species revealed that SOF was extensively metabolized by hydrolase activity that led to low systemic exposure of SOF and predominant systemic exposure to 2 major metabolites in humans: GS-566500 and the primary circulating metabolite GS-331007. These findings were confirmed in a mass balance study such that SOF, GS-566500, and GS-331007 accounted for approximately 4%, 7%, and >90% of drug-related material respectively. Because the active triphosphate moiety is not measurable in plasma, GS-331007 was considered to be the primary analyte of interest in clinical pharmacology studies for purposes of PK analyses and interpretation of results. GS-566500 PK profiles are similar to SOF and thus PK for GS-566500 was not analyzed in the later studies. SOF and GS-331007 were characterized in all clinical pharmacology studies and used for exposure-response analysis.

2.2.4 Exposure-response

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

Exposure-response analyses were based on LDV, SOF, and GS-331007 AUC_{tau} for genotype 1 subjects from the three Phase III trials (GS-US-337-0102, GS-US-337-0108, and GS-US-337-0109). Subjects in Phase III had only sparse sampling, and samples were obtained pre-dose on days of on-treatment virologic assessment. The primary endpoint evaluated in these analyses was sustained virologic response at week 12 of follow-up (SVR12). Response was assessed based on treatment duration, prior treatment status (treatment-naïve and treatment-experienced), and administration with or without RBV based on quartile of exposure for each of the above analytes.

Treatment-naïve non-cirrhotics

There was a numeric trend of increased response with respect to increased SOF, GS-331007, and LDV exposure from GS-US-337-0108 for LDV/SOF or LDV/SOF/RBV over 8 weeks (Table 2). The SVR rates in subjects administered LDV/SOF for 8 weeks were numerically higher in subjects in the highest exposure quartile (Q4) for SOF (1550-2380 ng•h/mL; 97% SVR12), GS-331007 (14600-29100 ng•h/mL; 96% SVR12) and LDV (10600-49100; 97% SVR12) compared to the lowest exposure quartiles (Q1) SOF (679-1090 ng•h/mL; 89% SVR12), GS-331007 (6840-9830 ng•h/mL; 91% SVR12) and LDV (1350-4810; 89% SVR12). Similar trends were observed for subjects administered LDV/SOF/RBV for 8 weeks with numerically higher SVR rates in the highest exposure quartile for SOF (1550-2250 ng•h/mL; 97% SVR12), GS-331007 (14600-22700 ng•h/mL; 100% SVR12) and LDV (10600-25800; 96% SVR12) compared to the lowest exposure quartiles SOF (730-1110 ng•h/mL; 91% SVR12), GS-331007 (5450-9880 ng•h/mL; 91% SVR12) and LDV (867-4870; 87% SVR12). No impact of RBV on treatment response was observed between the treatment arms. [Note: the quartile exposures described above for SOF, GS-331007, and LDV are applicable to the scenarios discussed below where

the treatment is extended to 12-weeks in treatment naïve subjects and 12-weeks and 24-weeks in treatment-experienced subjects].

Table 2: Percentage of Treatment-Naïve Non-cirrhotic HCV-Infected Subjects who Achieved SVR12 in GS-US-337-0108 by Population Quartiles of SOF, GS-331007, or LDV AUC_{tau}

Regimen		LDV/SOF 8 weeks (GS-US-337-0108)			LDV/SOF/RBV 8 weeks (GS-US-337-0108)		
Compound		SOF	GS-331007	LDV	SOF	GS-331007	LDV
Exposure Quartile (or combination of quartiles) ^a	Q4	97%	96%	97%	97%	100%	96%
	Q3	92%	96%	95%	92%	98%	98%
	Q2	88%	92%	93%	95%	93%	98%
	Q1	89%	91%	89%	91%	91%	87%
	Q12 SOF/ Q12 LDV	88%			90%		

^a Q1, Q2, Q3, Q4, and Q12: first quartile, second quartile, third quartile, fourth quartile, and first 2 quartiles

Treatment-naïve cirrhotics

In genotype 1 treatment-naïve subjects administered LDV/SOF or LDV/SOF/RBV for 12 weeks from GS-US-337-0102 and GS-US-337-0108, no differences were observed in SVR12 between the highest exposure quartiles (LDV/SOF: 96-99%; LDV/SOF/RBV: 91-100%) and the lowest exposure quartiles (LDV/SOF: 97-100%; LDV/SOF/RBV: 95-100%) (Table 3). These observations continue to support that RBV does not improve response in genotype 1 treatment-naïve subjects with at least 12-week treatment duration of LDV/SOF. In addition, the observations from GS-US-337-0102 and GS-US-337-0108 suggest that any of the numeric trends of decreased response with lower SOF, GS-331007, and LDV exposures observed from LDV/SOF and LDV/SOF/RBV (GS-US-337-0108) no longer were evident with a 12-week treatment duration.

Table 3: Percentage of Treatment-Naïve HCV-Infected Subjects who Achieved SVR12 in GS-US-337-0108 and GS-US-337-0102 by Population Quartiles of SOF, GS-331007, or LDV AUC_{tau}

Regimen		LDV/SOF 12 weeks (GS-US-337-0108, GS-US-337-0102)			LDV/SOF/RBV 12 weeks (GS-US-337-0102)		
Compound		SOF	GS-331007	LDV	SOF	GS-331007	LDV
Exposure Quartile (or combination of quartiles) ^a	Q4	99%	97%	96%	91%	100%	98%
	Q3	96%	98%	95%	100%	98%	97%
	Q2	98%	94%	98%	97%	98%	96%
	Q1	97%	100%	99%	100%	95%	98%
	Q12 SOF/ Q12 LDV	100%			97%		

^a Q1, Q2, Q3, Q4, and Q12: first quartile, second quartile, third quartile, fourth quartile, and first 2 quartiles

Treatment-experienced (both cirrhotic and non-cirrhotic)

No consistent trends in SVR12 was observed with respect to SOF, GS-331007, or LDV exposures in genotype 1 treatment-experienced subjects administered LDV/SOF or LDV/SOF/RBV for 12 or 24 weeks (GS-US-337-0109) (Table 4). SVR12 rates were between 92-100% and 100% for LDV/SOF 12-weeks and LDV/SOF/RBV 12-weeks, respectively, in the highest quartile compared to 88-100% and 93-95% for the same regimens in the lowest quartile. With respect to the 12-week duration, the 24-week duration had a higher overall response rate (99% compared to 94-96% for LDV/SOF or LDV/SOF/RBV for 12-weeks) and no exposure-response analyses could be performed for the 24-week duration as 238/240 subjects achieved SVR12. In both regimens, there were no differences in the response rate between regimens with RBV (12-weeks: 96%; 24-weeks: 99%) and without RBV (12-weeks: 96%; 24-weeks: 99%), continuing to support that RBV is not increasing response in the LDV/SOF regimen.

Table 4: Percentage of Treatment-Experienced HCV-Infected Subjects who Achieved SVR12 in GS-US-337-0109 by Population Quartiles of SOF, GS-331007, or LDV AUC_{tau}

Regimen		LDV/SOF 12 weeks (GS-US-337-0109)			LDV/SOF/RBV 12 weeks (GS-US-337-0109)		
Compound		SOF	GS-331007	LDV	SOF	GS-331007	LDV
Exposure Quartile (or combination of quartiles) ^a	Q4	96%	92%	100%	100%	100%	100%
	Q3	100%	93%	100%	95%	96%	97%
	Q2	88%	94%	89%	96%	97%	97%
	Q1	92%	100%	88%	93%	94%	95%
	Q12 SOF/ Q12 LDV	83%			92%		

Regimen		LDV/SOF 24 weeks (GS-US-337-0109)			LDV/SOF/RBV 24 weeks (GS-US-337-0109)		
Compound		SOF	GS-331007	LDV	SOF	GS-331007	LDV
Exposure Quartile (or combination of quartiles) ^a	Q4	96%	100%	100%	100%	100%	100%
	Q3	100%	100%	100%	100%	100%	100%
	Q2	100%	95%	100%	100%	100%	100%
	Q1	100%	100%	97%	100%	98%	98%
	Q12 SOF/ Q12 LDV	97%			98%		

^a Q1, Q2, Q3, Q4, and Q12: first quartile, second quartile, third quartile, fourth quartile, and first 2 quartiles

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

Exposure-response safety analyses were conducted based on a pooled analysis of Phase III subjects administered LDV 90 mg and SOF 400 mg. Analyses were conducted for the most common adverse events observed from the Phase III trials and included headaches, nausea, insomnia, and fatigue. In each of these analyses, exposure-response relationships could not be identified between LDV, SOF, or GS-331007 AUC_{tau} and the most common adverse events observed during the Phase III trials.

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

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

Following administration of LDV/SOF to healthy subjects, SOF plasma exposures were low, with the majority of drug exposure obtained from GS-331007. Following a single dose, SOF was absorbed at median T_{max} of 0.8 hours and eliminated with a median t_{1/2} of 0.5 hours. GS-331007 median T_{max} occurred later (3.5 hours) relative to SOF with a plasma half-life of 27 hours.

Cross-study comparisons of SOF PK revealed that SOF and GS-331007 exhibited similar PK characteristics upon single and multiple dosing of LDV/SOF (AUC_{inf} for a single fasted dose was comparable to AUC_{tau} for multiple fasted doses). Based on the short t_{1/2} and the principle of super-positioning, accumulation for SOF is not expected. Modest accumulation of GS-331007 of approximately 30% and 48% for C_{max} and AUC_{tau}, respectively, is observed.

Following administration of LDV/SOF, the median T_{max} for LDV was approximately 4 hours. The median half-life of 46 hours supports once-daily dosing. LDV exhibits time-independent PK. Consistent with a moderately long half-life relative to 24-hour dosing, an accumulation of LDV of approximately 69% for C_{max} and 129% for AUC_{tau} was observed following multiple dosing of LDV as a single agent.

2.2.5.2 How does the PK of the drug in healthy volunteers compare to that in patients?

SOF, GS-331007, and LDV exposures in healthy and HCV-infected subjects following multiple dose administration of LDV/SOF 90 mg/400 mg were analyzed by using population PK models. Based on population PK modeling, mean SOF and GS-331007 exposures (AUC_{tau} and C_{max}) achieved in HCV-infected subjects and healthy subjects were similar. Geometric mean LDV AUC_{tau}, C_{max}, and C_{tau} observed in HCV-infected subjects (N=2113) were slightly lower (24%, 32%, and 26%, respectively) than those observed in healthy subjects (N=191).

2.2.5.3 What are the characteristics of drug absorption?

SOF was stable in simulated gastric and intestinal fluids with half-lives of >20 hours in both fluids. Assessment of SOF permeability (concentration: 10-2800 µM) through Caco-2 cell monolayers revealed partially saturable efflux with a decreasing efflux ratio with increasing SOF concentration. In vitro screening for interactions with various membrane transporters revealed that SOF is a substrate for p-glycoprotein (P-gp) and breast cancer resistance protein (BCRP).

LDV is a substrate for P-gp and BCRP transport. Consistent with P-gp-dependent efflux, the amount of LDV in MDCKII-MDR1-transfected cells increased to levels near those observed in MDCKII-WT in the presence of 100 µM verapamil and 10 µM CsA. Consistent with BCRP-dependent efflux, the amount of LDV in MDCKII-BCRP cells was increased to levels similar to those observed in MDCKII-WT cells in the presence of 10 µM CsA.

The absorption potential of SOF and LDV in the context of LDV/SOF has been studied in vitro by assessing the effect of LDV on SOF permeability across Caco-2 cell monolayers. The apical to basolateral (forward) permeability of SOF was increased and the efflux ratio of SOF was decreased in the presence of LDV. These results suggest that SOF intestinal absorption may be increased in the context of the LDV/SOF FDC tablet due to inhibition of intestinal transporters by LDV. This finding was confirmed with in vivo studies demonstrating an approximately 2-fold

higher extent of absorption of SOF when coadministered with LDV as compared with dosing with SOF alone.

Overexpression of P-gp and BCRP in transfected cell lines in vitro resulted in a decrease in the apparent permeability of SOF and decreased accumulation of LDV in transfected cells. Although not studied, drugs that induce P-gp or BCRP may decrease SOF and LDV plasma concentrations leading to a potential reduction in delivery of the respective pharmacologically active species into the liver. It is recommended that LDV/SOF not be coadministered with inducers of intestinal P-gp (eg, phenytoin, St. John's Wort, RIF, tipranavir/ritonavir).

Following oral administration of the LDV/SOF FDC tablet, peak plasma concentrations of SOF were observed approximately 0.8 to 1 hour postdose (median T_{max}) in healthy subjects and in HCV-infected subjects. Peak plasma concentrations of GS-331007 were observed between 3.5 and 4 hours after LDV/SOF administration. For LDV, maximum plasma concentrations were achieved approximately 4 hours postdose (median T_{max}) following administration of LDV as a component of LDV/SOF.

Similar to the results seen with single-agent SOF dosing, administration of LDV/SOF with food resulted in a slower rate of absorption of SOF, with no clinically significant alteration in the extent of absorption (fed versus fasted; mean AUC_{inf} increased by 79% compared with fasted administration). When evaluated as GS-331007, a lower C_{max} (fed versus fasted; mean C_{max} decreased by <30%) was observed with no change in GS-331007 AUC. Since the changes in GS-331007 C_{max} and AUC parameters were modest, the effect of food on GS-331007 PK was not considered clinically significant.

In contrast to the food effect observed with single-agent LDV dosing, similar mean LDV plasma exposures (AUC_{inf} and C_{max}) were achieved upon administration of LDV/SOF under fed or fasted conditions, demonstrating that the PK of LDV within LDV/SOF is not altered by food. Of note, LDV/SOF has been administered without regard to food throughout the clinical development program.

LDV solubility decreases as pH increases. Drugs that increase gastric pH are expected to decrease plasma concentrations of LDV. The effect of acid-reducing agents on LDV absorption is discussed in Section 2.4.2 (Drug-Drug Interaction).

2.2.5.4 What are the characteristics of drug distribution?

Based on ultrafiltration studies, in vitro protein binding of SOF was low in human plasma (61-65%) and independent of protein concentration in human plasma); ex vivo plasma protein binding of SOF was approximately 82% and 85% in healthy subjects and subjects with end-stage renal disease (ESRD), respectively (Study P7977-0915). GS-331007 showed minimal binding to plasma proteins and there was no difference between subjects with normal renal function (unbound fraction: 93.3 ± 6.2%) and subjects with ESRD in Period 1 (unbound fraction: 95.5 ± 9.1%).

After a single 400-mg dose of [¹⁴C]-SOF in healthy male subjects, the blood to plasma ratio of ¹⁴C-radioactivity was approximately 0.71, indicating that SOF and its metabolites were predominantly distributed to plasma relative to the cellular components of blood (Study P7977-0312).

LDV is >99.8% bound to human plasma proteins when determined in vitro with equilibrium dialysis. In agreement with in vitro data, LDV protein binding was $\geq 98\%$ in healthy subjects and in subjects with renal or hepatic impairment. After a single 90 mg dose of [^{14}C]-LDV in healthy subjects, the blood to plasma ratio of ^{14}C -radioactivity ranged between 0.51 and 0.66.

Since coadministration of SOF and LDV is not anticipated to alter the distribution of either agent, distribution studies of LDV/SOF have not been conducted.

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

Elimination is primarily hepatic for SOF and renal for GS-331007. Following administration of [^{14}C]-SOF, mean total recovery of the radioactive dose was > 92%, consisting of approximately 80%, 14%, and 2.5% recovered in urine, feces, and respired air, respectively. The majority (78%) of the dose recovered in the urine was as GS-331007 (Study P7977-0312). Recovery of SOF, as unchanged drug, in the urine and feces was low, suggesting SOF is mainly metabolized to form GS-331007. Consistent with substantial excretion of GS-331007 in the urine, clinically significant changes in GS-331007 PK were noted with marked renal impairment (Study P7977-0915).

Biliary excretion of parent drug is a major route of elimination for LDV. Following single dose oral administration of [^{14}C]-LDV, unchanged LDV contributed the largest proportion (> 98%) of circulating radioactivity through 24 hours post-dose in plasma, with 1.1% and 0.75% of the total radioactivity attributed to unidentified metabolites M1 and M12, respectively. Approximately 1% of the dose was recovered in urine through 24 hours post-dose. No unchanged parent drug was detected in urine. In agreement with these results, no alterations in LDV PK were observed in non-HCV-infected subjects with severe renal impairment as compared to healthy subjects.

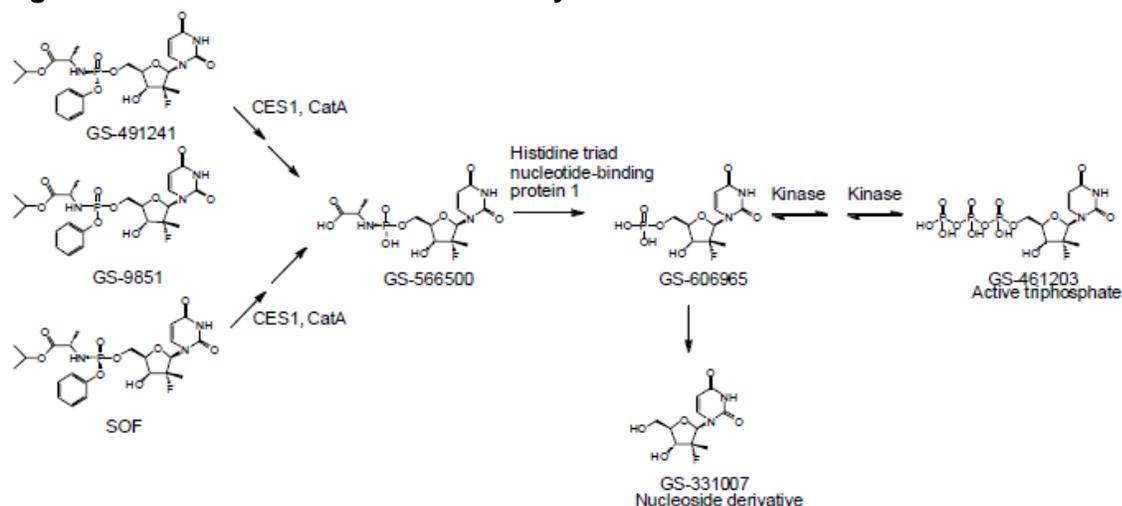
The major route of excretion of LDV (measured as radioactivity) was via feces, with approximately 86% of the administered dose recovered in feces. Unchanged LDV was the major component excreted in feces and accounted for a mean of 70% of the administered dose. Oxy-LDV-3 (M19) was also identified in feces, accounting for a mean of 2.21% of the dose (GS-US-256-0108). These results are consistent with findings from nonclinical studies where biliary excretion of parent drug was a major route of elimination. The enzymes responsible for the slow biotransformation of LDV are currently unknown.

2.2.5.6 What are the characteristics of drug metabolism and excretion?

Screening assays demonstrated that SOF, GS-566500, and GS-331007 were minimally metabolized by CYP, flavin monooxygenase (FMO), and uridine diphosphate glucuronosyltransferase (UGT) enzymes; therefore, SOF and its major metabolites should not be affected (victim drug) by coadministration with inhibitors of CYP isozymes, FMO enzymes, or UGT enzymes.

The primary metabolic route of SOF is via hydrolase cleavage, which ultimately results in the formation of GS-331007. Sequential intracellular activation by generally low affinity and high capacity hydrolase ([carboxyl esterase 1 [CES1], cathepsin A [CatA], histidine triad nucleotide binding protein 1[HINT1]) and nucleotide phosphorylation (uridine monophosphate-cytidine monophosphate [UMP-CMP] kinase, nucleoside diphosphate [NDP] kinase) pathways resulted in the formation of the pharmacologically active nucleoside analog triphosphate GS-461203 (Figure 1).

Figure 1: Intracellular Metabolic Pathway of Sofosbuvir



The metabolism of LDV, including metabolic routes leading to elimination, was characterized in vitro in hepatic microsomes and cryopreserved hepatocytes. No LDV turnover was detected in hepatic microsomes and hepatocytes, with estimated hepatic clearance values of < 0.17 L/h/kg for human. LDV was also stable under study conditions with tested CYPs, including CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4.

2.2.5.7 What are the characteristics of drug excretion?

See Section 2.2.5.5.

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

A cross-study analysis of SOF and GS-331007 AUC_{inf} and C_{max} was performed to investigate the dose linearity of SOF (power model regression) using data from Study P7977-0613 that evaluated the PK of single therapeutic (400 mg) and suprathreshold (1200 mg) doses of SOF in fasted healthy subjects and Study P7977-0111 that evaluated the 200-mg single-dose SOF in fasted healthy subjects. The power model mean slope and 90% CIs indicated that near dose linearity was observed for SOF AUC_{inf} and C_{max}, and GS-331007 AUC_{inf}, with GS-331007 C_{max} demonstrating less than dose proportional increases. Similar results were observed in HCV-infected subjects following single and multiple doses (once daily) of SOF 100-400 mg in Study P7977-0221 (Section 2.2.5.1).

The PK of LDV have been evaluated following single- and multiple-dose administration of LDV single agent. Power model regression results from Study GS-US-256-0101 indicate that the PK of single doses of LDV under fasted conditions are linear with dose for AUC_{inf} and C_{max} over the 3- to 100-mg range, as shown by the power model mean slope (near 1). The 3-day monotherapy study in genotype 1a and 1b HCV-infected subjects (GS-US-256-0102) also show that LDV exhibited a near-linear PK profile across the evaluated once daily dose range of 1 mg to 90 mg.

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

SOF and its metabolites exhibited time-independent PK with minimal accumulation (accumulation ratio is near 1) and similar CL_r over time as shown in Study P7977-0221 (Section 2.2.5.1).

In the 3-day monotherapy study in genotype 1a and 1b HCV-infected subjects, LDV exhibited time-independent PK across the evaluated dose range of 1 mg to 90 mg (GS-US-256-0102). Across all dosage strengths, accumulation ratios (%) of greater than 100 (152 to 483) were observed, a finding consistent with the long half-life of LDV.

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

SOF

Overall, in both healthy and HCV-infected subjects, moderate inter-individual variability was observed for SOF. Based on the population pharmacokinetic analysis, the inter-individual variability for the apparent oral clearance (CL/F) and apparent volume of distribution (V_d/F) was 40% and 33%. A significant difference between apparent oral clearance was identified between healthy subjects and HCV-infected patients (10% lower CL/F in HCV-infected patients). CrCL was identified to have a minor impact on clearance with a 30 mL/min decrease in CrCL predicted to result in a 7% decrease in SOF CL/F. No additional major causes of SOF variability were identified from the population pharmacokinetic analysis. There was insufficient data available to characterize the intra-subject variability of SOF from the available pharmacokinetic data.

GS-331007:

The inter-individual variability for GS-331007 was low (23%) for apparent oral clearance (CL/F) and moderate (53%) for apparent volume of distribution based on a population pharmacokinetic analysis. This analysis identified a significant impact of creatinine clearance (14% decrease in GS-331007 clearance for a 30 mL/min decrease in creatinine clearance), gender (16% lower CL/F in women), race (13% higher clearance in patients listed as non-white), and RBV use (21% increase in CL/F) on the clearance of CL/F. Similarly, the analysis identified significant impact of disease status (178% increase in V_d/F in HCV-infected subjects), RBV use (40% increase in V_d/F), and CrCL (13% decrease in V_d/F for a 30 mL/min decrease in CrCL) on V_d/F. No additional major causes of GS-331007 variability were identified from the population pharmacokinetic analysis. There was insufficient data available to characterize the intra-subject variability of GS-331007 from the available pharmacokinetic data.

LDV:

The inter-individual variability for LDV was 48% for apparent oral clearance (CL/F), 56% for apparent central volume of distribution (V_c/F) and 78% for apparent peripheral volume of distribution (V_p/F). Sex, body weight, RBV usage and disease status (healthy volunteer versus HCV-infected) were identified as significant covariates on CL/F and body weight was identified as a significant covariate on V_d/F. Female gender was associated with a 33% lower CL/F compared to males, RBV usage was associated with an 18% higher CL/F compared to use without RBV, healthy volunteers had a 27% higher CL/F compared to HCV-infected subjects, and a 20 kg increase in body weight was associated with an 11% increase in CL/F. A 20 kg increase in body weight was also associated with a 30% increase in V_d/F.

2.3 Intrinsic Factors

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

Based on population PK analyses, age (18 - 80 years), race, BMI, treatment status (treatment-naive or treatment-experienced), presence of RBV in the treatment regimen, or the presence or absence of cirrhosis had no clinically relevant effects on the exposure of SOF, GS-331007, or LDV.

LDV AUC_{tau}, C_{max}, and C_{tau} were approximately 77%, 58%, and 75% higher in female subjects compared with male subjects. These results are consistent with the covariate assessment during the population PK analysis, which identified sex as a statistically significant covariate for LDV apparent oral clearance (CL/F). Based on a population PK sensitivity analysis, 49%, 47%, and 29% of the increase in AUC_{tau}, C_{max}, and C_{tau}, respectively, are explained by gender. Of the remaining difference in exposure between female subjects and male subjects, differences in body weight explain 10% of the difference in exposure. Considering the higher interindividual variability in the PK of LDV (48% CV for CL/F; AUC_{tau} range: 416 - 49,140 ng•h/mL), favorable safety profile of LDV/SOF, and high response rates (> 90% SVR12) in male and female subjects across Phase 3 trials, the relationship between sex and LDV exposures was not considered clinically relevant.

Renal Impairment:

SOF:

Dose adjustment of SOF 400 mg is not warranted in patients with mild or moderate renal impairment. The safety and efficacy of SOF have not been established in patients with severe renal impairment (estimated glomerular filtration rate [eGFR] < 30 mL/min) or ESRD requiring hemodialysis.

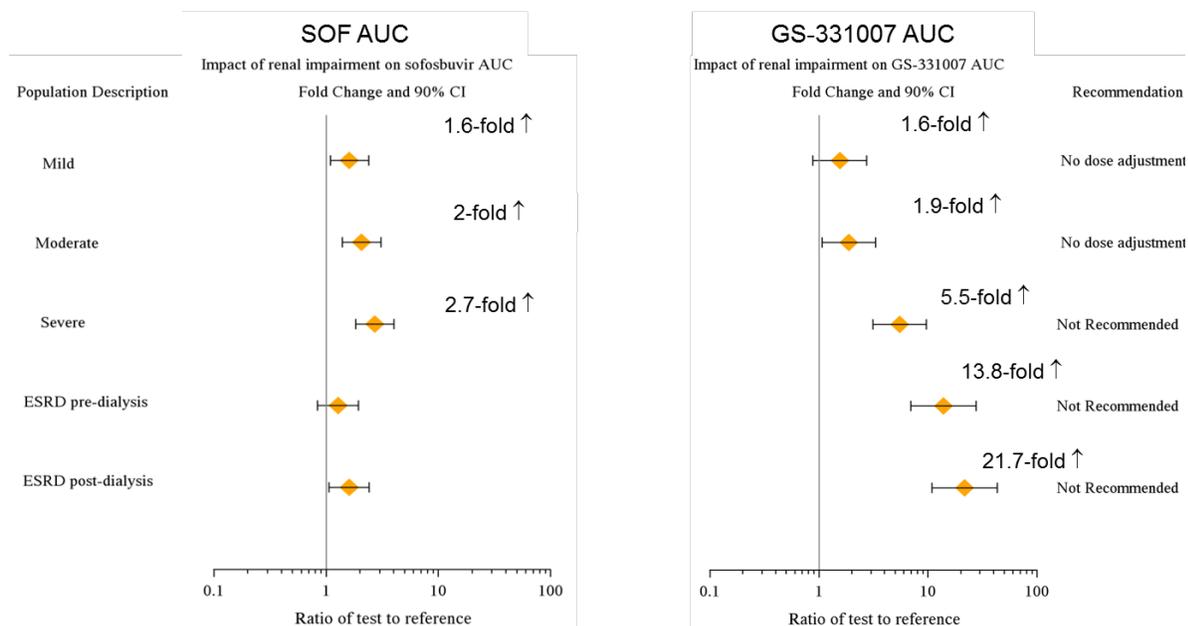
In Study P7977-0915, single-dose PK was evaluated in non-HCV-infected subjects with normal renal function; mild, moderate, or severe renal impairment or ESRD. SOF and GS-331007 plasma exposures were higher in subjects with mild and moderate renal impairment compared with subjects with normal renal function. As shown in Figure 2, relative to subjects with normal renal function (defined as GFR >80 mL/min/1.73m²), SOF AUC_{inf} was 61% and 107% higher in mild and moderate renal impairment, while AUC_{inf} for GS-331007 was 55% and 88% higher, respectively. As GS-331007 is primarily renally eliminated, an increase in GS-331007 exposure with decrease in renal function was expected.

For SOF, the increase in exposure was unlikely a result of a decrease in renal clearance (CL_r) because renal excretion of SOF is a minor pathway for its elimination (CL/F range: 1.4–3.3%). These results were confirmed in population-based analyses of HCV-infected subjects that identified creatinine clearance as a statistically significant covariate for GS-331007 and not for SOF.

Unlike subjects with mild or moderate renal impairment, markedly higher GS-331007 exposures were achieved in subjects with severe renal impairment or ESRD. As shown in Figure 2, relative to subjects with normal renal function, SOF AUC_{inf} was 171% higher in severe renal

impairment, while GS-331007 AUCinf was 451% higher. In subjects with ESRD, SOF and GS-331007 AUCinf were 28% and 1280% higher, respectively, when SOF was dosed 1 hour before hemodialysis compared with 60% and 2070% higher, respectively, when SOF was dosed 1 hour after hemodialysis. A 4-hour hemodialysis removed approximately 18% of the administered dose. A Phase 2 study evaluating the safety and efficacy of administration of 200 or 400 mg SOF plus RBV treatment for 24 weeks in HCV-infected subjects with severe renal impairment is ongoing (GS-US-334-0154).

Figure 2: The Effect of Renal Impairment on the AUC of SOF and GS-331007 Following Administration of Single Dose of SOF in Non-HCV-infected Subjects



LDV:

The effect of severe renal impairment (eGFR: < 30 mL/min) on the PK of LDV was evaluated in non-HCV-infected subjects and a matching cohort of subjects with normal renal function (matched for age, sex, and BMI; eGFR ≥ 90 mL/min) in Study GS-US-344-0108. In agreement with the results from the mass balance study, which demonstrated that renal clearance is a minor pathway for LDV elimination (GS-US-256-0108), LDV plasma exposure parameters (AUCinf, AUC0-last, and Cmax) were similar in subjects with severe renal impairment and control subjects with normal renal function who received a single 90-mg dose of LDV. No differences in LDV protein binding in the 2 groups and no statistically significant correlation between CLcr and primary LDV PK parameters (AUC or Cmax) were observed. Since the exposure of LDV was not impacted in the setting of severe renal impairment, evaluation of LDV PK in subjects with mild or moderate renal impairment was not specifically conducted.

LDV/SOF:

Although not directly studied, the effect of renal impairment on the PK of SOF and LDV when administered as individual agents or as LDV/SOF FDC tablet is expected to be similar. However, for LDV/SOF FDC, since LDV increases SOF AUC by approximately 2.3-fold, SOF AUC could be up to 4.5-fold higher (assuming the effects on SOF AUC are additive), for mild and moderate renal impairment, as compared to SOF alone. In the LDV/SOF Phase 3 trials, subjects with an eGFR of <60 mL/min at screening were excluded; therefore, no clinical data on these subjects are available. However, subjects with mild renal impairment were included, with

31.4% of subjects having an eGFR of 60 -<90 mL/min at baseline. Based on a population pharmacokinetic analysis of data from the Phase III trials, SOF AUCtau was approximately 17% higher in subjects with mild renal impairment at baseline compared to patients with normal renal function at baseline, suggesting that the increase in exposure may be less in patients. This phenomenon was also observed in the Phase 3 trials for SOF as a single agent, where SOF AUC was 8% higher in subjects with mild renal impairment compared to subjects with normal renal function.

Although patients with moderate renal impairment were not included in the Phase 3 trials, the efficacy and safety data generated from the inclusion of patients with mild renal impairment support the use of LDV/SOF FDC in patients with moderate renal impairment (the fold change in SOF AUC was similar between milds and moderates in the dedicated renal impairment study). Thus, LDV/SOF FDC may be administered to HCV-infected patients with mild or moderate renal impairment. The safety and efficacy of LDV/SOF have not been established in patients with severe renal impairment (eGFR < 30 mL/min) or ESRD requiring hemodialysis and thus, is not recommended for administration in these patient populations.

Hepatic Impairment

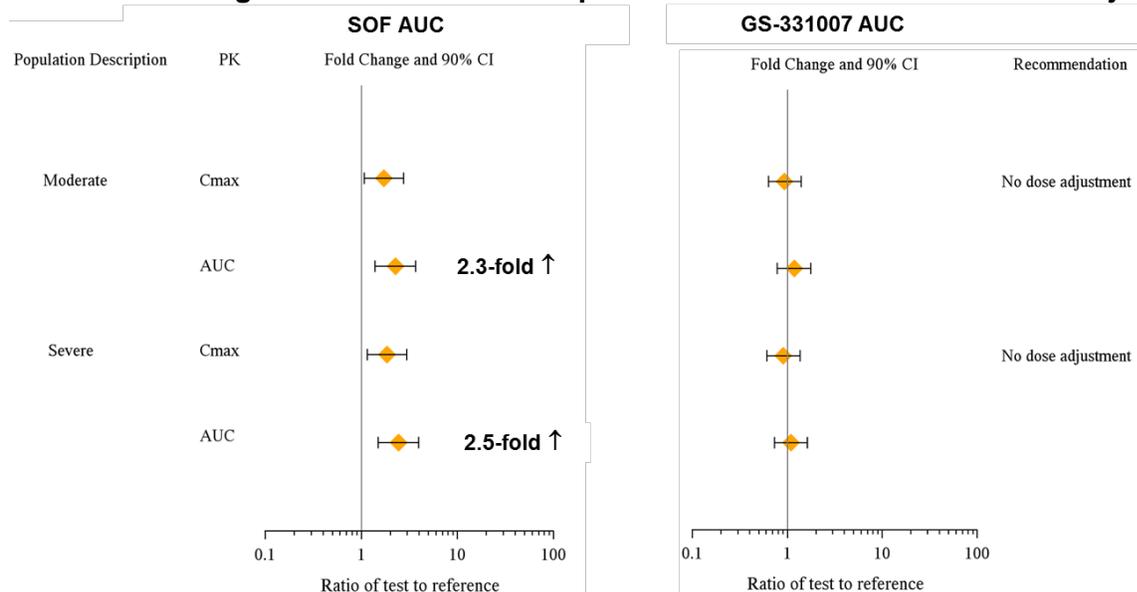
SOF:

In Study P2938-0515, multiple-dose PK was evaluated in HCV-infected subjects with moderate (Child-Pugh-Turcotte [CPT] Classification B) and severe (CPT Classification C) hepatic impairment after administration of SOF 400 mg for 7 days.

GS-331007 systemic exposure was comparable between subjects with moderate or severe hepatic impairment and historical control subjects from Study P2938-0212 (NUCLEAR) with normal hepatic function. SOF mean exposure parameters (AUCtau and Cmax) were similar between subjects with moderate or severe hepatic impairment (CPT Classifications B and C, respectively) but were higher (AUCtau: 126–143%↑; Cmax: 72–85%↑) than those achieved in subjects with normal hepatic function.

In the Phase 3 program, compensated cirrhotic subjects (CPT Classification A; N = 202 [20% of study population]) and noncirrhotic subjects had comparable mean GS-331007 exposure (AUCtau: 7150 vs 7210 ng·h/mL; Cmax: 582 vs 581 ng/mL, respectively) and mean SOF AUCtau (816 vs 871 ng·h/mL, respectively). Cirrhosis was also not identified as a relevant covariate based on population PK analyses. In summary, based on PK and PD results, no dose adjustment of SOF 400 mg is recommended in the setting of hepatic impairment as a single agent.

Figure 3: The Impact of Hepatic Impairment on Pharmacokinetics of Sofosbuvir and GS-331007 Following Administration of Multiple Doses of SOF in HCV-infected Subjects



LDV:

The effect of moderate (CPT Classification B) and severe (CPT Classification C) hepatic impairment on the PK of LDV was evaluated in Studies GS-US-248-0117 (LDV was combined with VDV ± TGV; not reviewed) and GS-US-344-0101, respectively. Moderate or severe hepatic impairment had no clinically relevant impact on LDV PK. LDV mean exposure parameters (AUC_{tau}, C_{max}, and C_{tau}) were similar between subjects with moderate hepatic impairment and subjects with normal hepatic function following administration of LDV 30 mg QD for 12 days in combination with an investigational protease inhibitor VDV. Similar AUC_{0-last} and AUC_{inf} and a lower C_{max} (~35%) were observed in subjects with severe hepatic impairment as compared to subjects with normal hepatic function who received a single dose of LDV 90 mg. A reduction in C_{max} in the absence of a change in AUC is not considered clinically significant.

The mean (SD) percent free fraction (unbound concentrations) for LDV was similar in subjects with moderate or severe hepatic impairment and normal hepatic function and indicated the lack of effect of hepatic impairment on LDV protein binding. Since the exposure of LDV was not impacted in the setting of severe or moderate hepatic impairment, evaluation of LDV PK in subjects with mild hepatic impairment was not specifically conducted.

LDV/SOF:

The effect of hepatic impairment on the PK of SOF and LDV when administered as individual agents or as the LDV/SOF FDC tablet is expected to be similar. In the LDV/SOF Phase 2 and 3 program, subjects with compensated cirrhosis (CPT Classification A) and noncirrhotic subjects achieved similar mean SOF, GS-331007, and LDV exposures. Cirrhosis was not identified as a relevant covariate based on population PK analyses. For patients with moderate and severe hepatic impairment, available intensive PK data presented in Table 5 for 25 subjects (CPT B and C) who have not received a liver transplant and 8 subjects (CPT B and C) who received a liver transplant confirm the observation from the SOF single agent hepatic impairment study, which demonstrated a 2.7- and 2.8-fold higher SOF AUC (CPT B vs. C) and 3.2 and 2.8-fold higher in SOF C_{max} (CPT B vs. C) in subjects with advanced liver disease compared to HCV-

infected subjects without cirrhosis. The PK data from these 25 subjects who have not received a liver transplant show that the exposures in subjects with moderate hepatic impairment (CPT B) and exposures in subjects with severe hepatic impairment (CPT C) are similar. AUC_{tau} values are approximately 2.8-fold higher and 4.6-fold higher as compared to non-cirrhotic subjects following administration of LDV/SOF, and HCV-infected subjects following administration of SOF+RBV (±pegylated interferon (peg-IFN)), respectively.

Table 5: SOF Pharmacokinetics in Subjects With/Without Cirrhosis (LDV/SOF Pooled Phase 2 and 3 Studies) and Subjects with Decompensated Cirrhosis (GS-US-337-0122 and GS-US-337-0123)

SOF Mean (%CV) PK Parameters	Non-Cirrhotic Subjects (N=1845)	CPT B (N = 20)	CPT C (N=5)	CPT B (N=7) Post-Transplant	CPT C (N=1) Post-Transplant
AUC _{tau} (ng•h/mL)	1360 (33.2)	3700 (44.4)	3860 (67.7)	2730 (19.6)	1880
C _{max} (ng/mL)	658 (32.7)	2120 (52.3)	1820 (53.2)	1810 (30.1)	377

Upon review of the limited safety data from these subjects, the Medical Officer indicates that there is no clustering of events and observed increased incidence of AEs, SAEs, or deaths in patients with moderate to severe hepatic impairment does not raise any safety concerns at this time. Although the applicant should continue collecting safety data for this population, the totality of data collected at this time supports the use of the LDV/SOF FDC tablet in patients with any degree of hepatic impairment.

2.4 Extrinsic Factors

2.4.1 What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence exposure -response and what is the impact of any differences in exposure on response?

Only drug-drug interactions have been assessed. See section 2.4.2.

2.4.2. Drug-Drug Interactions

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

Yes. *In vitro* studies suggest that both SOF and LDV are substrates for P-gp and BCRP. Drugs that are P-gp inducers in the intestine, although not studied in vivo, may decrease SOF and LDV plasma concentrations leading to reduced therapeutic effect and thus should not be used with LDV/SOF. LDV is an inhibitor of drug transporter P-gp and breast cancer resistance protein (BCRP) and may increase intestinal absorption of coadministered substrates for these transporters. The absorption potential of SOF and LDV in the context of LDV/SOF has been studied in vitro by assessing the effect of LDV on SOF permeability across Caco-2 cell monolayers. The apical to basolateral (forward) permeability of SOF was increased and the efflux ratio of SOF was decreased in the presence of LDV. Results suggest that SOF intestinal absorption may be increased in the context of the LDV/SOF FDC tablet due to inhibition of intestinal transporters by LDV.

The drug interaction between SOF and LDV as well as between LDV/SOF and P-gp/BCRP inhibitors, were assessed in vivo. See sections 2.4.2.4 and 2.4.2.8 for details.

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

Pathways involving CYP isozymes are not likely to be important considerations in the disposition of SOF, its metabolites, GS-566500, GS-606965, and GS-331007 based on in vitro microsome assay results. These findings were further confirmed in drug interaction trials with known CYP inhibitors and inducers.

When SOF was incubated in microsomes in the presence of 1-aminobenzotriazole (a nonspecific CYP inhibitor), an apparent decrease in the disappearance of SOF was observed compared to the control (incubation without 1-aminobenzotriazole). This suggests potential involvement of CYP isoforms in the metabolism of SOF. However, the following observations suggest that CYP isoforms do not play a clinically relevant role in SOF metabolism; in vitro and clinical studies have shown that SOF is rapidly metabolized to GS-566500 by high capacity esterases (Cat A and CES1). No other metabolite directly derived from SOF was detected in vitro or in vivo. In vivo drug interaction studies with ritonavir and ketoconazole indicated no clinically relevant changes of those drugs on the metabolism of SOF. Based on these observations, the sponsor did not additionally characterize the roles of individual CYP isoforms on the metabolism of SOF using purified CYP isozymes. GS-566500, GS-606965, and GS-331007 were stable in microsome mixtures for an hour, indicating that these metabolites are not further metabolized by CYP isoforms. In vivo interaction studies with efavirenz (in Atripla®, no significant effect) and DRV/RTV (up to 45% and 34% increase on SOF C_{max} and AUC, respectively, possibly due to P-gp inhibition by ritonavir) indicated no *clinically relevant* changes in the metabolism of SOF and its metabolites caused by these drugs.

The in vitro metabolic stability of LDV is consistent with the slow rates of hepatic biotransformation observed during PK studies. No metabolism of LDV was detected in vitro during incubations with hepatic microsomes from mice, rats, dogs, monkeys, and humans and in cryopreserved human hepatocytes under the conditions tested (AD-256-2138; AD-256-2095). No detectable turnover by human CYP1A2, 2C8, 2C9, 2C19, 2D6, and 3A4 was observed in reaction phenotyping studies (AD-256-2098).

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

SOF slightly increased the mRNA expression levels of CYP2B6 and CYP3A4 (2.0- and 2.7-fold respectively) and the CYP2B6 activity (2.7-fold) at 100 µM. The induction effects of SOF on CYP3A4 and CYP2B6 are not considered clinically relevant (<15% of positive controls, RIF or phenobarbital). SOF caused little to no induction in CYP1A2 in vitro.

LDV caused little to no induction of CYP, UGT1A1, and P-gp mRNA or CYP activities when assessed in cultured human hepatocytes from 3 separate donors (AD-256-2146). Small increases in CYP2B6 and 3A4 mRNA levels were observed at the highest concentration tested (10 µM). However, these increases were less than 4-fold that caused by vehicle (a threshold level indicating a possible clinically relevant effect) and less than 15% of those caused by the positive controls. Thus, LDV is unlikely to be a clinical inducer of these CYP enzymes. Furthermore, clinical studies with CYP3A and CYP2B6 substrates of various sensitivities (e.g., EFV, RPV, ATV/RTV, DRV/RTV) confirmed that LDV does not cause clinically relevant induction of CYP3A or CYP2B6.

No concentration-dependent increases in CYP2C9 mRNA, P-gp mRNA, or UGT1A1 mRNA were observed. Results in human hepatocytes are consistent with the lack of induction through

the aryl hydrocarbon receptor and weak induction through the pregnane X receptor detected in reporter cell lines (AD-256-2097).

SOF and its metabolites (GS-566500, GS-606965, GS-331007, and GS-461203) are not inhibitors (IC₅₀ >100 μM) of human CYP isozymes CYP3A4, CYP1A2, CYP2C19, CYP2C9, CYP2C8, and CYP2D6.

LDV did not inhibit the activity of CYP1A2, 2B6, 2C8, 2C9, 2C19 and 2D6 (IC₅₀ >25 μM; AD-256-2096 and AD-256-2133). LDV had an IC₅₀ of 9.9 μM for CYP3A-catalyzed testosterone metabolism but did not inhibit midazolam metabolism (IC₅₀ > 25 μM; AD-256-2096). LDV had an inhibitory effect on the activity of UGT1A1, with an IC₅₀ value of 7.95 μM in vitro (AD-256-2132). Based on IC₅₀ values greatly exceeding plasma C_{max} (409 nM total; < 1 nM unbound), LDV is unlikely to be a clinically relevant inhibitor of UGT1A1 or CYP3A enzymes and is not expected to inhibit the clearance of drugs metabolized by these enzymes in the systemic circulation. These findings were further confirmed in *in vivo* drug interaction studies with a UGT substrate (RAL) and several CYP3A substrates (darunavir (DRV), methadone, EE, etc).

2.4.2.4. Is the drug a substrate and/or an inhibitor of transport processes?

In vitro studies suggest that SOF is a substrate for P-gp and BCRP but not OCT1, OATP1B1, or OATP1B3; GS-331007 is not a substrate for P-gp, BCRP, or the renal transporters OAT1, OAT3, OCT2, and MATE1. SOF is not a clinically relevant inhibitor of any of these transporters.

LDV is a substrate for P-gp and BCRP in vitro (AD-256-2144 and AD-256-2150). No evidence for LDV transport by the hepatic uptake transporters OCT1, OATP1B1, and OATP1B3 has been observed in vitro (AD-256-2143 and AD-256-2139). LDV did not inhibit MRP2, but was found to inhibit P-gp and BCRP-mediated transport (approximately 50% inhibition at 1 μM; AD-256-2109). LDV did not inhibit the hepatic uptake transporter OCT1 (AD-256-2143) but showed dose-dependent inhibition of OATP1B1 and OATP1B3, with IC₅₀ values of 3.5 μM and 6.5 μM, respectively (AD-256-2134). However, based on the draft Guidance for Industry (Drug Interaction Studies — Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations), although calculated C_{max}/IC₅₀ is >0.1, the R-value is <1.25, which indicates that the potential for LDV drug interactions with OATP1B1 or OATP1B3 substrates is low. No inhibition of the renal transporters MRP4, OCT2, OAT1, OAT3, and MATE1 was detected (AD-256-2140). LDV showed minimal potential to inhibit the hepatic efflux pump for endogenous bile acids, BSEP, with an IC₅₀ of approximately 6 μM (AD-256-2140). Similar to the potential for a clinically relevant interaction with OATP inhibitors, the potential for LDV to inhibit these transporters are low. These results suggest that LDV may inhibit the efflux transport of P-gp and BCRP substrates during intestinal absorption but has a limited potential to cause clinically relevant transport inhibition because LDV is highly protein bound.

2.4.2.5. Are there other metabolic pathways that may be important?

The intracellular metabolic activation pathway of SOF is mediated by generally low affinity and high capacity hydrolase (CES1, CatA, histidine triad nucleotide-binding protein 1 [HINT1]) and nucleotide phosphorylation (UMP-CMP kinase, NDP kinase) pathways that are less likely affected by commonly coadministered drugs given to HCV-infected subjects.

Telaprevir (a relatively nonspecific protease inhibitor) and boceprevir have been reported to inhibit SOF activation in vitro via inhibition of CatA. The applicant indicated because CatA is a

low affinity and high capacity hydrolase, boceprevir and telaprevir are not expected to be involved in a clinically relevant DDI with SOF. More data may be needed to support this conclusion. However, at this time, SOF is not likely to be coadministered with telaprevir or boceprevir and no drug interaction studies have been performed with these two drugs.

The applicant conducted in vitro studies to determine whether FMO and UGTs are involved in the metabolism of SOF and its metabolites. No evidence for the metabolism of SOF, GS-566500, and GS-331007 by FMO and UGTs was observed. Evidence for a minor UGT component in the metabolism of GS-606965 was observed; a slight increase in the rate of disappearance of GS-606965 (going from stable to approximately 30% degradation over 60 minutes) was observed in the presence of uridine 5'-diphosphate glucuronic acid (UDPGA). However, no glucuronide products of GS-606965 were detected in vivo, suggesting that the involvement of UGT on the metabolism of GS-606965 is not likely clinically relevant.

2.4.2.6. Does the label specify co-administration of another drug (e.g., combination therapy in oncology) and, if so, has the interaction potential between these drugs been evaluated?

No. LDV/SOF is used as a FDC indicated for the treatment of chronic genotype 1 HCV infection. No other drugs are to be coadministered with LDV/SOF FDC for this indication.

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

Other medications that are likely to be co-administered in HCV-infected patients include antiretroviral agents for the treatment of HIV, analeptics, anticonvulsants, antimycobacterials, methadone therapy for the treatment of opioid addiction, CsA and TAC in the prevention of organ rejection following liver transplant, antidepressants and other mood-stability medications, combined oral contraceptives in women, acid-reducing agents, and some herbal supplements. Drug interaction studies have been conducted with SOF, LDV, LDV/SOF in combination with representative antiretrovirals, methadone, CsA and TAC, Ortho Tri-Cyclen Lo® (OC), an H2RA, and a PPI. DDI studies were conducted with acid-reducing agents because LDV solubility decreases as pH increases. Drugs that increase gastric pH are expected to decrease concentration of LDV (Sections 2.2.5.3 and 2.4.2.8).

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

Drug interaction (DDI) studies have been conducted with SOF, LDV, or LDV/SOF in combination with representative antiretrovirals, and other drugs as victims or perpetrators. No clinical meaningful DDI's have been identified.

LDV/SOF, SOF, or LDV as a victim:

- **LDV/SOF FDC tablets** have been studied with: ABC/3TC, ATR, CPA, ATV/RTV, DRV/RTV, EVG+COBI, acid-reducing agents.
 - These drugs have no clinically significant effects on the PK of SOF or GS-331007, the magnitude of the effects is similar to those observed for SOF tablets.
 - The effects of these drugs on the exposures of LDV (AUC and C_{max}) ranged from 34% reduction caused by ATR to 100% increase caused by ATV/RTV.

- Famotidine was studied either with simultaneous administration or with a 12-hour stagger with LDV/SOF, while omeprazole was studied only with simultaneous administration with LDV/SOF. After oral administration, the onset of the antisecretory effect for H2RA occurs within one hour; the maximum effect is dose-dependent, generally occurs within one to three hours. The duration of inhibition of acid secretion is 10 to 12 hours. Therefore, staggered administration of LDV/SOF with H2RA 1 to 10 hours may result in lower LDV concentrations as compared to when LDV/SOF is coadministered with H2RAs simultaneously or 12 hours apart. Thus, H2RAs should only be administered simultaneously or 12 hours apart.
- Compared to H2RAs, PPIs, such as omeprazole, have prolonged onset of the antisecretory effect (up to 10 hours) , but the effect may last up to 3 days, and it reaches plateau after 3-5 days of once daily administration. Staggered administration of LDV/SOF with PPIs may result in lower LDV concentrations. As shown in Study GS-US-256-0110, a substantial decrease in LDV plasma exposure (~42% to 48% lower AUC and Cmax) was observed upon administration of omeprazole 2 hours earlier than LDV as a *single agent*. Therefore, PPI should only be administered simultaneously with LDV/SOF at doses comparable to omeprazole 20 mg once daily (or lower).
- **SOF as a single agent** has been evaluated with ATR, DRV/RTV, RAL, RPV, CsA, TAC and a combined oral hormonal contraceptive (Ortho Tri-Cyclen® Lo).

The largest magnitude of effect on SOF is caused by CsA (SOF AUC ↑353%, Cmax ↑154%), but was not deemed clinically significant. LDV causes about a 2.5-fold increase in SOF exposure. Therefore, higher SOF exposures may be achieved in the context of LDV/SOF. However, in Study GS-US-334-0126 in post-transplant subjects receiving SOF+RBV (N=40), SOF exposure data are available for 9 out of 10 HCV-infected subjects who received a CsA containing immunosuppressive regimen and 26 out of 30 subjects who did not receive CsA as part of their immunosuppressive regimen. For SOF, specifically, exposures (AUCtau and Cmax) were slightly increased approximately 15% and 4%, respectively, in subjects on CsA-containing regimens compared with those on a non-CsA-containing regimen. In contrast to the results from the Phase 1 drug-drug interaction study (P7977-1819) where a 4.5-fold increase in SOF exposure was observed with a single dose of CsA 600 mg, PK data from this study demonstrate that administration of clinically relevant doses of CsA (75 mg to 225 mg) are not associated with substantial increases in SOF. Therefore, SOF in the context of LDV/SOF may be coadministered with CsA without dose adjustment.

The applicant is collecting samples for PK analysis for LDV and SOF exposures in the ongoing post-transplant Studies GS-US-337-0123 and GS-US-337-0124. These data will provide additional insight into the mechanism of higher SOF exposures in the setting of P-gp and BCRP inhibitors and provide direct support for actual SOF exposures in this patient population.

- **LDV as a single agent** has been evaluated with ATR, DRV/RTV, RAL, SMV, and OC. The data show that:
 - SMV increases LDV AUC by 92%, Cmax by 81%
 - DRV/RTV increases LDV AUC by 39%, Cmax by 45%
 - No significant effect by RAL or OC

- **LDV + VDV + TGV** was studied with RIF, verapamil, and CsA. The data show that:
 - RIF (a P-gp inducer) decreases LDV AUC by 59%, Cmax by 35%.
 - Verapamil (a P-gp inhibitor) increases LDV AUC by 100%, Cmax by 59%
 - CsA (a P-gp inhibitor) had no significant effect on LDV AUC or Cmax

Because there is a gender effect on LDV (LDV AUCtau, Cmax, and Ctau were approximately 77%, 58%, and 75% higher in female subjects compared with male subjects), these DDI's were evaluated in the context of the gender effect.

Lower LDV exposure when coadministered with ATR is not likely to impact efficacy of LDV/SOF in male, HCV-infected subjects receiving ATR based on assessment of these exposures relative to the established Emax model for LDV. Specifically, LDV exposures, at the 90 mg dose, in the Phase 3 population reside on the near-maximal portion of dose-response curve, with the mean (SD) predicted maximal HCV RNA suppression (% of Emax) estimated as 99.8% (0.1%). Additionally, the use of LDV/SOF with ATR is supported by available clinical efficacy data from the NIAID-sponsored co-infection study ERADICATE (GS-US-337-0116, NIAID IND 117444). Interim efficacy data are available in 11 male and 2 female HIV/HCV infected subjects receiving ATR and LDV/SOF for 12 weeks. All 13 subjects achieved SVR 4, suggesting no impact on efficacy of LDV/SOF when administered with ATR.

The use of P-gp inhibitors, including verapamil, has been permitted in all HCV-infected subjects in the LDV/SOF Phase 2/3 clinical program. In the Phase 2/3 clinical program, 12 subjects were identified as taking a P-gp inhibitor chronically (azithromycin: N=1; erythromycin: N= 1; verapamil: N=7; ketoconazole: N = 1; amiodarone: N=1; fluvoxamine: N= 1). PK data on subjects who received chronic P-gp inhibitors are limited. A descriptive comparison reveals a 1.3-1.7-fold higher LDV exposure (overall and by gender) as compared to subjects not on P-gp inhibitors (Table 6). LDV exposures in subjects who received P-gp inhibitors short-term and subjects who did not receive P-gp inhibitors were similar.

Table 6: LDV PK Parameters in HCV-Infected Subjects Who Did or Did Not Receive a P-gp Inhibitor in the LDV/SOF Pooled Phase 2 and 3 Studies (Integrated Phase 2 and Phase 3 Studies)

LDV Mean (%CV) PK Parameters ^a	Male and Female Subjects		
	With Chronic P-gp Inhibitors	With Short-term P-gp Inhibitors	Not on P-gp Inhibitors
	N= 12	N=59	N=2042
AUCtau (ng•h/mL)	14000 (65.4)	8800 (50.3)	8490 (60.8)
Cmax (ng/mL)	485 (46.6)	376 (43.5)	363 (51.6)
	Male Subjects		
	With Chronic P-gp Inhibitors	With Short-term P-gp Inhibitors	Not on P-gp Inhibitors
	N= 5	N=26	N=1252
AUCtau (ng•h/mL)	11100 (111)	6150 (41.6)	6640 (57.4)
Cmax (ng/mL)	402 (71.9)	281 (41.6)	299 (48.5)
	Female Subjects		
	With Chronic P-gp Inhibitors	With Short-term P-gp Inhibitors	Not on P-gp Inhibitors
	N= 7	N=33	N=790
AUCtau (ng•h/mL)	16100 (39.5)	10900 (41.3)	11400 (49.4)
Cmax (ng/mL)	544 (30.7)	450 (35.0)	464 (43.5)

Note: Includes all LDV/SOF-treated HCV-infected subjects in Studies P7977-0523, GS-US-337-0118, GS-US-337-0102, GS-US-337-0108, and GS-US-337-0109.

^a Pharmacokinetic parameters are shown to 3 significant digits.

For the 12 subjects chronically taking P-gp inhibitors orally, seven of the subjects were female, and 5 of the subjects were male. No serious AEs were reported within this cohort.

In the thorough QT study (GS-US-344-0109) for LDV, 120 mg of LDV was given twice daily for 10 consecutive days. LDV plasma exposures at this suprathreshold dose were approximately 4.2-fold higher for Cmax and 3.7-fold higher for AUC0-24, relative to the LDV 90-mg dose as a component of LDV/SOF administered to HCV-infected subjects. Sixty subjects were enrolled and randomized in this study. Eighteen were female (30%). No Grade 3 or 4 or serious AEs occurred during the study. The estimated absolute AUC0-24 and Cmax in this study is expected to be higher than LDV exposures in female subjects when coadministered with a P-gp inhibitor.

Table 7: LDV PK Summary following administered 120 mg BID in Healthy Volunteers as Compared to LDV 90-mg Dose as a Component of LDV/SOF Administered to HCV-infected Subjects

LDV PK Parameter Mean (%CV)	LDV 120 mg BID (N=59) (GS-US-344-0109)	LDV/SOF 90 mg/400 mg (N=48) (GS-US-337-0118)
AUC _{tau} (ng·h/mL)	15932.9 (27.0) ^a	--
AUC ₀₋₂₄ (ng·h/mL)	31865.8 (27.0) ^a	9472.8 (51.8) ^b
C _{max} (ng/mL)	1519.5 (27.9)	500.3 (44.1)

^a AUC_{tau}: AUC₀₋₁₂; AUC₀₋₂₄: calculated as AUC_{tau} x 2

^b N = 47

The totality of the PK and safety data supports the coadministration of ATV/RTV, verapamil, or other P-gp inhibitors with LDV/SOF in both men and women.

LDV/SOF, SOF, or LDV as a perpetrator:

- The effects of **LDV/SOF FDC** on other drugs have been evaluated for EVG+COBI, ATV/RTV, ATR, CPA, ABC/3TC. The most significant results are presented in the following table:

LDV/SOF FDC in combination with:	AUC	C _{max}	C _{tau}
TFV in ATR ^a	↑ 98%	↑ 79%	↑ 163%
TFV in CPA	↑ 40%	↑ 32%	↑ 91%
COBI	↑ 59%	↑ 25%	↑ 325%

^a The use of LDV/SOF with ATR may be supported by safety data from study CO-US-337-0116 (ERADICATE), and study GS-US-337-0115 (ION-4). These safety assessments were still ongoing by the clinical review team at the time of this review.

No significant changes in PK were observed for other drugs studied.

- The effects of **SOF (as a single agent)** on other drugs have been evaluated for ATR, TVD+ATV/RTV, 3TC+ZDV+EFV, methadone, RAL, RPV, CsA, TAC and OC. No clinically significant effect has been observed, except for OC: SOF increased EE C_{max} by 40%, AUC by 20%. However, after consulting the Repro-Uro Clinical Pharmacology Team, the effect would not be a significant safety concern based on the relatively short duration of treatment with LDV/SOF (up to 24 weeks).
- The effects of **LDV (as a single agent)** on other drugs have been evaluated for DRV/RTV, OC, RAL, and SMV. The data show that:
 - LDV 30 mg once daily increased SMV C_{max} and AUC by 161% and 169%, respectively, similar magnitude as the effect of DRV/RTV on SMV, and is not recommended to be coadministered.
 - No significant changes in PK for DRV/RTV, RAL or OC

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

Although not directly studied, there is no known mechanistic basis for pharmacodynamic drug-drug interactions. No significant efficacy or safety-based exposure-response relationship has been identified for SOF, GS-31007, or LDV.

2.5 General Biopharmaceutics

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

As shown in the Clinical Pharmacology review in NDA 204671, SOF is a high-solubility, low-permeability (BCS 3) compound.

The applicant claims that LDV is a low solubility, high-permeability (BCS 2) compound. LDV exhibits a pH-dependent solubility profile, and is very slightly soluble at pH 2 and practically insoluble from pH 4 to pH 7.5. See the Biopharmaceutics Reviewer's review for details. The dose-dependent permeability of LDV was assessed in vitro using Caco-2 cell monolayers (AD-256-2108). Permeability at 1 μM of LDV in the apical to basolateral (forward) and basolateral to apical (reverse) directions was assessed. While LDV showed high apical to basolateral permeability across Caco-2 cell monolayers (1.76×10^{-6} cm/second) and no efflux transport, evidence for association between LDV and the trans-well apparatus was observed, and this may have adversely affected the predictive value of the assay. In addition, the study showed there was no efflux transport, while studies in MDCKII cells show that LDV is a substrate of P-gp and BCRP. Therefore, this study cannot be reliably used to assess permeability. In the absence of evidence suggesting high permeability in vitro and instability in the gastrointestinal tract, a drug substance is considered to be *highly permeable only* when the extent of absorption in humans is determined to be 90% or more of an administered dose based on a mass balance determination or in comparison to an intravenous reference dose. The absolute BA of LDV has not been evaluated in humans, but it is expected to be modest (< 30%) based on evaluation of LDV PK following coadministration with inhibitors of P-gp and/or BCRP drug transporters, for which LDV is a substrate. Therefore, there is no clear evidence to suggest LDV is a highly permeable drug.

2.5.2 What is the relative bioavailability (BA) of the proposed to-be-marketed formulation to the pivotal clinical trial? Is clinical and analytical inspection required?

No pivotal bioequivalence study is required and no clinical and analytical inspection is needed for the relative BA study. The LDV/SOF FDC tablet was developed prior to the initiation of Phase 3 clinical studies using SOF (b)(4) drug substance (the commercialized form of SOF) and LDV (b)(4) to support Phase 3 clinical studies. LDV (b)(4) (30- and 90-mg strength) was developed during late Phase 2 clinical studies to improve the biopharmaceutical performance of LDV. The tablet contains 90 mg of LDV and 400 mg of (b)(4) SOF (b)(4). The LDV/SOF tablet formulation demonstrated similar PK performance to the 2 coadministered single-agent tablets, LDV (b)(4) 90-mg strength and SOF tablet 400-mg strength in Study GS-US-337-0101. The LDV/SOF FDC tablet formulation was subsequently used in all Phase 3 clinical studies (GS-US-337-0102 [ION-1], GS-US-337-0109 [ION-2], and GS-US-337-0108 [ION-3]) and no additional changes will be made to the proposed to-be-marketed product.

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

The effect of food on the PK of the proposed commercial formulation of LDV/SOF was evaluated in Study GS-US-337-0101. Compared with fasted administration, food (moderate fat or high calorie/high fat) slowed the rate of absorption of SOF with 79% and 15% increases in AUC and C_{max}, respectively, similar to the results obtained from the SOF single agent tablet. For GS-331007, an approximately 18% to 30% lower C_{max} was observed upon LDV/SOF administration with food, with no significant change in AUC. Since the decrease in GS-331007 C_{max} was modest and the AUC parameters met the “no effect” criteria, the effect of food on GS-331007 PK was not considered clinically significant. The PK of LDV administered within the FDC are not altered by food. Based on these results, LDV/SOF has been administered without regard to food in the Phase 3 clinical development.

2.5.4 If different-strength formulations are not bioequivalent based on standard criteria, what clinical safety and efficacy data support the approval of the various strengths of the to-be-marketed product?

Not applicable.

2.6 Analytical Section

2.6.1 How are the active moieties identified and measured in the plasma in the clinical pharmacology and biopharmaceutics studies? What bioanalytical methods are used to assess concentrations?

The active metabolite (GS-461203) is converted from prodrug SOF intracellularly and is not detectable in plasma. Nonclinical characterization of the disposition of SOF across species revealed that SOF was extensively metabolized by hydrolase activity that led to low systemic exposure of SOF and predominant systemic exposure to the major metabolite GS-331007 in humans, but not GS-461203. These findings were confirmed in a mass balance study such that SOF and GS-331007 accounted for approximately 4% and > 90% of drug-related material respectively. GS-331007 was considered to be the primary analyte of interest in clinical pharmacology studies for purposes of PK analyses and interpretation of results, and was characterized in clinical pharmacology studies and used for exposure-response analysis.

Liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) with positive ionization was utilized to determine the concentration of SOF and its metabolites in plasma, urine and dialysate. Calibration curves for SOF ranged from 5 (LLOQ) to 5000 ng/mL. Calibration curves for GS-331007 ranged from 10 (LLOQ) to 5000 ng/mL. Standards, quality control solutions, blank matrix, and study samples (as applicable) were prepared according to the validated methods. All samples were analyzed within the time frame supported by long-term storage stability data. The standard curve and QC data indicated that the plasma and urine assay methods for SOF and GS-331007 were precise and accurate. Details of the analytical methods for each study were reviewed in the individual study reviews.

LDV itself is an active moiety and following single dose oral administration of [¹⁴C]LDV, unchanged LDV contributed the major portion (> 98%) of circulating radioactivity through 24 hours postdose in plasma, with 1.1% and 0.75% of the total radioactivity attributed to

unidentified metabolites M1 and M12, respectively. LDV was characterized in clinical pharmacology studies and used for exposure-response analysis.

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

The total (bound+unbound) moiety of SOF and its metabolites were measured. This is acceptable because protein binding of SOF (62%) is independent of concentration, and protein binding of GS-331007 was minimal in human plasma.

The total (bound+unbound) moiety of LDV and its metabolites were measured. This is acceptable because LDV is highly protein bound (>99.8%) and is independent of concentration. In addition, the fraction of protein binding is not altered by renal or hepatic impairment.

3. DETAILED LABELING RECOMMENDATIONS

Although details of the labeling are still under active negotiation, some general clinical pharmacology proposals can be made based on the clinical pharmacology results from studies with SOF, LDV, and LDV/SOF and the additional PK, efficacy and safety results provided by the applicant:

- In accordance with the Sovaldi® label, no dose recommendation can be given for patients with severe renal impairment (estimated Glomerular Filtration Rate (eGFR) <30 mL/min/1.73m²) or with end stage renal disease (ESRD) due to higher exposures (up to 20-fold) of the predominant sofosbuvir metabolite.
- No dose adjustment of LDV/SOF is required for patients with mild or moderate renal impairment.
- No dose adjustment of LDV/SOF is required for patients with mild, moderate or severe hepatic impairment (Child-Pugh Class A, B or C).
- Drugs that are P-gp inducers in the intestine (e.g. RIF or St. John's Wort) may decrease LDV and sofosbuvir plasma concentrations leading to reduced therapeutic effect of LDV/SOF and should not be used with LDV/SOF.
- LDV solubility decreases as pH increases. Drugs that increase gastric pH are expected to decrease concentrations of LDV
 - LDV/SOF should only be coadministered with proton pump inhibitors (PPI) simultaneously
 - LDV/SOF should be coadministered with H₂-receptor antagonist (H₂RA) either simultaneously or 12 hours apart.
- No dose adjustment is needed for LDV/SOF and the following drugs based on ADME profiles of SOF and LDV and/or the results from the DDI studies with LDV/SOF or its components: ABC, ATV/r, CsA, DRV/RTV, FTC, EFV, 3TC, methadone, RAL, RPV, TAC, TDF (excluding TDF when used as part of Stribild® because there are insufficient data to make a dosing recommendation), or verapamil.

4. APPENDICES

4.1 Individual Study Review

4.2.1 Biopharmaceutics

4.2.1.1 GS-US-337-0101: A Phase 1 Single-Dose Study to Evaluate the Relative Bioavailability and the Effect of Food on GS-7977 and GS-5885 Fixed-Dose Combination Tablets in Healthy Volunteers

Objectives:

- To evaluate the relative bioavailability of the to-be-marketed formulation of LDV/SOF FDC tablet relative to existing individual tablet formulations in healthy subjects.
- To evaluate the effect of food on the pharmacokinetics (PK) of the to-be-marketed formulation of a LDV/SOF FDC tablet in healthy subjects

Study Design: This is a Phase 1, randomized, open-label, single-center, single-dose, crossover Study in 2 cohorts.

In Cohort 1, the relative bioavailability of the to-be-marketed formulation of SOF/LDV FDC tablet was evaluated relative to concurrent administration of the individual tablet formulations (SOF 400 mg taken as 1 tablet plus LDV 90 mg taken as 1 tablet; SOF+LDV). The relative bioavailability was assessed using a two-period, two-sequence crossover design.

In Cohort 2, the PK of the to-be-marketed formulation of SOF/LDV FDC tablet was assessed under fasted conditions and 2 different fed conditions (high-calorie/high-fat meal and moderate-fat meal) using a three-period, six-sequence crossover design.

Within a cohort, eligible subjects were enrolled and randomized to a treatment sequence. Following screening procedures and baseline assessments (Day 0), subjects received doses of study drug with 9-day washout periods between doses.

Formulation: SOF (b) (4) tablets (400 mg), LDV (b) (4) tablets (90 mg), LDV/SOF 90/400 mg to-be-marketed FDC tablets

PK Sampling: Blood samples were collected relative to the dosing of SOF or LDV. In Cohort 1, blood samples were collected on Days 1 and 11; in Cohort 2, blood samples were collected on Days 1, 11, and 21. Regardless of study day, blood samples for SOF, GS-566500, GS-331007, and LDV plasma concentrations were collected predose, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 14, 16, 20, 24, 48, 72, 96 and 120 hours postdose.

Analytical Methods: Concentrations of SOF, GS-566500, GS-331007, and LDV in human plasma samples were determined using fully validated high-performance liquid chromatography-tandem mass spectroscopy (LC/MS/MS) bioanalytical methods. All samples were analyzed in the timeframe supported by frozen stability storage data. The assays for SOF, GS-566500, GS-331007, and LDV were all performed and validated (b) (4)

The standard curve and QC data indicated that the plasma assay methods for SOF, GS-566500, GS-331007 and LDV were precise and accurate as shown in the following table.

Table 1: Bioanalytical Assay Validation for Sofosbuvir and Ledipasvir and Metabolites in Human Plasma

Parameter	SOF	GS-566500	GS-331007	LDV
Linear range (ng/mL)	5–5000	10–5000	10–5000	1–2000
LLOQ (ng/mL)	5	10	10	1
Intraassay precision range (%CV)	1.3 to 7.4	3.3 to 9.1	0.8 to 5.6	1.0 to 8.8 ^a
Intraassay accuracy range (%RE)	-3.3 to 8.2	-5.2 to 5.9	-0.1 to 7.5	-7.1 to 3.6 ^a
Stability in frozen matrix (days)	377 days at -70°C and 36 days at -20°C	308 days at -70°C and 25 days at -20°C	308 days at -70°C and 25 days at -20°C	351 days at -20°C and -70°C

CV = coefficient of variation; LLOQ = lower limit of quantitation; RE = relative error

^a Interday ranges reported

Pharmacokinetic Results:

In Cohort 1, plasma exposures of SOF, its metabolites GS-566500 and GS-331007 (predominant circulating nucleoside metabolite), and LDV are not expected to be significantly different upon administration of SOF/LDV FDC from SOF+LDV coadministered as individual components. The lower bounds of the 90% CIs for the primary PK parameters (AUC and C_{max}) of SOF, GS-566500, and LDV were greater than 70% (Table 2). The 90% CIs of the GLSM ratios for all GS-331007 primary PK parameters were contained within bioequivalence bounds of 80% to 125%.

Table 2: Statistical Comparison of PK Parameters Following Administration of SOF/LDV FDC or SOF+LDV

PK Parameter	GLSM		%GLSM Ratio (90%CI)
	SOF+LDV (N=28)	SOF/LDV FDC (N=28)	SOF/LDV FDC vs SOF+LDV (N=28)
SOF			
AUC _{last} (h·ng/mL)	1438.05	1258.23	87.50 (77.80, 98.40)
AUC _{inf} (h·ng/mL)	1444.96	1264.94	87.54 (77.93, 98.33)
C _{max} (ng/mL)	1399.00	1151.56	82.31 (71.04, 95.37)
GS-566500			
AUC _{last} (h·ng/mL)	1840.28	1565.57	85.07 (76.09, 95.12)
AUC _{inf} (h·ng/mL)	1896.72	1613.24	85.05 (76.72, 94.29)
C _{max} (ng/mL)	475.18	401.67	84.53 (74.26, 96.22)
GS-331007			
AUC _{last} (h·ng/mL)	10,843.68	11,392.88	95.18 (89.50, 101.22)
AUC _{inf} (h·ng/mL)	11,543.32	12,105.63	95.35 (89.99, 101.04)
C _{max} (ng/mL)	725.51	735.82	98.60 (90.81, 107.05)
LDV			
AUC _{last} (h·ng/mL)	7325.92	7043.59	96.15 (79.34, 116.51)
AUC _{inf} (h·ng/mL)	8556.59	8240.56	96.31 (79.21, 117.10)
C _{max} (ng/mL)	285.66	280.53	98.21 (81.89, 117.77)

In Cohort 2, as shown in Table 3, food increased SOF and GS-566500 mean plasma exposure (AUC and C_{max}) by < 2-fold. For GS-331007, an approximately 18% to 30% lower C_{max} was observed upon SOF/LDC FDC administration with food, with no change in AUC. These results were consistent with the data from previous Phase 1 studies (Studies P7977-1318 and P7977-0111) with SOF as a single agent, which demonstrated that SOF as a component of the LDV/SOF FDC could be administered without regard to food.

Similar LDV plasma exposures (AUC and C_{max}) were achieved upon administration of SOF/LDV FDC under fasted or fed conditions. The 90% CIs of the GLSM ratios (fed/fasted treatments) were contained within 70% to 143% (Table 2). A food effect has been previously observed with LDV (single-agent, conventional formulation, Study GS-US-256-0101): administration of LDV with a high fat meal delayed LDV absorption and decreased LDV plasma exposure by approximately 45% in mean C_{max}, AUC_{last}, and AUC_{inf}. However, the PK of LDV administered within the FDC ((b) (4) formulation) is not significantly altered by food.

Therefore, SOF/LDV FDC was studied in the Phase 3 studies without regard to food.

Table 3: Statistical Comparison of PK Parameters Following Administration of SOF/LDV FDC under Fasted, Moderate-Fat, or High-Calorie/High-Fat Conditions

PK Parameter	GLSM			%GLSM Ratio (90% CI)	
	High-Calorie (N=28)	Moderate-Fat (N=29)	Fasted (N=29)	High-Calorie/Fasted (N=28)	Moderate-Fat/Fasted (N=29)
SOF					
AUC _{last} (h·ng/mL)	2487.13	2718.58	1393.58	178.47 (161.08,197.74)	195.08 (176.28, 215.88)
AUC _{inf} (h·ng/mL)	2504.08	2729.75	1399.32	178.95 (161.66,198.09)	195.08 (176.44, 215.68)
C _{max} (ng/mL)	1279.71	1405.34	1112.45	115.04 (98.65,134.14)	126.33 (108.52,147.06)
GS-566500					
AUC _{last} (h·ng/mL)	2436.96	2381.41	1326.14	183.76 (168.11, 200.87)	179.57 (164.46,196.08)
AUC _{inf} (h·ng/mL)	2488.65	2435.72	1378.15	180.58 (166.11, 196.31)	176.74 (162.74, 191.94)
C _{max} (ng/mL)	489.58	477.94	317.32	154.29 (138.90, 171.38)	150.62 (135.77,167.10)
GS-331007					
AUC _{last} (h·ng/mL)	12082.00	12584.75	11024.66	109.59 (103.49,116.06)	114.15 (107.87, 120.80)
AUC _{inf} (h·ng/mL)	12895.73	13524.55	11518.21	111.96 (106.66, 117.52)	117.42 (111.93, 123.18)
C _{max} (ng/mL)	586.73	681.09	835.51	70.22 (65.03, 75.83)	81.52 (75.56, 87.94)
LDV					
AUC _{last} (h·ng/mL)	7258.61	7996.14	6997.22	103.74 (88.78, 121.21)	114.28 (97.98, 133.29)
AUC _{inf} (h·ng/mL)	8648.33	9700.46	8412.86	102.80 (88.46,119.47)	115.31 (99.40, 133.76)
C _{max} (ng/mL)	246.25	303.22	279.18	88.21 (75.80,102.64)	108.61 (93.50,126.17)

Conclusion:

- Similar plasma exposures of SOF, its metabolites GS-566500 and GS-331007 (predominant circulating nucleoside metabolite), and LDV are achieved following administration of the to-be-marketed SOF/LDV FDC formulation and SOF and LDV administered together as single agents.
- The SOF/LDV FDC can be administered without regard to food

4.2.1.2 GS-US-256-0110: A Phase 1 Study to Evaluate the Relative Bioavailability and Safety of a New Tablet Formulation of GS-5885 and the Effect of Acid Reducing Agents on the New Tablet Formulation

Objectives:

- To compare the relative bioavailability of a new tablet formulation of GS-5885 relative to the existing tablet formulation in healthy subjects
- To evaluate the effect of a representative acid reducing agent, omeprazole (OME), on the pharmacokinetics of GS-5885 (LDV) administered as the new tablet formulation to healthy subjects
- To evaluate the pharmacokinetics of the LDV test formulation following multiple dose administration to healthy subjects

Study Design:

This Phase 1 study evaluated the bioavailability, safety, and tolerability of a new formulation of LDV in 2 cohorts. Cohort 1 of this study had a randomized, open-label, 2-sequence, single-dose, 3-period crossover design, while Cohort 2 had an open-label, multiple-dose design.

Eligible Cohort 1 subjects (n=18) were randomized to one of two 3-period treatment sequences (Treatment Sequence 1 = ABC or Treatment Sequence 2 = BAC), where Treatment A was LDV conventional formulation (reference formulation) 30 mg administered orally as a single dose; Treatment B was LDV (b) (4) formulation (test formulation) 30 mg administered orally as a single dose; and Treatment C was OME 20 mg administered orally as once daily for 6 days, followed by LDV (b) (4) formulation) 30 mg administered orally as a single dose 2 hours after the last OME dose. All LDV doses were administered after a meal. Omeprazole was administered under fasting conditions. Each treatment period within a sequence was separated by 7 days of washout.

Eligible Cohort 2 subjects (n=15) were enrolled into a single treatment group (Treatment D). Subjects received LDV (b) (4) formulation) 120 mg administered orally twice a day (BID) for 11 days with a moderate fat meal. The last dose of LDV was given in the morning on Day 11.

Formulation:

LDV: 1) 30-mg tablets, (b) (4) formulation (test), Lot No. CF1109B1
2) 30-mg tablets, conventional formulation [reference], Lot No. CF1105B1
OME 20-mg capsules, Lot No. G002265

PK Sampling: Serial blood samples were collected at the following time points on Days 1, 8 and 20 in Cohort 1 relative to the LDV dose: 0 (predose, ≤ 5 min before dose), 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 14, 16, 20, 24, 48, 72, 96, 120 and 144 hours post dose. On Day 20, when both OME and LDV drugs were given, samples were collected relative to the LDV dose.

Analytical Methods: Concentrations of LDV in human plasma samples were determined using fully validated high-performance liquid chromatography-tandem mass spectroscopy (LC/MS/MS) bioanalytical methods. All samples were analyzed in the timeframe supported by frozen stability storage data. The assay for LDV was all performed and validated by (b) (4)

The standard curve and QC data indicated that the plasma assay method for LDV was precise and accurate as shown in the following table.

Table 1: Bioanalytical Assay Validation for LDV in Human Plasma

Parameter	LDV
Linear range (ng/mL)	1-2000 ng.mL
LLOQ ^a (ng/mL)	1 ng.mL
Interassay precision range	1.0 to 8.8 (%CV)
Interassay accuracy range	-7.1 to -3.6 (%RE)
Stability in frozen matrix (days)	351 days at -20°C and -70°C

^a Lower limit of quantitation

Pharmacokinetic Results: Single dose PK parameters of LDV after oral administration of LDV conventional (Treatment A) and (b) (4) (Treatment B) formulations alone and upon staggered administration of the (b) (4) formulation and OME (Treatment C) are presented in Table 2.

Table 2: LDV PK Parameters Following Administration of LDV Reference Formulation or (b) (4) Formulation Alone or 2 Hours after Administration of Omeprazole, Cohort 1

LDV PK Parameter Mean (%CV)	LDV Reference Formulation (Treatment A) (N=18)	LDV Test Formulation (Treatment B) (N=17)	LDV Test Formulation + Omeprazole (Treatment C) (N=16)
AUC _{last} (ng*hr/ml)	1508.3 (43.9)	1845.2 (33.5)	1069.1 (45.5)
AUC _{inf} (ng*hr/ml)	1838.1 (50.5)	2141.4 (38.8)	1298.2 (50.7)
AUC _{exp} (%)	16.2 (56.0)	12.1 (48.9)	15.1 (64.6)
C _{max} (ng/ml)	54.6 (39.5)	64.8 (32.9)	36.2 (55.9)
C _{last} (ng/ml)	3.59 (61.15)	3.53 (55.02)	2.34 (66.31)
T _{max} (hr) ^a	4.50 (4.50, 5.00)	4.50 (4.50, 5.00)	6.00 (5.00, 8.00)
T _{1/2} (hr) ^a	49.37 (39.52, 67.34)	50.70 (39.08, 59.90)	54.29 (37.40, 67.21)
T _{last} (hr) ^a	144.00 (120.00, 144.00)	144.00 (144.00, 144.00)	144.00 (144.00, 144.00)

^a Median (Q1, Q3)

Statistical comparisons of LDV primary PK parameters AUC_{inf}, AUC_{last} and C_{max}, following administration of LDV (b) (4) formulation alone (Treatment B) versus conventional formulation (Treatment A) or (b) (4) formulation alone (Treatment B) versus (b) (4) formulation administered 2 hours post OME (Treatment C) are presented in Table 3.

Table 3: Statistical Comparisons of LDV PK Parameters Following Administration of LDV Reference and (b) (4) Formulations (Treatments A and B), and Following Administration of (b) (4) Formulation Alone and 2 hours post Omeprazole (Treatments B and C)

GS-5885 PK Parameters	Geometric Least Squares Means		Geometric Least Squares Means Ratio (90% CI)
	LDV Test Formulation (Treatment B) (N=17)	LDV Reference Formulation (Treatment A) (N=18)	Treatment B/Treatment A
C _{max} (ng/mL)	61.35	49.83	123.11 (107.11, 141.50)
AUC _{inf} (ng·h/mL)	2000.93	1622.23	123.34 (107.11, 142.04)
AUC _{last} (ng·h/mL)	1729.66	1350.35	128.09 (110.06, 149.07)
GS-5885 PK Parameters	LDV Test Formulation + Omeprazole (Treatment C) (N=16)	LDV Test Formulation (Treatment B) (N=17)	Treatment C/Treatment B
C _{max} (ng/mL)	31.87	61.03	52.23 (41.38, 65.92)
AUC _{inf} (ng·h/mL)	1155.07	1975.76	58.46 (48.31, 70.75)
AUC _{last} (ng·h/mL)	974.36	1730.57	56.30 (46.42, 68.30)

Administration of the LDV (b) (4) formulation achieved approximately 23% higher AUC_{inf} and C_{max}, and 28% higher AUC_{last}, as compared to the exposures observed upon dosing of the conventional formulation.

Administration of LDV (b) (4) formulation 2 hours after OME administration resulted in a substantial decrease in the relative bioavailability of LDV, as assessed by an approximately 42% lower AUC_{inf}, 44% lower AUC_{last} and 48% lower C_{max}, relative to LDV (b) (4) formulation, administered alone.

Single (first dose) and multiple dose PK parameters of LDV after oral administration of the (b) (4) formulation of LDV 120 mg BID for 11 days are presented in Table 4.

Table 4: LDV PK Parameters Following BID Administration of GS-5885, Cohort 2

LDV PK Parameter Mean (%CV) (N=13)	LDV Test Formulation (Day 1)	LDV Test Formulation (Day 11)
AUC (ng·hr/ml) ^a	2166.5 (33.6)	17171.4 (34.8)
C _{max} (ng/ml)	295.1 (33.3)	1727.0 (34.8)
C _{last} (ng/ml) ^b	182.34 (25.89)	1223.3 (37.2)
T _{max} (hr) ^c	5.00 (4.50, 5.00)	4.50 (4.00, 4.50)
T _{last} (hr) ^c	12.00 (12.00, 12.00)	12.00 (12.00, 12.00)

a AUC: AUC_{last} (Day 1), AUC_{tau} (Day 11)

b C_{last}= C_{tau} (Day 11)

c Median (Q1, Q3);

Supratherapeutic exposures of LDV were achieved upon BID dosing. LDV mean exposure parameters AUC and C_{max} were approximately 8.5-fold and 6-fold higher on Day 11 than those observed on Day 1, consistent with the long t_{1/2} of LDV.

Descriptive comparison of LDV 120 mg Day 1 AUC_{last} and C_{max} values to AUC₀₋₁₂ (data on file) and C_{max} values upon administration of LDV 30 mg (Cohort 1: (b) (4) formulation), revealed approximately dose proportional increases in exposure, suggestive of near linear PK of (b) (4) formulation over this dose range. These LDV exposures are similar to those observed in the thorough QT study (GS-US-344-0109).

Conclusions:

- Administration of LDV (b) (4) formulation resulted in modestly higher (~23% to 28%) plasma exposure (AUC and C_{max}) relative to the conventional formulation. Accordingly, the (b) (4) formulation has been selected for clinical development.
- A substantial decrease in LDV plasma exposure (~ 42% to 48% lower AUC and C_{max}) was observed upon administration of omeprazole 2 hours earlier.
- Supratherapeutic exposures of LDV ((b) (4) formulation) were achieved upon administration of multiple doses of LDV 120 mg BID.

4.2.2 General Pharmacokinetics/Pharmacodynamics

4.2.2.1 GS-US-334-0111: A Phase 1 Single Dose Study to Investigate the Pharmacokinetics, Safety and Tolerability of GS-7977 and GS-7977/GS-5885 FDC in Healthy Japanese and Caucasian Subjects

Objectives:

- To investigate the pharmacokinetics (PK) of sofosbuvir (SOF, GS-7977) and metabolites (GS-566500 and GS-331007) following administration of SOF in healthy Japanese and Caucasian subjects
- To investigate the PK of SOF, metabolites (GS-566500 and GS-331007), and ledipasvir (LDV, GS-5885) following administration of SOF/LDV fixed-dose combination (FDC) tablet in healthy Japanese and Caucasian subjects
- To assess the safety and tolerability of SOF and SOF/LDV FDC in healthy Japanese and Caucasian subjects

Study Design: This is a Phase 1, open-label, single-dose study. A total of 64 subjects were enrolled into 1 of 4 treatment groups of 16 healthy subjects each (8 Japanese and 8 Caucasian subjects per group). Japanese subjects were first generation. Subjects were born in Japan, had not lived outside Japan for > 10 years, and could trace their maternal and paternal Japanese ancestry of parents and grandparents. Their lifestyle, including diet, had not significantly changed since leaving Japan. Eligible subjects received a single oral dose of study drug on Day 1 under fasting conditions, corresponding to their assigned group, as follows:

- Group 1: SOF 200 mg (1 × 200-mg tablet)
- Group 2: SOF 400 mg (1 × 400-mg tablet)
- Group 3: SOF 800 mg (2 × 400-mg tablets)
- Group 4: SOF/LDV FDC 400 mg/90 mg (1 × 400-mg/90-mg FDC tablet)

All the medications were administered under fasting conditions.

Formulation:

SOF 200- mg tablets (Lot # 11G086-P1)

SOF 400- mg tablets (Lot # DC1203B1)

SOF/LDV FDC 400-mg/90-mg tablets (Lot # DK1201B1)

PK Sampling: For Groups 1, 2, and 3, serial blood samples for analysis of plasma SOF and metabolite (GS-566500 and GS-331007) concentrations were collected at the following time points: predose (within 5 minutes), 0.5, 1, 1.5, 2, 3, 4, 4.5, 5, 6, 8, 10, 12, 24, 36, 48, 72, and 96 hours postdose. Urine samples for analysis of urine SOF and metabolite (GS-566500 and GS-331007) concentrations were collected at the following time points: empty bladder predose and at the 0–6, 6–12, 12–24, 24–48, 48–72, and 72–96 hour collection intervals postdose.

For Group 4, serial blood samples for analysis of plasma SOF, metabolite (GS-566500 and GS-331007), and LDV concentrations were collected at the following time points: predose (within 5 minutes), 0.25, 0.5, 1, 1.5, 2, 3, 4, 4.5, 5, 6, 8, 10, 12, 24, 36, 48, 72, 96, 120, and 144 hours postdose.

Analytical Methods: Concentrations of SOF, GS-566500, GS-331007, and LDV in human plasma were determined using fully validated high-performance liquid chromatography-tandem mass spectroscopy (LC/MS/MS) bioanalytical methods. Concentrations of SOF, GS-566500, and GS-331007 in human urine were determined using fully validated LC/MS/MS bioanalytical methods. The assays were all performed (b) (4)

All samples were analyzed in the timeframe supported by frozen stability storage data. The standard curve and QC data indicated that the plasma and urine assay methods for SOF, GS-566500, and GS-331007 were precise and accurate as shown in the following table.

Table 1: Summary of Quality Control (QC) Results

Parameter	SOF	GS-566500	GS-331007	LDV
Linear range (ng/mL)	5 to 5000	10 to 5000	10 to 5000	1 to 2000
Lower limit of quantitation (ng/mL)	5	10	10	1
Interassay precision range (%CV)	1.3 to 7.4 ^a	3.3 to 9.1 ^a	0.8 to 5.6 ^a	1.0 to 8.8
Interassay accuracy range (%RE)	-3.3 to 8.2 ^a	-5.2 to 5.9 ^a	-0.1 to 7.5 ^a	-7.1 to -3.6
Stability in frozen matrix (Days)	377 Days at -70°C and 36 Days at -20°C	308 Days at -70°C and 25 Days at -20°C	308 Days at -70°C and 25 Days at -20°C	351 Days at -20°C and -70°C

a Intraday ranges reported

Note: RE = relative error; CV = coefficient of variance

Pharmacokinetic Results: Tables 2, 3, and 4 present SOF, GS-566500 and GS-331007 PK parameters, respectively, following administration of a single dose of SOF 200 mg, 400 mg, or 800 mg, or the SOF/LDV FDC (400 mg/90 mg) to Japanese and Caucasian subjects.

Table 2: Sofosbuvir Pharmacokinetic Parameters

SOF PK Parameter	SOF 200 mg		SOF 400 mg		SOF 800 mg		SOF/LDV FDC (400 mg/90 mg)	
	Japanese (N=8)	Caucasian (N=8)	Japanese (N=8)	Caucasian (N=8)	Japanese (N=8)	Caucasian (N=8)	Japanese (N=8)	Caucasian (N=8)
AUC _{last} (ng•h/mL)	288.7 (48.2)	288.9 (41.6)	643.2 (47.0)	491.8 (16.9)	1205.2 (27.4)	1140.9 (30.2)	1569.4 (51.8)	1609.5 (46.5)
AUC _{inf} (ng•h/mL)	297.0 (46.2)	300.0 (41.7)	648.7 (46.5)	499.0 (16.8)	1212.2 (27.1)	1146.9 (30.0)	1576.0 (51.5)	1615.7 (46.3)
AUC _{exp} (%)	3.85 (105.9)	3.63 (64.6)	0.97 (52.3)	1.49 (35.2)	0.64 (51.7)	0.57 (55.6)	0.74 (140.6)	0.47 (59.8)
C _{max} (ng/mL)	349.4 (51.3)	319.2 (47.4)	639.3 (28.6)	631.3 (39.1)	1148.5 (45.3)	1218.8 (35.2)	1316.0 (34.1)	1412.0 (33.8)
C _{last} (ng/mL)	14.4 (45.6)	19.7 (59.5)	9.1 (60.4)	13.3 (53.0)	12.4 (41.9)	10.2 (60.3)	10.9 (45.0)	8.2 (43.4)
T _{max} (h)	0.50 (0.50, 0.52)	1.00 (0.50, 1.00)	0.50 (0.50, 0.50)	0.50 (0.50, 0.51)	0.55 (0.50, 0.85)	0.50 (0.50, 1.00)	0.53 (0.50, 1.25)	0.54 (0.51, 0.79)
T _{last} (h)	2.00 (2.00, 3.00)	2.00 (2.00, 3.00)	3.00 (3.00, 4.25)	3.00 (2.00, 3.51)	3.00 (3.00, 4.25)	4.00 (3.00, 4.27)	4.26 (3.00, 5.00)	4.52 (4.00, 5.01)
t _{1/2} (h)	0.37 (0.34, 0.48)	0.32 (0.29, 0.43)	0.43 (0.38, 0.53)	0.41 (0.29, 0.50)	0.39 (0.38, 0.42)	0.43 (0.38, 0.52)	0.38 (0.36, 0.45)	0.49 (0.42, 0.60)
CL/F (L/h)	821.8 (47.8)	788.5 (46.0)	709.9 (33.1)	828.8 (22.8)	704.1 (27.0)	751.5 (28.8)	376.9 (89.0)	294.2 (42.7)
CL _r (L/min)	0.189 (30.4)	0.161 (27.5)	0.230 (27.2)	0.227 (32.1)	0.205 (22.4)	0.201 (16.6)	—	—
% Excreted in Urine	1.60 (44.1)	1.42 (42.9)	2.08 (27.9)	1.71 (38.2)	1.82 (27.4)	1.73 (34.5)	—	—

Note: All parameters are reported as mean (%CV), except for T_{max}, T_{last}, and t_{1/2}, which are reported as median (Q1, Q3).

Table 3: GS-566500 Pharmacokinetic Parameters

GS-566500 PK Parameter	SOF 200 mg		SOF 400 mg		SOF 800 mg		SOF/LDV (400 mg/90 mg)	
	Japanese (N=8)	Caucasian (N=8)	Japanese (N=8)	Caucasian (N=8)	Japanese (N=8)	Caucasian (N=8)	Japanese (N=8)	Caucasian (N=8)
AUC _{last} (ng•h/mL)	731.6 (49.3)	440.3 (25.1)	1434.3 (24.2)	966.0 (28.1)	2340.3 (20.5)	1882.9 (24.0)	1896.3 (38.6)	1675.9 (42.9)
AUC _{inf} (ng•h/mL)	767.7 (47.7)	476.8 (23.1)	1471.3 (23.7)	1003.7 (27.0)	2386.5 (20.4)	1931.0 (23.7)	1940.4 (37.9)	1728.1 (42.2)
AUCexp (%)	5.12 (24.5)	8.00 (31.5)	2.64 (27.9)	4.04 (39.9)	1.95 (19.7)	2.55 (32.1)	2.60 (43.6)	3.33 (32.3)
C _{max} (ng/mL)	242.6 (39.1)	153.2 (28.8)	391.8 (22.5)	286.6 (28.7)	647.3 (20.8)	547.3 (18.1)	546.4 (32.4)	425.8 (37.3)
C _{last} (ng/mL)	13.5 (8.9)	13.4 (22.1)	13.1 (14.4)	13.0 (18.4)	16.3 (27.1)	16.5 (26.6)	15.5 (13.0)	17.2 (27.2)
T _{max} (h)	1.00 (1.00, 1.25)	1.00 (1.00, 1.50)	1.00 (1.00, 1.00)	1.00 (1.00, 1.50)	1.00 (1.00, 1.75)	1.25 (1.00, 1.75)	1.50 (1.25, 1.87)	1.00 (1.00, 1.51)
T _{last} (h)	8.00 (8.00, 8.01)	8.00 (7.00, 8.12)	11.00 (10.00, 12.00)	10.00 (9.00, 11.00)	12.00 (12.00, 12.00)	12.00 (10.00, 12.01)	12.00 (10.02, 12.00)	12.00 (10.01, 12.00)
t _{1/2} (h)	1.78 (1.71, 1.85)	1.93 (1.78, 1.99)	1.99 (1.86, 2.01)	2.02 (1.94, 2.11)	1.96 (1.90, 2.04)	2.04 (1.85, 2.11)	2.00 (1.90, 2.06)	2.04 (1.99, 2.22)
CL _r (L/min)	0.111 (20.9)	0.128 (20.2)	0.127 (13.5)	0.133 (21.8)	0.109 (18.4)	0.126 (14.1)	—	—
% Excreted in Urine	3.23 (43.3)	2.39 (33.6)	3.59 (27.0)	2.55 (31.3)	2.51 (24.6)	2.35 (25.6)	—	—

Note: All parameters are reported as mean (%CV), except for T_{max}, T_{last}, and t_{1/2}, which are reported as median (Q1, Q3).

Table 4: GS-331007 Pharmacokinetic Parameters

GS-331007 PK Parameter	SOF 200 mg		SOF 400 mg		SOF 800 mg		SOF/LDV (400 mg/90 mg)	
	Japanese (N=8)	Caucasian (N=8)	Japanese (N=8)	Caucasian (N=8)	Japanese (N=8)	Caucasian (N=8)	Japanese (N=8)	Caucasian (N=8)
AUC _{last} (ng•h/mL)	5997.6 (28.7)	7285.5 (18.6)	10,410.9 (23.2)	11,038.3 (24.3)	16,398.0 (17.5)	19,769.9 (11.4)	11,484.7 (30.7)	13,556.0 (36.1)
AUC _{inf} (ng•h/mL)	6462.0 (26.0)	7719.8 (17.5)	10,988.7 (21.9)	11,478.1 (23.3)	17,415.1 (14.6)	20,751.0 (11.4)	12,074.2 (29.8)	14,269.2 (34.8)
AUC _{exp} (%)	8.09 (43.0)	5.77 (28.8)	5.45 (21.7)	3.96 (30.1)	6.15 (73.2)	4.65 (73.8)	5.10 (43.5)	5.33 (54.1)
C _{max} (ng/mL)	736.3 (29.6)	988.2 (20.3)	1437.9 (32.2)	1232.1 (17.8)	1808.6 (34.1)	1747.9 (32.2)	876.9 (35.8)	904.3 (33.2)
C _{last} (ng/mL)	12.6 (14.6)	13.0 (11.4)	15.6 (14.6)	12.6 (21.0)	21.9 (37.9)	24.5 (51.4)	13.4 (23.6)	14.8 (35.8)
T _{max} (h)	2.00 (1.77, 2.50)	1.75 (1.25, 2.50)	2.09 (1.50, 3.00)	2.00 (1.75, 2.50)	2.50 (1.75, 3.00)	3.00 (2.00, 3.00)	2.50 (1.25, 3.00)	4.25 (3.01, 4.50)
T _{last} (h)	72.00 (48.00, 72.00)	48.00 (48.00, 72.00)	72.00 (72.00, 84.00)	84.00 (72.00, 96.01)	96.00 (96.00, 96.00)	96.00 (96.00, 96.02)	96.01 (96.00, 132.00)	108.00 (96.00, 144.00)
t _{1/2} (h)	24.38 (22.17, 27.51)	22.42 (19.41, 25.13)	24.94 (23.17, 28.22)	23.00 (20.45, 28.32)	26.45 (24.68, 37.04)	24.86 (23.54, 26.63)	27.66 (25.38, 28.63)	33.18 (27.61, 35.23)
CL _r (mL/h)	0.145 (28.0)	0.147 (23.4)	0.176 (12.8)	0.160 (25.2)	0.174 (16.1)	0.152 (26.7)	—	—
% Excreted in Urine	53.9 (15.9)	67.1 (14.8)	57.9 (13.4)	54.1 (18.3)	46.1 (20.7)	47.3 (23.9)	—	—

Note: All parameters are reported as mean (%CV), except for T_{max}, T_{last}, and t_{1/2}, which are reported as median (Q1, Q3).

The PK parameters for SOF, GS-566500 and GS-331007 following administration of a single dose of SOF 200 mg, 400 mg, or 800 mg, or the SOF/LDV FDC (400 mg/90 mg) to Caucasian subjects are in the range of observed values for Caucasian healthy subjects in other studies.

The power model mean slope and 90% CIs indicate that near dose linearity was observed for SOF (Japanese and Caucasian subjects) AUC_{inf}, AUC_{last}, and C_{max} across the evaluated dose range of SOF 200 mg to 800 mg. GS-331007 (Japanese and Caucasian subjects) AUC_{inf}, AUC_{last}, and C_{max} showed modestly less than dose-proportional increases over the evaluated dose range. These results are consistent with historical data (ie., Studies P7977-0111 and P7977-0613).

Table 5 presents LDV PK parameters following administration of a single dose of the SOF/LDV FDC (400 mg/90 mg) to Japanese and Caucasian subjects.

Table 5: Ledipasvir Pharmacokinetic Parameters

LDV PK Parameter	SOF/LDV FDC (400 mg/90 mg)	
	Japanese (N=8)	Caucasian (N=8)
AUC _{last} (ng•h/mL)	12339.2 (53.2)	11022.5 (39.4)
AUC _{inf} (ng•h/mL)	13958.9 (53.6)	12427.8 (39.8)
AUC _{exp} (%)	11.5 (34.6)	10.9 (45.2)
C _{max} (ng/mL)	420.7 (49.0)	308.1 (29.0)
C _{last} (ng/mL)	21.9 (63.0)	19.2 (49.3)
T _{max} (h)	5.00 (5.00, 5.00)	5.00 (4.75, 5.02)
T _{last} (h)	144.00 (144.00, 144.00)	144.00 (144.00, 144.00)
t _{1/2} (h)	50.04 (45.81, 55.93)	45.73 (42.18, 51.11)
CL/F (mL/h)	8374.5 (58.4)	8477.0 (44.6)

Note: All parameters are reported as mean (%CV), except for T_{max}, T_{last}, and t_{1/2}, which are reported as median (Q1, Q3).

Table 6 shows the statistical comparisons of SOF, GS-566500, GS-331007, and ledipasvir primary PK parameters in Japanese versus Caucasian subjects. The data indicate no clinically significant differences in the PK of SOF, its metabolites GS-566500 and GS-331007, or LDV were observed between Japanese and Caucasian subjects.

Table 6: Geometric Least-Squares Mean Ratios (90% Confidence Intervals) for Sofosbuvir, GS-566500, GS-331007, and Ledipasvir Primary Pharmacokinetic Parameters in Japanese versus Caucasian Subjects

	SOF 200 mg %GLSM Ratio (90% CI)	SOF 400 mg %GLSM Ratio (90% CI)	SOF 800 mg %GLSM Ratio (90% CI)	SOF/LDV FDC (400 mg/90 mg) %GLSM Ratio (90% CI)
SOF PK Parameter				
AUC _{last} (ng•h/mL)	97.02 (63.62, 147.96)	122.62 (92.54, 162.47)	106.20 (82.63, 136.49)	90.52 (54.44, 150.50)
AUC _{inf} (ng•h/mL)	97.30 (64.77, 146.16)	121.98 (92.30, 161.20)	106.27 (82.84, 136.33)	90.77 (54.90, 150.07)
C _{max} (ng/mL)	101.56 (62.88, 164.02)	107.05 (76.31, 150.18)	96.37 (62.23, 149.23)	93.82 (64.53, 136.42)
GS-566500 PK Parameter				
AUC _{last} (ng•h/mL)	158.37 (119.80, 209.34)	149.64 (117.13, 191.17)	125.17 (103.74, 151.03)	114.13 (78.15, 166.67)
AUC _{inf} (ng•h/mL)	153.51 (117.65, 200.30)	147.47 (116.45, 186.74)	124.39 (103.30, 149.79)	113.27 (78.24, 163.98)
C _{max} (ng/mL)	154.48 (116.12, 205.51)	138.62 (108.12, 177.72)	117.59 (98.72, 140.06)	130.16 (93.88, 180.44)
GS-331007 PK Parameter				
AUC _{last} (ng•h/mL)	80.29 (64.62, 99.77)	94.44 (78.30, 113.90)	82.32 (71.98, 94.16)	85.63 (63.56, 115.36)
AUC _{inf} (ng•h/mL)	82.37 (67.59, 100.36)	95.93 (80.14, 114.82)	83.68 (74.25, 94.30)	85.40 (64.04, 113.89)
C _{max} (ng/mL)	72.72 (57.86, 91.40)	113.48 (91.12, 141.32)	102.44 (73.52, 142.73)	94.34 (68.03, 130.82)
LDV PK Parameter				
AUC _{last} (ng•h/mL)	—	—	—	106.07 (68.95, 163.18)
AUC _{inf} (ng•h/mL)	—	—	—	106.66 (69.13, 164.59)
C _{max} (ng/mL)	—	—	—	125.63 (83.83, 188.26)

Conclusion: No clinically significant differences in the PK of SOF, its metabolites GS-566500 and GS-331007, or LDV were observed between Japanese and Caucasian subjects, supporting the use of SOF 400 mg or SOF/LDV FDC (400 mg/90 mg) in Japanese subjects.

4.2.2.2 GS-US-256-0101: A Phase 1, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety, Tolerability and Pharmacokinetics of Escalating, Single, Oral Doses of GS-5885 in Healthy Subjects

Objectives:

- To characterize the pharmacokinetics (PK) of GS-5885 in plasma following administration of escalating, single, oral doses in healthy subjects
- To evaluate preliminarily the effect of concomitant food intake on GS-5885 PK

Study Design: The study enrolled 6 cohorts: 5 cohorts with 10 subjects and 1 cohort with 11 subjects. In each cohort, 2 of the subjects were randomly assigned to treatment with placebo. Five cohorts were dosed under fasting conditions with GS-5885 3 mg or placebo, GS-5885 10 mg or placebo, GS-5885 30 mg or placebo, GS-5885 60 mg or placebo, and GS-5885 100 mg or placebo. Following a review of the safety and PK data (through Day 5) for subjects under fasted conditions, the sixth cohort was dosed under fed conditions with GS-5885 30 mg or placebo.

Formulation:

GS-5885 tablets: 1 mg (Lot No. CF1002B1) and 10 mg (Lot No. CF1003B1)
Placebo tables: Lot No. CF1001B1

PK Sampling: On Day 1, serial blood samples for analysis of plasma concentrations of GS-5885 were collected as follows: predose (≤ 5 min) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24 (Day 2), 36 (Day 2), 48 (Day 3), 72 (Day 4) and 96 (Day 5) hours postdose.

Analytical Methods: Concentrations of LDV in human plasma samples were determined using fully validated high-performance liquid chromatography-tandem mass spectroscopy (LC/MS/MS) bioanalytical methods. All samples were analyzed in the timeframe supported by frozen stability storage data. The assay for LDV was all performed and validated by Bioanalytical group at Gilead Sciences in Durham.

The standard curve and QC data indicated that the plasma assay method for LDV was precise and accurate as shown in the following table.

Table 1: Bioanalytical Assay Validation for GS-5885 in Human Plasma

Parameter	GS-5885
Linear range (ng/mL)	1 to 2000
Lower limit of quantitation (ng/mL)	1
Interassay precision range	3.0 to 9.1 (%CV)
Interassay accuracy range	0.0 to 10.0 (%Bias)
Stability in frozen matrix (-80°C) (days)	69 days

Pharmacokinetic Results:

Table 2 presents the mean GS-5885 plasma PK parameters following oral administration of single escalating doses of GS-5885. The GS-5885 exposure as measured by AUClast, AUCinf, and Cmax increased with escalating doses of GS-5885 under fasted conditions.

Table 2: GS-5885 Plasma PK Parameters Following Single-Dose Administration of GS-5885 by Treatment

PK Parameter	Mean (%CV)				
	Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5
	GS-5885 3 mg (N = 8)	GS-5885 10 mg (N = 8)	GS-5885 30 mg (N = 8)	GS-5885 60 mg (N = 8)	GS-5885 100 mg (N = 8)
C _{max} (ng/mL)	6.0 (37.2)	18.9 (36.4)	73.1 (50.8)	118.3 (50.0)	215.5 (34.6)
T _{max} (h) ^a	6.00 (4.00, 6.00)	5.00 (4.00, 6.00)	6.00 (5.00, 6.00)	6.00 (5.00, 6.00)	6.00 (5.04, 6.00)
AUC _{last} (ng·h/mL)	124.4 (62.3)	496.4 (34.2)	1988.2 (58.2)	3562.4 (55.3)	6265.9 (33.1)
AUC _{inf} (ng·h/mL)	218.0 (60.6)	618.0 (32.3)	2415.9 (60.3)	4711.1 (58.2)	7697.1 (34.3)
t _{1/2} (h) ^a	32.18 (28.84, 65.33)	42.34 (33.80, 57.71)	39.82 (33.15, 41.65)	48.05 (42.99, 58.14)	44.12 (35.92, 51.56)
CL/F (mL/h)	16,894.7 (41.9)	18,656.1 (50.2)	17,034.5 (58.6)	19,883.8 (83.6)	15,271.9 (53.8)
V _z /F (mL)	958,201.6 (34.3)	1,204,954 (55.3)	876,546.3 (44.2)	1,256,090 (60.7)	893,026.3 (35.0)
C _{last} (ng/mL)	1.4 (15.6)	1.7 (32.4)	6.8 (68.0)	15.2 (64.1)	21.8 (38.9)

a Median (Q1, Q3)

Note: Plasma concentrations below lower limit of quantification were treated as 0 for summary statistics and missing for log-normalized data.

Table 3 presents the dose proportionality analysis based on the power model. GS-5885 exposure, assessed as AUC_{inf}, AUC_{last}, and C_{max}, was dose proportional over the dose range of 3 mg to 100 mg, under fasted conditions, indicating that GS-5885 exhibits linear PK.

Table 3: Assessment of Dose Proportionality Using the Power Model

Parameter	Degrees of Freedom	Slope of Ln (Dose)	90% CI
Dose range of 3 to 100 mg			
AUC _{last} (ng·h/mL)	38	1.033	0.922, 1.143
AUC _{inf} (ng·h/mL)	38	1.126	1.017, 1.235
C _{max} (ng/mL)	38	1.015	0.917, 1.112

Note: Analysis of dose proportionality under fasting conditions: 3 mg, 10 mg, 30 mg, 60 mg, and 100 mg

Table 4 summarizes PK parameters of GS-5885 following a single dose of GS-5885, 30 mg, under fasted and fed conditions.

Table 4: Plasma GS-5885 PK Parameters Following Single-dose Administration of GS-5885 by Concomitant Food Intake Status

PK Parameter	Mean (%CV)	
	Cohort 3	Cohort 6
	GS-5885 30 mg (N = 8)	GS-5885 30 mg Fed (N = 8)
C _{max} (ng/mL)	73.1 (50.8)	36.5 (22.6)
T _{max} (h) ^a	6.00 (5.00, 6.00)	8.00 (7.00, 8.00)
AUC _{last} (ng·h/mL)	1988.2 (58.2)	996.5 (21.6)
AUC _{inf} (ng·h/mL)	2415.9 (60.3)	1175.0 (25.3)
t _{1/2} (h) ^a	39.82 (33.15, 41.65)	36.83 (22.19, 49.08)
CL/F (mL/h)	17,034.5 (58.6)	26,917.9 (23.6)
V _z /F (mL)	876,546.3 (44.2)	1,386,469 (24.9)
C _{last} (ng/mL)	6.8 (68.0)	3.1 (42.2)

^a Median (Q1, Q3)

Note: Plasma concentrations below lower limit of quantification (BQL) were treated as 0 for summary statistics and missing for log-normalized data.

Table 5 presents the ratio of the GLSMs (GS-5885 30 mg under fasted conditions/GS-5885 30 mg under fed conditions) for the primary PK parameters.

Table 5: Statistical Evaluations of GS-5885 PK Parameters for Food Effect

PK Parameter	Geometric Least Squares Mean (GLSM)		GLSM Ratio (Fed/Fasted) (%)	90% Confidence Interval
	Cohort 3	Cohort 6		
	GS-5885 30 mg, Fed (N = 8)	GS-5885 30 mg, Fasted (N = 8)		
C _{max} (ng/mL)	35.87	65.33	54.90	39.10, 77.08
AUC _{last} (ng·hr/mL)	977.76	1724.28	56.71	38.87, 82.73
AUC _{inf} (ng·hr/mL)	1143.64	2058.78	55.55	36.88, 83.67

Note: GLSMs calculated using PROC MIXED model.

The data show that high-fat meal delays GS-5885 absorption, prolong T_{max}, and decreases approximately 45% of GS-5885 plasma exposure.

Conclusions:

- Plasma PK profile of GS-5885 following single oral doses under fasting conditions shows that GS-5885 exhibits linear PK over the dose range of 3 to 100 mg.
- Peak concentrations of GS-5885 are measured approximately 6 hours postdose in all cohorts administered GS-5885 under fasting conditions.

- A high-fat meal, prolonged median Tmax to 8 hours and decreases exposure as assessed by AUClast and Cmax by approximately 45%.

4.2.2.3 GS-US-256-0102: A Phase 1, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Antiviral Activity of Escalating, Multiple, Oral Doses of GS-5885 in Treatment Naive Subjects with Chronic Genotype 1 Hepatitis C Virus Infection

Objectives:

- To evaluate the safety and tolerability of escalating, multiple, oral doses of GS-5885 in subjects with chronic genotype 1 HCV infection.
- To evaluate the antiviral activity of GS-5885 against genotype 1 HCV following administration of multiple oral doses.
- To characterize the plasma pharmacokinetics of GS-5885 following administration of escalating, multiple, oral doses in genotype 1 HCV-infected subjects.
- To assess the PK/pharmacodynamic (PD) relationship between HCV viral load change and GS-5885 plasma concentrations following multiple-dose administration.
- To compare GS-5885 antiviral activity in genotype 1a versus 1b infections.

Study Design: Multiple doses of GS-5885 or placebo were administered under fasted conditions once a day for 3 consecutive days as follows:

- **Cohort 1** (N = 12 genotype 1a subjects): GS-5885 3 mg or placebo once daily (total daily dose [TDD] = 3 mg) for 3 days;
- **Cohort 2** (N = 12 genotype 1a subjects): GS-5885 10 mg or placebo once daily (TDD = 10 mg) for 3 days;
- **Cohort 3** (N = 12 genotype 1a subjects): GS-5885 30 mg or placebo once daily (TDD = 30 mg) for 3 days;
- **Cohort 4** (N = 12 genotype 1a subjects): GS-5885 1 mg or placebo once daily for 3 days;
- **Cohort 5** (N = 12 genotype 1b subjects): GS-5885 10 mg or placebo once daily for 3 days
- **Cohort 6** (N = 12 genotype 1a subjects): GS-5885 90 mg or placebo once daily for 3 days

Formulation: GS-5885 tablets: 1 mg (Lot No. CF1002B1) and 10 mg (Lot No. CF1003B1)
Placebo tables: Lot No. CF1001B1

PK Sampling: PK samples for GS-5885 were obtained prior to dosing (≤ 10 minutes) and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 16 hours after the first dose on Day 1, prior to the morning dosing on Day 2 (24 hours after the first dose), and prior to the morning dosing on Day 3 (≤ 10 minutes) and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 16 hours after morning dosing on Day 3. Additional PK samples were obtained at 24 (Day 4), 36 (Day 4), 48 (Day 5), 72 (Day 6), 96 (Day 7), 120 (Day 8), and 168 (Day 10) hours after the last dose.

Analytical Methods: Concentrations of GS-5885 in plasma samples were determined using fully validated high-performance liquid chromatography/tandem mass spectroscopy (LC/MS/MS) bioanalytical methods. All samples were analyzed in the timeframe supported by frozen stability

storage data. The assays for GS-5885 were performed and validated

(b) (4)

The standard curve and QC data indicated that the plasma assay method for LDV was precise and accurate as shown in the following table.

Table 1: Bioanalytical Assay Validation for GS-5885 in Human Plasma

Parameter	GS-5885
Linear range (ng/mL)	1-2,000
LLQ (ng/mL)	1
Interassay precision range (%CV)	1.0 to 8.8
Interassay accuracy range (%RE)	-7.1 to -3.6
Stability in frozen matrix (days)	351 Days at -20°C and -70°C

Pharmacokinetic Results: Table 2 presents PK parameters of GS-5885 after single-dose and multiple-dose administration of GS-5885 in subjects with genotype 1a and 1b chronic HCV infection. For GS-5885, the C_{max} and AUC increased in an approximately dose-proportional manner over the dose range of 1 to 90 mg once daily. Median time of maximal concentration (T_{max}) ranged between 4.00 to 6.00 hours and 5.00 to 6.00 hours after the first dose and multiple doses, respectively.

Table 2: GS-5885 Pharmacokinetic Parameters Following Single-Dose and Multiple-Dose Administration of GS-5885

PK Parameter	Mean (%CV)					
	GS-5885 1 mg GT 1a (N = 10)	GS-5885 3 mg GT 1a (N = 10)	GS-5885 10 mg GT 1a (N = 9)	GS-5885 10 mg ^a GT 1b (N = 10)	GS-5885 30 mg GT 1a (N=10)	GS-5885 90 mg GT 1a (N = 10)
Single Dose (Day 1)						
C _{max} (ng/mL)	1.6 (20.2) ^b	4.5 (33.3)	18.1 (49.6)	19.4 (24.3)	67.0 (45.9)	166.6 (44.5)
T _{max} (h) ^a	4.00 (4.00, 5.00) ^b	5.00 (4.00, 6.00)	6.00 (4.00, 6.00)	5.00 (4.00, 6.00)	6.00 (6.00, 6.00)	6.00 (6.00, 6.00)
C _{last} (ng/mL)	1.13 (11.9) ^b	1.54 (43.7)	5.08 (41.4)	6.36 (40.6)	22.6 (53.1)	59.16 (52.9)
T _{last} (h) ^a	9.00 (7.00, 11.04) ^b	24.00 (16.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
AUC _{last} (ng·h/mL)	9.0 (52.4) ^b	50.9 (48.9)	212.5 (39.3)	259.6 (31.3)	894.4 (48.5)	2163.4 (45.8)
AUC _{inf} (ng ^h /ml)	30.9 (51.8) ^c	103.1 (85.1)	359.8 (40.7)	413.6 (38.4)	1491.0 (57.0)	4137.4 (67.1)
CL/F (ml/hr)	38,587.4 (40.5) ^c	45,509.5 (61.4)	31,564.8 (36.3)	27,491.7 (36.7)	32,641.7 (95.2)	29,409.8 (52.7)
t _{1/2} (h) ^a	10.15 (5.57, 15.11) ^c	13.51 (11.47, 22.03)	16.84 (13.37, 22.59)	15.15 (13.18, 20.27)	14.21 (11.53, 20.08)	17.98 (14.81, 26.10)
Multiple Dose (Day 3)						
C _{max} (ng/mL)	2.2 (39.7)	6.1 (56.6)	22.2 (31.4)	28.0 (42.7)	103.3 (57.5)	247.7 (45.4)
T _{max} (h) ^a	6.00 (6.00, 6.00)	6.00 (6.00, 6.00)	6.00 (6.00, 6.00)	6.00 (6.00, 6.00)	5.00 (4.00, 6.00)	6.00 (4.00, 8.00)
C _{tau} (ng/mL)	0.3 (161.0)	2.4 (73.6)	8.3 (33.1)	10.9 (43.0)	46.5 (62.7)	115.9 (42.6)
T _{last} (h) ^a	14.05 (10.00, 24.00)	48.00 (36.00, 48.00)	120.00 (120.00, 122.00)	120.00 (96.00, 120.57)	168.00 (120.00, 168.00)	168.00 (168.00, 168.98)
AUC _{tau} (ng·h/mL)	34.0 (29.8) ^b	89.7 (54.6)	323.6 (27.9)	409.5 (42.5)	1592.4 (59.5)	3815.5 (42.1)
CL/F (mL/hr)	31996.5 (31.7) ^b	43585.1 (54.5)	33219.8 (29.0)	28699.4 (42.4)	31284.6 (89.1)	27705.2 (41.7)
t _{1/2} (h) ^a	13.01 (7.76, 17.83) ^b	22.82 (13.05, 36.81)	40.51 (38.72, 48.66)	36.20 (23.02, 43.71)	41.69 (25.84, 53.39)	49.67 (37.76, 54.33)

Note: Subject 2011(GS-5885 10 mg 1a) was excluded from the PK analysis due to dosing error. Subjects 4004 and 4006 (GS-5885 1 mg 1a) did not have quantifiable concentrations at any timepoints on Day 1.

GT= genotype of HCV infection (1a or 1b)

a Median (Q1, Q3)

b N=8

c N=6

In the dose proportionality analysis using the power model (Table 3), AUC and C_{max} values were approximately dose proportional over the dose range of 1 mg to 90 mg.

Table 3: Statistical Analysis of Dose Proportionality of GS-5885 Following Single- and Multiple-Dose Administration of GS-5885

GS-5885 PK Parameter	Day	Degrees of Freedom	Slope of ln (dose)	90% CI Around Slope
AUC _{inf} (ng*hr/ml)	1	53	1.099	1.008, 1.189
C _{max} (ng/ml)	1	55	1.046	0.981, 1.110
AUC _{tau} (ng*hr/ml)	3	55	1.080	1.004, 1.157
C _{max} (ng/ml)	3	57	1.079	1.006, 1.152

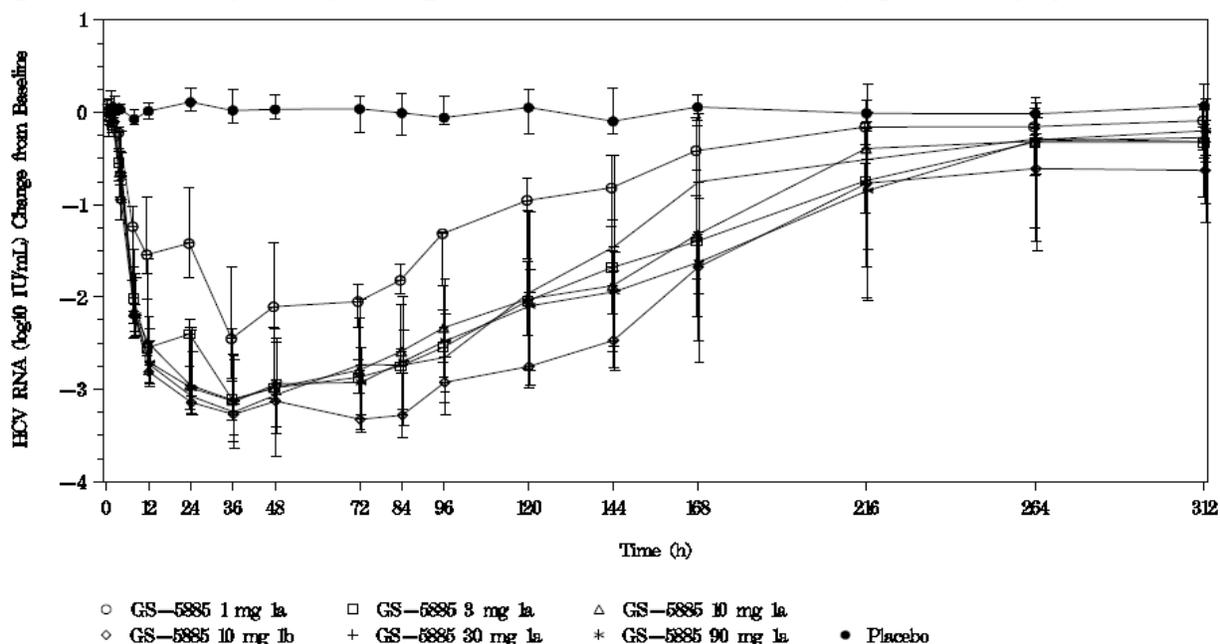
Accumulation indices were calculated by comparing the AUC_{last} value following single-dose administration of GS-5885 on Day 1 with the AUC_{tau} value following multiple-dose administration of GS-5885 for 3 days. Across all dosage strengths, accumulation ratios (%) of greater than 100 (152 to 483) were observed, a finding consistent with the long half-life of GS-5885.

Efficacy:

The primary efficacy endpoint was the change from baseline in HCV RNA level (log₁₀) at each postdose assessment, using both continuous and categorical analysis methods. Baseline was defined as the predose HCV RNA level on Day 1.

The median change from baseline in plasma HCV RNA levels is illustrated over time and by treatment in Figure 1 and summarized by postdose assessment in Table 4.

Figure 1: Median (Q1, Q3) Change from Baseline in HCV RNA (log₁₀ IU/mL) by Treatment



Note: Undetectable HCV RNA (< 25 IU/mL) was treated as 24 IU/mL for summary statistics

Table 4: Median (Q1, Q3) Change from Baseline in HCV RNA (log10 IU/mL) over Time by Treatment

Median (Q1, Q3) Change from Baseline in HCV RNA (log ₁₀ IU/mL)	GS-5885 1 mg 1a (N=10)	GS-5885 3 mg 1a (N=10)	GS-5885 10 mg 1a (N=10)	GS-5885 10 mg 1b (N=10)	GS-5885 30 mg 1a (N=10)	GS-5885 90 mg 1a (N=10)	Placebo (N=12)
Baseline (Day 1)	7.01	6.47	6.63	6.48	6.18	6.61	6.80
Change at 8 hrs	-1.24 (-1.82, -1.02)	-2.01 (-2.28, -1.67)	-2.16 (-2.44, 1.48)	-2.21 (-2.44, -2.07)	-2.20 (-2.37, -1.75)	-2.20 (-2.42, -1.79)	-0.07 (-0.14, 0.03)
Change at 24 hrs	-1.42 (-1.79, -0.82)	-2.4 (-2.75, -2.33)	-2.95 (-3.22, -2.24)	-3.15 (-3.27, -2.36)	-3.08 (-3.26, -2.33)	-2.99 (-3.27, -2.59)	0.11 (0.02, 0.26)
Change at 36 hrs	-2.45 (-2.89, -1.68)	-3.11 (-3.34, -2.92)	-3.12 (-3.25, -2.35)	-3.27 (-3.57, -2.62)	-3.25 (-3.49, -2.64)	-3.13 (-3.64, -2.68)	0.02 (-0.11, 0.25)
Change at 48 hrs	-2.11 (-2.33, -1.42)	-2.98 (-3.10, -2.94)	-2.98 (-3.13, -2.34)	-3.13 (-3.73, -2.89)	-3.06 (-3.41, -2.45)	-2.94 (-3.48, -2.49)	0.03 (-0.07, 0.19)
Change at 72 hrs	-2.05 (-2.32, -1.86)	-2.87 (-3.04, -2.67)	-2.79 (-3.04, -2.23)	-3.33 (-3.44, -2.85)	-2.73 (-3.27, -2.03)	-2.92 (-3.46, -2.56)	0.04 (-0.22, 0.17)
Change at Day 5	-1.31 (-1.87, -1.30)	-2.54 (-2.71, -2.14)	-2.33 (-2.87, -1.87)	-2.92 (-3.15, -2.65)	-2.65 (-3.03, -1.81)	-2.48 (-3.28, -2.19)	-0.06 (-0.13, 0.17)
Change at Day 6	-0.95 (-1.59, -0.72)	-2.04 (-2.41, -1.61)	-2.02 (-2.79, -1.07)	-2.75 (-2.99, -2.03)	-1.95 (2.79, -1.08)	-2.10 (-2.95, -1.70)	0.05 (-0.24, 0.25)
Change at Day 7	-0.82 (-1.23, -0.47)	-1.68 (-2.19, -1.17)	-1.88 (-2.60, -1.49)	-2.47 (-2.77, -1.71)	-1.45 (-2.54, -0.47)	-1.94 (-2.80, -1.51)	-0.10 (-0.23, 0.26)
Change at Day 10	-0.16 (-0.35, -0.11)	-0.74 (-1.10, -0.56)	-0.39 (-1.67, -0.18)	-0.77 (-2.02, -0.20)	-0.51 (-1.48, 0)	-0.85 (-2.03, 0.30)	-0.01 (-0.11, 0.13)
Change at Day 14	-0.09 (-0.41, 0.08)	-0.32 (-0.92, -0.16)	-0.28 (-0.49, 0.03)	-0.63 (-0.98, -0.47)	-0.32 (-0.54, -0.08)	-0.20 (-1.20, 0.15)	0.07 (-0.13, 0.30)

Note: GS-5885 3 mg: N = 8 at Day 10; GS-5885 10 mg: N = 9 at Days 6 and 14 and N = 8 at Days 7 and 10; GS-5885 1 mg: N = 9 at 36 hours; Placebo: N = 11 at Days 6 and 10

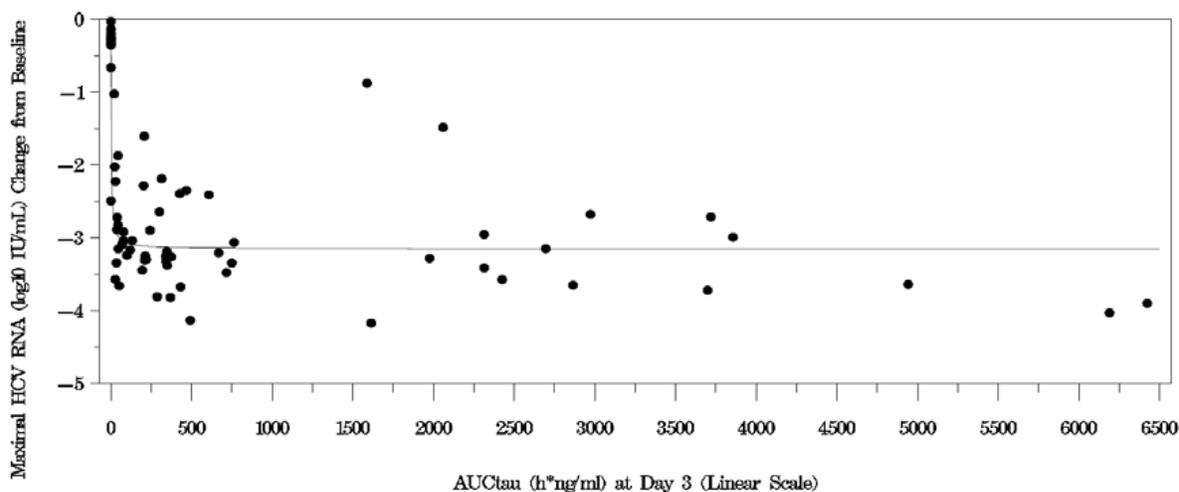
The greatest reductions from baseline in median HCV RNA were observed on Day 2 (36 hours), and were > 3 log₁₀ IU/mL for doses of GS-5885 ≥3 mg. Median HCV RNA reductions at Day 2 (36 hours) were as follows in the GS-5885 dose groups: 1 mg: -2.45; 3 mg: -3.11; 10 mg (genotype 1a): -3.12; 10 mg (genotype 1b): -3.27; 30 mg: -3.25; and 90 mg: -3.13 log₁₀ IU/mL. The median change from baseline in HCV RNA for the placebo group was 0.02 log₁₀ IU/mL at Day 2; there were no meaningful changes from baseline in median HCV RNA at any postdose assessment for the placebo group.

Median maximal HCV RNA reductions were similar for subjects with genotype 1a HCV (-3.22 log₁₀ IU/mL) and 1b (-3.34 log₁₀ IU/mL) who received GS-5885 10 mg. However, HCV RNA suppression appeared to be more sustained in subjects with genotype 1b, as HCV RNA reductions remained ≥ 2 log₁₀ IU/mL through Day 7 (-2.47 log₁₀ IU/mL) compared to subjects with genotype 1a (-1.88 log₁₀ IU/mL).

Pharmacodynamic Results:

Similar and maximal antiviral responses (median ~ 3 log₁₀ reduction) were observed following GS-5885 doses of 10, 30 or 90 mg. The relationship between GS-5885 exposure and anti-HCV activity was explored using a pharmacologically simple Emax model (Phoenix WinNonlin™, v.6.0) as illustrated in Figure 2. The Emax model adequately described the relationship between GS-5885 exposure (assessed as AUC_{tau} on Day 3) and maximal reductions in HCV RNA concentrations. The Emax values predicted by the model were close to the observed values. Emax modeling indicated that exposures (>100 ng.hr/mL) achieved following administration of GS-5885 doses equal or greater than 30 mg provide > 95% of maximal antiviral response in genotype 1a-infected subjects.

Figure 2: AUCtau Exposure-Response Relationship of Anti-HCV Activity of GS-5885 following 3-Day Monotherapy



Note: $E = E_0 + (E_{max} - E_0) * AUC_{tau} / (EC_{50} + AUC_{tau})$

$EC_{50} = -2.5344 \mu\text{g}\cdot\text{h/mL}$ (CV% = 36.83%); $E_{max} = -3.1513 \log_{10} \text{IU/mL}$ (CV% = 3.14%)

Conclusions:

- Pharmacokinetic parameters for GS-5885 were approximately dose-proportional over the dose range of 1 mg to 90 mg.
- E_{max} modeling indicated that exposures to GS-5885 ≥ 30 mg provided > 95% of maximal antiviral response; therefore, GS-5885 doses of 30 mg and 90 mg once daily were selected for continued clinical evaluation.
- GS-5885 resulted in rapid reductions in plasma HCV RNA of $\geq 2 \log_{10} \text{IU/mL}$ as early as 8 hours and reductions > 3 $\log_{10} \text{IU/mL}$ on Day 2 (36 hours) following administration of 3 mg through 90 mg.
- Although reductions in HCV RNA were generally similar between the 2 genotypes, HCV RNA suppression was more sustained in subjects with genotype 1b HCV infection.

4.2.2.4 GS-US-344-0109: A Phase 1, Partially-Blinded, Randomized, Placebo- and Positive-Controlled Study to Evaluate the Effect of Ledipasvir (GS-5885) on the QT/QTc Interval in Healthy Subjects

This study was reviewed by Interdisciplinary Review Team for QT Studies Consultation, dated 3/21/2014. This review only summarizes the PK data from this study to aid the discussion of PK results from other studies.

Summary: This is a Phase 1, partially-blinded, randomized, placebo- and positive-controlled, 3-period, 6-treatment sequence, single- and multiple-dose crossover study. LDV 120 mg BID (supratherapeutic dose) and LDV placebo were administered for 10-day in a double-blind fashion and moxifloxacin 400 mg single dose was administered as an active control in an open-label fashion. No significant QTc prolongation effect of ledipasvir 120 mg BID was detected in this TQT study.

A summary of LDV PK following 10 days of 120 mg BID administration of LDV as Compared to LDV 90-mg Dose as a Component of LDV/SOF Administered to HCV-infected Subjects are presented in the table below.

Table 1: LDV PK Summary following administered 120 mg BID in Healthy Volunteers as Compared to LDV 90-mg Dose as a Component of LDV/SOF Administered to HCV-infected Subjects

LDV PK Parameter Mean (%CV)	LDV 120 mg BID (N=59) (GS-US-344-0109)	SOF/LDV 400 mg/90 mg (N=48) (GS-US-337-0118)
AUC _{tau} (ng•h/mL)	15932.9 (27.0) ^a	--
AUC ₀₋₂₄ (ng•h/mL)	31865.8 (27.0) ^a	9472.8 (51.8) ^b
C _{max} (ng/mL)	1519.5 (27.9)	500.3 (44.1)

^a AUC_{tau}: AUC₀₋₁₂; AUC₀₋₂₄: calculated as AUC_{tau} x 2

^b N = 47

LDV plasma exposures at this supratherapeutic dose were approximately 4.2-fold higher for C_{max} and 3.7-fold higher for AUC₀₋₂₄, relative to the LDV 90-mg dose as a component of LDV/SOF administered to HCV-infected subjects. Sixty subjects were enrolled and randomized in this study. Eighteen were female (30%). No Grade 3 or 4 or serious AEs occurred during the study.

4.2.2.5 GS-US-256-0108 (Leslie): A Phase 1 Study to Evaluate the Pharmacokinetics, Metabolism and Excretion of GS-5885

Trial Period and Site

27 Mar to 17 May 2012; (b) (4)

Trial Rationale

In this study, the mass balance of a single oral dose of ledipasvir (LDV, formerly known as GS-5885) was determined using a dose of radiolabeled [¹⁴C]-LDV. In addition, the pharmacokinetics of LDV and its metabolites were determined.

Trial Design

This was an open-label, nonrandomized, single-dose, mass balance study. Eight healthy subjects received a single oral dose of LDV 90 mg containing a mixture of unlabeled and [¹⁴C]-labeled LDV. Study drug was administered on Day 1 within 5 minutes of consuming a standardized breakfast (approximately 400 calories and 13 g fat) following an overnight fast. Subjects were confined to the clinic from Day 0 until 96 h postdose (Day 5).

Rationale for Dose Selection

The dose of LDV 90 mg was selected based on antiviral activity and a favorable safety profile in patients infected with HCV genotype 1a in study GS-US-256-0102. This dose is being evaluated (in combination with sofosbuvir 400 mg) in Phase 3 studies.

Investigational Product

Ledipasvir 90 mg capsules contained a mixture of unlabeled LDV (88.35 mg) and [¹⁴C]-LDV (1.65 mg). Both were supplied by Gilead Sciences, Inc. (Foster City, California) and the lot numbers were 5885-03-A-1 and GS002-010-0599-B-20120305-JOH, for the unlabeled and [¹⁴C]-labeled LDV, respectively.

Key Inclusion and Exclusion Criteria

Subjects were healthy males between the ages of 18 and 45 years, inclusive, with a BMI between 19 and 30 kg/m² and creatinine clearance ≥80 mL/min. Subjects had to agree to use a highly effective method of contraception. Exclusion criteria included history of nicotine use (within 90 days), any serious or active medical or psychiatric illness, positive test result for HIV-1 antibody, hepatitis C antibody, or hepatitis B surface antigen, or a positive urine screen for drugs of abuse.

Concomitant Medications

The following medications and substances were disallowed while subjects were participating in the study:

- all prescription and over-the-counter medications (with the exception of acetaminophen, ibuprofen, or vitamins) from within 3 months prior to and during dosing
- systemic steroids, immunosuppressant therapies, or chemotherapeutic agents from within 3 months of screening and during dosing

Sample Collection

Whole blood, plasma, urine, and stool were collected to assess LDV concentrations up to the morning of Day 22 (504 h postdose) or until the stopping rule is met (i.e. when liquid scintillation counting [LSC] radioanalysis indicates that radioactivity in [a] two consecutive whole blood and/or plasma samples decreased to ≤2-fold the level of background radioactivity [b] two consecutive 24-hour collection intervals were ≥1% of the administered dose and the cumulative [¹⁴C]-radioactivity recovered was ≥90% of the administered dose).

Whole blood and plasma were collected predose and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 12, 24, 36, 48, 72, and 96 h postdose, and at 24-hour intervals thereafter up to the morning of Day 22 or until the stopping rule was met.

Urine was collected and pooled starting 12 h predose and 0-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-72, and 72-96 h postdose, and for successive 24-hour intervals thereafter up to the morning of Day 22 or until the stopping rule was met.

Stool was collected starting 24 h predose and 0-24, 24-48, 48-72, and 72-96 h postdose, and for successive 24-hour intervals thereafter up to the morning of Day 22 or until the stopping rule was met.

Analytical Methods

Concentrations of LDV in plasma samples were measured by LC-MS/MS (b) (4) (b) (4) validation report (b) (4) 60-1028; study report (b) (4) 60-1229A). Sample receipt commenced on 15 May 2012 (storage temperature: -70°C) and analysis was completed on 29 Aug 2012. The first day of sample collection was 19 Apr, so the maximum storage sample time was 132 days, which is within the validated long-term frozen stability duration of 351 days for plasma. The calibration standards ranged from 1-2000 ng/mL and the quality control (QC) concentrations were 3, 75, 750, and 1600 ng/mL. All inter-assay accuracy and precision estimates were within the acceptable range (Table 1).

Table 1: Bioanalytical assay validation for LDV in plasma

Parameter	LDV
Linear range (ng/mL)	1–2000
LLOQ (ng/mL)	1
Interassay precision range	1.0 to 8.8
Interassay accuracy range	-7.1 to -3.6
Stability in frozen matrix (days)	351 days @ -20°C and -70°C

Source: Appendix 16.1.10

Concentrations of LDV in urine samples were measured by LC-MS/MS (b) (4) (validation report (b) (4) 60-1217; study report (b) (4) 60-1229B). Sample receipt commenced on 15 May 2012 (storage temperature: -70°C) and analysis was completed on 7 Aug 2012. The first day of sample collection was 19 Apr, so the maximum storage sample time was 110 days, which is within the validated long-term frozen stability duration of 119 days for urine. The calibration standards ranged from 5-5000 ng/mL and QC concentrations were 15, 500, 2500, and 4000 ng/mL. All inter-assay accuracy and precision estimates were within the acceptable range (data not shown). Quantification of radioactivity in whole blood, plasma, urine, and feces samples was performed (b) (4) (b) (4) study report 8259249).

Results

Trial Population

Eight healthy male subjects between the ages of 19 and 41 were enrolled in the study and received study drug. The majority of subjects were white (62.5%); the remainder were African American (25%) or self-identified as other race (12.5%). One subject discontinued early (Day 10) due to an AE (abdominal discomfort secondary to constipation) that was judged by the investigatory to be unrelated to study drug. Pharmacokinetic data from this subject are included in the analyses because 85% of the total radioactive dose had been recovered at the time of discontinuation, which is comparable to the total recovery across all subjects.

For all subjects, whole blood and plasma collection was terminated 48 h postdose when the stopping rule was met. In addition, collection of urine and stool samples was completed prior to Day 21 for four subjects; the other three subjects remained in the clinic until Day 21.

Blood-to-Plasma Ratio

The mean blood-to-plasma ratio of concentration of [¹⁴C]-radioactivity ranged from 0.513 to 0.661 up to 24 h postdose, suggesting that radioactivity did not accumulate in erythrocytes. Therefore, PK analyses were performed using plasma concentrations.

Whole Blood and Plasma Pharmacokinetics

Quantifiable concentrations of [¹⁴C]-radioactivity were detectable by LSC for up to 36 h in whole blood and plasma in four and eight subjects, respectively. Maximal concentrations of [¹⁴C]-radioactivity were observed at 5.5 h postdose and the estimated half-life was 32.2 h (Table 2). Concentrations of [¹⁴C]-radioactivity peaked at 7.0 h in plasma, with an estimated half-life of 19.9 h (Table 2). Concentrations of LDV (measured using the more sensitive LC-MS/MS assay) were detected in plasma for up to 96 h postdose, with a t_{max} of 6.0 h and an estimated half-life of 31.4 h (Table 2).

Table 2: PK parameters for total [¹⁴C]-radioactivity and LDV in whole blood and plasma (source: Study Report Tables 10-1, 10-2, 10-3)

PK Parameter	Total Radioactivity using LSC [mean (%CV)]		LDV using LC-MS/MS [mean (%CV)]
	Whole Blood (N=4) ^b	Plasma (N=8)	Plasma (N=8)
C _{max} (ng eq/g)	77.0 (12.2)	118 (30.9)	127 (34.5)
T _{max} ^a (h)	5.5 (4.8, 6.0)	7.0 (5.5, 8.0)	6.0 (4.5, 8.0)
C _{last} (ng eq/g)	57.5 (13.6)	52.6 (14.1)	12.1 (36.0)
T _{last} ^a (h)	18.0 (9.0, 24.0)	24.1 (18.0, 36.0)	96.0 (96.0, 96.0)
AUC _{last} (ng eq.h/g)	812 (82.0)	1840 (62.6)	3960 (35.2)
AUC _{inf} (ng eq.h/g)	3880 (21.0) ^c	3220 (41.4)	4550 (35.2)
AUC _{exp} (%)	69.8 (25.6) ^c	47.9 (39.0)	13.3 (37.8)
t _{1/2} ^a (h)	32.2 (24.1, 46.4) ^c	19.9 (15.6, 21.2)	31.4 (29.6, 37.4)

^a median (Q1, Q3)

^b Only four subjects had whole blood total [¹⁴C]-radioactivity concentrations above the limit of quantitation using LSC

^c N=3

Urine Pharmacokinetics

The pharmacokinetics of LDV in urine were not determined because all but three individual samples had LDV concentrations that were BLQ.

Recovery from Urine and Feces

The mean cumulative urinary recovery of [¹⁴C]-radioactivity was 1.24% (SD 0.08%), with approximately 1% of the dose recovered in the first 24 h postdose. The mean cumulative fecal recovery of [¹⁴C]-radioactivity was 85.9% (SD 7.8%), with approximately 85% of the dose recovered in the first 216 h (nine days) postdose. The majority of the radioactive dose was recovered in feces.

Analysis of Metabolites

Plasma samples obtained at 4, 4.5, 5, 6, 8, 12, and 24 h postdose were pooled by subject to generate AUC-representative pooled samples. Plasma radioactivity was comprised primarily of LDV, with minor metabolites M1 and M12 also detected (Table 3).

Table 3: [¹⁴C]-LDV and [¹⁴C]-metabolite profiling in AUC pooled samples (source: Study Report Table 10-4)

Mean (CV) PK Parameter	[¹⁴ C]-Total Radioactivity (N = 8)	[¹⁴ C]-LDV (N = 8)	[¹⁴ C]-M1 (N = 7)	[¹⁴ C]-M12 (N = 3)
AUC ₀₋₂₄ (h·ng eq/g)	1646 (52%)	1619 (52%)	19.6 (94%)	14.3 (65%)
% of total [¹⁴ C] AUC (%)	N/A	98.3 (1.0%)	1.12 (95%)	0.75 (6.8%)

Source: Section 15.1, Table 4.3

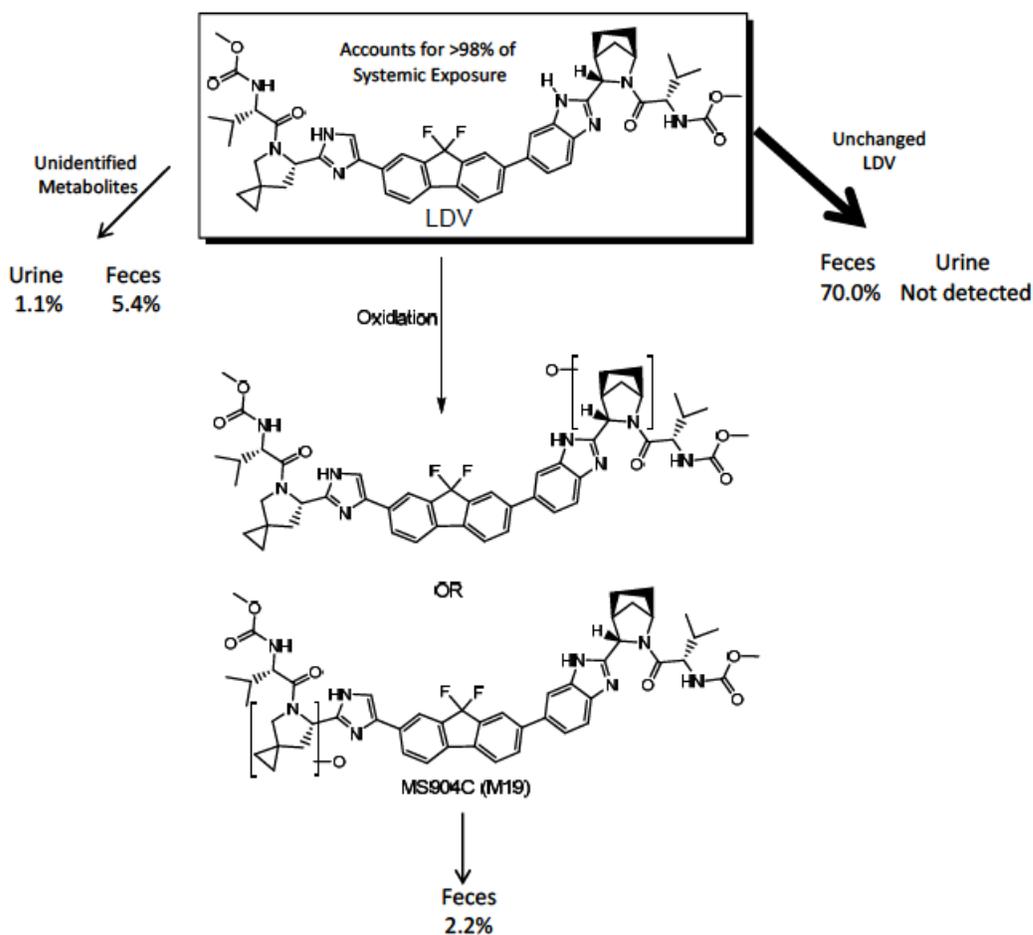
Similarly, plasma samples obtained at 6 and 8 h postdose were pooled by subject to generate C_{max}-representative pooled samples. The majority of [¹⁴C]-radioactivity was attributed to LDV (97%), with minor contributions by M1 and M12 (1.7% and 0.42%, respectively).

Urine samples collected within 24 h postdose were pooled by subject. Nine unknown compounds were detected, each of which accounted for less than 1% of the dose. Unchanged LDV was not detected in urine.

Feces samples collected up to 216 h (nine days; mean (SD) 77.6% (6.8%) of total dose recovered) postdose were pooled by subject and combusted and analyzed by LSC and radiochromatography. Nine radioactive peaks were observed. Unchanged LDV was the major component excreted in feces (70% of radioactive dose) and M19 (oxy-LDV-3) was the most abundant metabolite identified in feces (2.21% of radioactive dose). The remaining unidentified compounds each represented less than 2% of the radioactive dose.

The Sponsor's proposed biotransformation pathway is displayed in Figure 1.

Figure 1. Proposed major biotransformation and excretion pathways of LDV (source: Study Report Figure 10-7)



Values reported are % of dose administered
Total [¹⁴C]-radioactivity Recovery ~87%: ~86% in Feces, ~1% in Urine

Source: Appendix 16.1.10 with modifications based on data from the current study

Conclusion

In the study, the pharmacokinetics of LDV in whole blood, plasma, urine, and feces were evaluated following a single oral dose of [¹⁴C]-LDV 90 mg. Unchanged LDV was the major component (more than 98%) of circulating radioactivity in plasma through 24 h postdose. Renal elimination was minor (approximately 1% of dose) and no unchanged LDV was detected in urine. The majority of radioactivity was excreted in the feces (approximately 86%) as unchanged LDV (approximately 70% of dose). M19 (oxy-LDV-3) was the most abundant metabolite in feces (approximately 2.21% of dose). LDV appears to be metabolically stable in humans and is predominantly excreted unchanged in the feces.

4.2.3 Intrinsic Factors

4.2.3.1 GS-US-344-0101: A Phase 1 Open-Label, Parallel-Group, Single-Dose Study to Evaluate the Pharmacokinetics of GS-5885 in Subjects with Normal Hepatic Function and Severe Hepatic Impairment

Objectives: To evaluate the single-dose pharmacokinetics (PK) of ledipasvir (LDV) in subjects with normal hepatic function and severe hepatic impairment

Study Design: Eligible subjects were enrolled in one of the 2 treatment groups (Group 1 = severe hepatic impairment; Group 2 = normal hepatic function) and received a single oral dose of LDV (1 × 90-mg tablet) with a moderate fat meal.

Formulation: 90-mg tablet (Lot No.: CF1203B1)

PK Sampling: LDV PK samples were collected predose (within 5 minutes prior to dosing) and 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 48, 72, 96, 120, 144, and 168 hours postdose. Additional aliquots for determination of percent protein binding of LDV were collected 4 and 6 hours postdose.

Analytical methods: Plasma concentrations of LDV were determined using fully validated high-performance liquid chromatography-tandem mass spectroscopy (LC/MS/MS) bioanalytical methods. All samples were analyzed in the timeframe supported by frozen stability storage data. All assays for LDV were performed and validated (b) (4)

Parameter	LDV
Linear range (ng/mL)	1 to 2000
Lower limit of quantitation (ng/mL)	1
Interday precision range (%CV)	1.4 to 3.2
Interday accuracy range (%RE)	0 to 2.8
Stability in frozen matrix (days)	366 at -20°C and -70°C

Pharmacokinetic Results: Table 1 presents single-dose LDV PK parameters following single-dose administration of LDV 90 mg in subjects with severe hepatic impairment and subjects with normal hepatic function. Mean LDV AUC_{inf} and AUC_{last} values were generally similar in both groups. Mean C_{max} was modestly lower, median t_{1/2} was prolonged, and median T_{max} was similar in subjects with hepatic impairment compared with control subjects with normal hepatic

function. Mean oral clearance (CL/F) was not affected, and mean apparent volume of distribution (V_z/F) was higher in hepatically impaired subjects relative to control subjects with normal hepatic function.

Table 10-1: Ledipasvir Plasma Pharmacokinetic Parameters Following Administration of a Single 90-mg Dose in Subjects with Severe Hepatic Impairment and Subjects with Normal Hepatic Function

LDV PK Parameter	Mean (%CV)	
	Group 1 (Severe Hepatic Impairment) (N=10)	Group 2 (Normal Hepatic Function) (N=10)
AUC _{exp} (%)	26.0 (45.6)	8.10 (42.0)
AUC _{inf} (h·ng/mL)	9567.2 (67.7)	7615.7 (30.9)
AUC _{last} (h·ng/mL)	6672.5 (51.8)	6976.4 (30.6)
C _{last} (ng/mL)	17.95 (70.1)	8.81 (44.6)
C _{max} (ng/mL)	134.3 (43.9)	197.4 (35.2)
t _{1/2} (h) ^a	84.25 (78.30, 112.21)	45.72 (43.30, 53.38)
T _{max} (h) ^a	6.00 (4.00, 8.00)	6.00 (6.00, 8.00)
T _{last} (h) ^a	168.00 (168.00, 168.00)	168.00 (168.00, 168.00)
CL/F (mL/hr)	14,784.4 (89.8)	13,137.6 (40.1)
V _z /F (mL)	187,571.0 (89.9)	88,993.0 (30.8)

a Median (Q1, Q3)

Table 2 presents statistical comparisons of the primary LDV PK parameters following administration of single-dose LDV in subjects with severe hepatic impairment and control subjects with normal hepatic function. The geometric least-squares mean (GLSM) values of AUC_{inf} and AUC_{last} for LDV were similar in both groups, with 90% CIs for the GLSM ratios crossing 100%. Ledipasvir C_{max} in subjects with severe hepatic impairment was approximately 35% lower than that in subjects with normal hepatic function, with the upper bound of the 90% CI < 100%.

Table 2: Statistical Comparisons of Ledipasvir Plasma Pharmacokinetic Parameters Following Administration of a Single 90-mg Dose in Subjects with Severe Hepatic Impairment and Subjects with Normal Hepatic Function

LDV PK Parameter	GLSM		%GLSM Ratio (90% CI) Group 1 / Group 2
	Group 1 (Severe Hepatic Impairment) (N=10)	Group 2 (Normal Hepatic Function) (N=10)	
AUC _{inf} (h·ng/mL)	7813.60	7256.56	107.68 (70.06, 165.49)
AUC _{last} (h·ng/mL)	5705.69	6664.89	85.61 (57.44, 127.60)
C _{max} (ng/mL)	120.49	186.41	64.64 (45.24, 92.36)

Reviewer's note: Results indicate that LDV absorption/bioavailability may be affected by hepatic impairment, but the magnitude of reduction on Cmax is not considered clinically significant.

The mean (SD) % free and % bound LDV values were 0.21% (0.07%) and 99.8% (0.07%), respectively, in subjects with severe hepatic impairment; and 0.11% (0.06%) and 99.9% (0.06%), respectively, in subjects with normal hepatic function. These results demonstrated a lack of alteration in LDV protein binding in subjects with severe hepatic impairment

Pharmacodynamics: The relationships between various measures of hepatic functional abnormalities and the primary PK parameters of LDV were explored using scatter plots and Spearman's rank correlation coefficients, with associated p-values calculated to assess the strength/statistical significance of the relationships.

No correlations or only weak correlations (ρ $<$ 0.40) were observed between LDV AUCinf, AUClast, and Cmax and baseline measurements of albumin or bilirubin. A moderate positive correlation was observed between LDV AUCinf and CPT score (ρ = 0.435) in subjects with severe hepatic impairment, and moderate negative correlations (ρ = -0.42) were observed between LDV Cmax and baseline prothrombin time or INR. These correlations were not statistically significant (p $>$ 0.05) and were not deemed clinically meaningful.

Conclusion: Dose modification of LDV is not warranted for patients with any degree of hepatic impairment.

4.2.3.2 GS-US-344-0108: A Phase 1, Open-Label, Parallel-Group, Single-Dose Study to Evaluate the Pharmacokinetics of Ledipasvir in Subjects with Normal Renal Function and Severe Renal Impairment

Objectives:

- To evaluate the single-dose pharmacokinetics (PK) of ledipasvir (LDV) in subjects with severe renal impairment and matched healthy control subjects
- To evaluate the safety and tolerability of a single dose of LDV in subjects with severe renal impairment and matched healthy control subjects

Study Design: Eligible subjects for Group 1 (severe renal impairment) were enrolled and dosed first. Once a renally impaired subject completed through Day 8, a healthy match to that subject was enrolled into Group 2. Each subject in the control group (Group 2) was matched for age (\pm 10 years), gender, and body mass index (BMI) (\pm 15%) with a subject in the severe renal impairment group. Subjects in each group received a single dose of LDV (90 mg) under fasted conditions on Day 1. A follow-up visit occurred 10 to 14 days after study drug dosing.

Formulation: 90-mg LDV tablet (Lot#: CF1203B1)

PK Sampling: Intensive PK sampling occurred relative to LDV dosing on Day 1. Blood PK samples were collected at the following time points: Predose (\leq 5 min), 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 48, 72, 96, 120, 144 and 168 hours postdose. Urine PK samples were collected as follows: predose, at 0-6, 6-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 hours collection intervals postdose.

Analytical methods: Concentrations of LDV in plasma samples were determined using a fully validated high-performance liquid chromatography-tandem mass spectroscopy (LC-MS/MS)

bioanalytical method. All samples were analyzed in the timeframe supported by frozen stability storage data. The assay for LDV was performed and validated (b) (4). The standard curve and QC data indicated that the plasma and urine assay method for LDV were precise and accurate as shown in the following table. Urine PK data were not analyzed.

Table 1: Bioanalytical Assay Validation for LDV

Parameter	Plasma LDV
Linear Range (ng/mL)	1–2000
Lower Limit of Quantitation (ng/mL)	1
Interassay Precision Range (%CV)	1.4–3.2
Interassay Accuracy Range (%RE)	0.0–2.8
Stability in Frozen Matrix (days)	366 days at –70°C and –20°C

Pharmacokinetic Results: Table 2 presents single-dose LDV PK parameters following single-dose administration of LDV 90 mg in subjects with severe renal impairment and normal renal function. Mean or median PK parameters were similar between both groups.

Table 2: LDV Plasma PK Parameters Following Administration of LDV in Subjects with Severe Renal Impairment and Normal Renal Function

LDV PK Parameter	Mean (%CV)	
	Severe Renal Impairment (N = 10)	Normal Renal Function (N = 9) ^b
%AUC _{exp} (%)	12.18 (40.9)	9.78 (32.7)
AUC _{last} (ng•h/mL)	11,403.9 (30.2)	11,608.2 (39.7)
AUC _{inf} (ng•h/mL)	13,162.0 (35.0)	12,875.1 (40.0)
C _{max} (ng/mL)	311.2 (30.2)	341.7 (32.7)
T _{max} (h) ^a	6.00 (4.00, 6.02)	6.00 (4.00, 6.00)
C _{last} (ng/mL)	18.49 (51.53)	15.77 (48.67)
T _{last} (h) ^a	168.00 (168.00, 168.02)	168.00 (168.00, 168.00)
t _{1/2} (h) ^a	59.50 (49.40, 67.89)	54.90 (52.07, 62.13)
V _z /F (mL)	640,348.1 (28.8)	687,698.9 (65.3)
Weight normalized V _z /F (mL/kg)	8058.9 (24.9)	8051.5 (58.0)
CL/F (mL/h)	7672.6 (38.4)	8515.9 (54.7)
Weight normalized CL/F (mL/h/kg)	97.1 (35.6)	101.3 (48.5)

^a Median (Q1, Q3)

^b Excludes Subject 7588-2002

Ledipasvir exposure for 1 subject in the control group (Subject 7588-2002, normal renal function) was unexpectedly lower (~30-fold) compared to the mean exposure of the group. Pharmacokinetic parameters dependent on AUC (eg, CL/F) were also correspondingly deviant from the group mean. Ledipasvir T_{max}, t_{1/2}, and %AUC_{exp} were comparable to the rest of the

group. The sponsor's investigation did not reveal any clinical or bioanalytical findings. Analyses have been conducted with and without this subject. Inclusion of this individual into analyses resulted in a modest increase in %GMR (renal impaired/normal renal function) and significantly increased variability (with upper 90% CI > 200%). However, evaluation of the paired subject with severe renal function showed results comparable to the rest of the severe renal impairment group as well as those with normal renal function. As such, summary statistics and comparisons are presented excluding this subject.

Table 3 presents statistical comparisons of the primary LDV PK parameters following administration of single-dose LDV in subjects with severe renal impairment and control subjects with normal renal function. The geometric least-squares mean (GLSM) values of AUCinf, AUClast, and Cmax were similar in both groups, with GLSM ratios near 100% and 90% confidence intervals (CIs) for the GLSM ratios crossing 100% for all parameters. Although the 90% CIs are wide, the data do not warrant LDV dose adjustment for renally impaired subjects.

Table 3: Statistical Evaluations of LDV Plasma PK Parameters Following Administration of LDV in Subjects with Severe Renal Impairment and Normal Renal Function

LDV PK Parameter	GLSM		%GLSM Ratio (Impaired/Normal)	90% CI
	Severe Renal Impairment (N = 10)	Normal Renal Function (N = 9) ^a		
C _{max} (ng/mL)	298.49	323.26	92.34	70.24, 121.39
AUC _{inf} (ng•h/mL)	12,447.25	11,787.17	105.60	75.32, 148.05
AUC _{last} (ng•h/mL)	10,914.68	10,628.88	102.69	74.05, 142.40

a Excludes Subject 7588-2002.

Mean LDV protein binding (% free and % bound) was determined in all subjects at 4 or 6 hours postdose; protein binding was assessed at T_{max} whenever possible or at the time point closest to T_{max} for each subject.

The mean (SD) % free and % bound LDV values were 0.01% (0.019%) and 99.99% (0.032%), respectively, for subjects with severe renal impairment, and 0.01% (0.043%) and 99.99 (0.032%), respectively, for subjects with normal renal function. These results demonstrated a lack of alteration of LDV protein binding in subjects with severe renal impairment.

Conclusion: No dose adjustment is necessary for LDV in subjects with any degree of renal impairment. Because LDV is highly protein bound, dialysis is not expected to significantly affect LDV exposure.

4.2.4 Extrinsic Factors

4.2.4.1 GS-US-344-0102: A Phase 1, Open-Label, Pharmacokinetic Drug-Drug Interaction Study Between GS-5885 and Antiretrovirals Efavirenz/Emtricitabine/Tenofovir Disoproxil Fumarate (EFV/FTC/TDF), a Boosted Protease Inhibitor, Darunavir plus Ritonavir (DRV/r), a Non-Nucleoside Reverse Transcriptase Inhibitor, Rilpivirine (RPV), an Integrase Inhibitor, Raltegravir (RAL), and Between Sofosbuvir/GS-5885 FDC and a Boosted Integrase Inhibitor Elvitegravir plus Cobicistat (EVG+COBI) or a Boosted Protease Inhibitor, Atazanavir plus Ritonavir (ATV+RTV)

Objectives: To evaluate the drug-drug interactions between LDV (formerly GS-5885) and efavirenz/emtricitabine/tenofovir disoproxil fumarate (EFV/FTC/TDF), darunavir plus ritonavir (DRV/r), rilpivirine (RPV), raltegravir (RAL), and Between SOF/LDV FDC and elvitegravir plus cobicistat (EVG+COBI) or atazanavir plus ritonavir (ATV+RTV)

Study Design: A total of 168 subjects were enrolled and randomized in the study. Eligible subjects were enrolled in 1 of the following 6 cohorts:

Cohort 1:

Subjects (n=30) were randomized to 1 of 2 treatment sequences and received 2 of the treatments described below:

Treatment A: LDV administered in the morning with food

Treatment B: EFV/FTC/TDF administered in the morning under fasted conditions

Treatment C: LDV administered in the morning with food plus EFV/FTC/TDF administered in the morning under fasted conditions (LDV was given 2 hours after EFV/FTC/TDF administration)

Cohort 1	Days 1–14	Days 16–29
Sequence: AC	Treatment A	Treatment C
Sequence: BC	Treatment B	Treatment C

Cohort 2:

Subjects (n=24) were randomized to 1 of 6 treatment sequences and received the 3 treatments as described below:

Treatment A: LDV administered in the morning with food

Treatment D: DRV/r administered in the morning with food

Treatment E: LDV+DRV/r administered in the morning with food

Treatment Sequence	Days 1–10	Days 11–20	Days 21–30
I	A	D	E
II	A	E	D
III	D	A	E
IV	D	E	A
V	E	A	D
VI	E	D	A

Cohort 3:

Subjects (n=24) were randomized to 1 of 6 treatment sequences and received the 3 treatments as described below:

Treatment A: LDV administered in the morning with food

Treatment F: RPV administered in the morning with food

Treatment G: LDV+RPV administered in the morning with food

Treatment Sequence	Days 1–10	Days 11–20	Days 21–30
I	A	F	G
II	A	G	F
III	F	A	G
IV	F	G	A
V	G	F	A
VI	G	A	F

Cohort 4:

Subjects (n=30) were randomized to 1 of 6 treatment sequences and received the 3 treatments as described below:

Treatment A: LDV administered in the morning with food

Treatment H: RAL administered in the morning and evening with food

Treatment I: LDV+RAL administered in the morning with food; RAL also administered in the evening with food

Treatment Sequence	Days 1–10	Days 11–20	Days 21–30
I	A	H	I
II	A	I	H
III	H	A	I
IV	H	I	A
V	I	A	H
VI	I	H	A

Cohort 5:

Subjects (n=30) were randomized to 1 of 6 treatment sequences and received the 3 treatments as described below:

Treatment J: SOF/LDV administered in the morning with food

Treatment K: EVG+COBI administered in the morning with food

Treatment L: SOF/LDV+EVG+COBI administered in the morning with food

Treatment Sequence	Days 1–10	Days 11–20	Days 21–30
I	J	K	L
II	J	L	K
III	K	J	L
IV	K	L	J
V	L	K	J
VI	L	J	K

Cohort 6:

Subjects (n=30) were randomized to 1 of 6 treatment sequences and received the 3 treatments as described below:

Treatment J: SOF/LDV administered in the morning with food

Treatment M: ATV/r administered in the morning with food

Treatment N: SOF/LDV+ATV/r administered in the morning with food

Treatment Sequence	Days 1–10	Days 11–20	Days 21–30
I	J	M	N
II	J	N	M
III	M	J	N
IV	M	N	J
V	N	M	J
VI	N	J	M

Formulation:

	LDV (tablet)	EVG (tablet)	SOF/LDV (tablet)	COBI (tablet)	ATR ^a (tablet)	ATV ^a (capsule)	DRV ^a (tablet)	RTV ^a (tablet)	RAL ^a (tablet)	RPV ^a (tablet)
Strength	90 mg	150 mg	SOF 400 mg LDV 90 mg	150 mg	EFV 600 mg FTC 200 mg TDF 300 mg	300 mg	400 mg	100 mg	400 mg	25 mg
Lot No.	CF1203 B1	AJ1101G1	DK1202B2	BB1004B2	000475	2G5022A	2EG854	141212E/ 200942E	H011486	BLLOE00
Expiration Date	October 2013	December 2014	May 2014	April 2013	December 2015	July 2014	February 2014	February 2014/June 2014	June 2014	May 2014
Manufacturer / Supplier	(b) (4)				Gilead Sciences td - Cork IDA Business Park, Carrigtohill, County Cork, Ireland	(b) (4)				
Site of Release in Europe	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

ATR = Atripla; ATV = atazanavir; COBI = cobicistat; DRV = darunavir; EVG = elvitegravir; LDV = ledipasvir; NA = not applicable; RAL = raltegravir; RPV = rilpivirine; RTV = ritonavir; SOF = sofosbuvir a Commercial product

PK Sampling:

Cohort 1:

Treatment Sequence AC:

- Day 14: Serial blood samples were collected relative to LDV administration in the morning as follows: predose (≤ 5 min) and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 20, and 24 hours postdose.
- Day 29: Serial blood samples were collected relative to EFV/FTC/TDF administered in the morning as follows: predose (≤ 5 min) and at 0.25, 0.5, 0.75, 1, 1.5, 2 (predose relative to LDV), 2.5, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, and 26 hours postdose.

Treatment Sequence BC:

- Day 14: Serial blood samples were collected relative to EFV/FTC/TDF administered in the morning as follows: predose (≤ 5 min) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 14, 16, 20, and 24 hours postdose.
- Day 29: Serial blood samples were collected relative to EFV/FTC/TDF administered in the morning as follows: predose (≤ 5 min) and at 0.25, 0.5, 0.75, 1, 1.5, 2 (predose relative to LDV), 2.5, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, and 26 hours postdose.

Cohorts 2 through 4:

All Treatment Sequences:

Days 10, 20, and 30: Serial blood samples were collected relative to study drug administration in the morning as follows: predose (≤ 5 min) and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 20, and 24 hours postdose.

Cohorts 5 and 6:

All Treatment Sequences:

Days 10, 20, and 30: Serial blood samples were collected relative to study drug administration in the morning as follows: predose (≤ 5 min) and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 20, and 24 hours postdose.

Analytical Methods: Concentrations of SOF, GS-566500, GS-331007, LDV, EVG, COBI, FTC, TFV, ATV, DRV, RTV, EFV, RPV, and RAL in plasma samples were determined using fully validated high-performance liquid chromatography-tandem mass spectroscopy bioanalytical methods. All samples were analyzed in the time frame supported by frozen stability storage data. The assays for SOF, GS-566500, GS-331007, LDV, EVG, COBI, FTC, TFV, ATV, DRV, RTV, EFV, RPV, and RAL were all performed and validated (b) (4)

The standard curve and QC data indicated that the plasma assay methods for SOF, GS-566500, GS-331007, LDV, EVG, COBI, FTC, TFV, ATV, DRV, RTV, EFV, RPV, and RAL were precise and accurate as shown in the following table.

Table 1 Summary of Quality Control (QC) Results

Parameter	SOF	GS-566500	GS-331007	LDV
Linear Range (ng/mL)	5–5000	10–5000	10–5000	1–2000
Lower Limit of Quantitation (ng/mL)	5	10	10	1
Interday Precision Range (%CV)	0.0 to 1.4	0.0 to 1.2	0.0 to 3.5	1.4 to 3.2
Interday Accuracy Range (%RE)	0.0 to 3.5	-0.3 to 2.8	-2.0 to 1.0	0.0 to 2.8
Stability in Frozen Matrix (days)	377 days at -70°C and 36 days at -20°C	308 days at -70°C and 25 days at -20°C	308 days at -70°C and 25 days at -20°C	366 days at -70°C and -20°C

Parameter	EVG	COBI	FTC	TFV	ATV
Linear Range (ng/mL)	20–10000	5–2500	5–3000	5–3000	10–5000
Lower Limit of Quantitation (ng/mL)	20	5	5	5	10
Interday Precision Range (%CV)	1.8 to 7.0	2.1 to 5.8	0.0 to 2.8	0.6 to 3.8	2.3 to 3.0
Interday Accuracy Range (%RE)	-6.5 to 1.6	2.0 to 5.0	-1.3 to 5.3	-3.3 to 0.0	-2.0 to -1.5
Stability in Frozen Matrix (days)	585 days at -70°C and 127 days at -20°C	365 days at -70°C and 121 days at -20°C	92 days at -70°C and -20°C	92 days at -70°C and -20°C	173 days at -70°C and 446 days at -20°C

Parameter	RTV	DRV	EFV	RPV	RAL
Linear Range (ng/mL)	5–2500	20–4000	5–5000	1–500	1–1000
Lower Limit of Quantitation (ng/mL)	5	20	5	1	1
Interday Precision Range (%CV)	2.4 to 3.4	0.0 to 2.1	0.0 to 1.8	0.0 to 3.9	1.4 to 2.1
Interday Accuracy Range (%RE)	–3.0 to –1.8	–1.3 to 2.7	–2.0 to 1.0	–2.3 to 4.3	–6.1 to 6.7
Stability in Frozen Matrix (days)	173 days at –70°C and 446 days at –20°C	68 days at –70°C and 84 days at –20°C	233 days at –70°C	27 days at –70°C and 20 days at –20°C	136 days at –70°C and –20°C

Pharmacokinetic Results:

HIV Antiretrovirals: Tables 2 to 12 show the PK parameters of HIV antiretroviral drugs with or without LDV or SOF/LDV.

Table 2: EFV Plasma Pharmacokinetic Parameters Following Administration of EFV/FTC/TDF Alone and in Combination with LDV (Cohort 1)

EFV PK Parameter	Mean (%CV)	
	EFV/FTC/TDF (N = 12)	LDV+EFV/FTC/TDF (N = 12)
AUC _{tau} (ng•h/mL)	85,927.1 (55.8)	80,508.6 (70.0)
C _{max} (ng/mL)	4949.2 (42.9)	4610.0 (56.0)
C _{tau} (ng/mL)	2922.8 (64.0)	2752.2 (79.8)
T _{max} (h) ^a	2.50 (2.23, 5.00)	3.50 (2.50, 5.50)
T _{last} (h) ^a	24.0 (24.0, 24.0)	26.00 (26.00, 26.00)
t _{1/2} (h) ^a	42.70 (30.03, 52.31)	44.05 (38.16, 56.86)
CL _{ss} /F (mL/h)	8637.1 (43.0)	9891.1 (44.0)

a Median (Q1, Q3)

Table 3: FTC Plasma Pharmacokinetic Parameters Following Administration of EFV/FTC/TDF Alone and in Combination with LDV (Cohort 1)

FTC PK Parameter	Mean (%CV)	
	EFV/FTC/TDF (N = 12)	LDV+EFV/FTC/TDF (N = 12)
AUC _{tau} (ng•h/mL)	9996.1 (18.0)	10033.4 (17.9)
C _{max} (ng/mL)	2017.5 (28.0)	1979.2 (37.4)
C _{tau} (ng/mL)	72.6 (27.6)	80.0 (24.4)
T _{max} (h) ^a	1.97 (1.50, 1.97)	1.50 (1.25, 1.95)
T _{last} (h) ^a	24.0 (24.0, 24.0)	26.00 (26.00, 26.00)
t _{1/2} (h) ^a	11.62 (9.79, 13.16)	13.97 (12.35, 16.31)
CL _{ss} /F (mL/h)	20,627.4 (18.7)	20,470.0 (16.2)

a Median (Q1, Q3)

Table 4: TFV Plasma Pharmacokinetic Parameters Following Administration of EFV/FTC/TDF Alone and in Combination with LDV (Cohort 1)

TFV PK Parameter	Mean (%CV)	
	EFV/FTC/TDF (N = 12)	LDV+EFV/FTC/TDF (N = 12)
AUC _{tau} (ng•h/mL)	2370.4 (24.8)	3256.1 (22.9)
C _{max} (ng/mL)	314.3 (29.0)	335.0 (29.7)
C _{tau} (ng/mL)	43.8 (26.0)	67.6 (25.2)
T _{max} (h) ^a	1.50 (0.88, 1.50)	1.25 (1.00, 1.50)
T _{last} (h) ^a	24.0 (24.0, 24.0)	26.00 (26.00, 26.00)
t _{1/2} (h) ^a	11.51 (9.06, 14.15)	12.15 (10.74, 14.08)
CL _{ss} /F (mL/h)	61,299.5 (30.1)	43,986.7 (24.5)

a Median (Q1, Q3)

Table 5: DRV Plasma Pharmacokinetic Parameters Following Administration of DRV/r Alone and in Combination with LDV (Cohort 2)

DRV PK Parameter	Mean (%CV)	
	DRV/r Alone (N = 24)	LDV+DRV/r (N = 23)
AUC _{tau} (ng•h/mL)	93,139.2 (28.9)	91,771.9 (46.8)
C _{max} (ng/mL)	8544.2 (26.7)	9092.6 (57.8)
C _{tau} (ng/mL)	2266.7 (47.6)	2127.7 (50.1)
T _{max} (h) ^a	4.00 (3.00, 4.00)	4.00 (3.00, 4.00)
T _{last} (h) ^a	24.0 (24.0, 24.0)	24.0 (24.0, 24.0)
t _{1/2} (h) ^a	16.53 (10.44, 25.35)	18.49 (12.33, 22.83)
CL _{ss} /F (mL/h)	9328.7 (29.7)	10,024.1 (33.8)

a Median (Q1, Q3)

Table 6: RTV Plasma Pharmacokinetic Parameters Following Administration of DRV/r Alone and in Combination with LDV (Cohort 2)

RTV PK Parameter	Mean (%CV)	
	DRV/r Alone (N = 24)	LDV+DRV/r (N = 23)
AUC _{tau} (ng•h/mL)	3902.8 (35.8)	5254.5 (33.8)
C _{max} (ng/mL)	498.3 (44.8)	631.0 (37.4)
C _{tau} (ng/mL)	46.5 (46.5)	60.3 (42.7)
T _{max} (h) ^a	4.00 (4.00, 6.00)	4.00 (4.00, 6.00)
T _{last} (h) ^a	24.0 (24.0, 24.0)	24.0 (24.0, 24.0)
t _{1/2} (h) ^a	9.05 (7.54, 11.13)	9.21 (8.08, 11.31)
CL _{ss} /F (mL/h)	29,265.8 (40.4)	21,581.8 (39.8)

a Median (Q1, Q3)

Table 7: RPV Plasma Pharmacokinetic Parameters Following Administration of RPV Alone and in Combination with LDV (Cohort 3)

RPV PK Parameter	Mean (%CV)	
	RPV Alone (N = 23)	LDV+RPV (N = 23)
AUC _{tau} (ng•h/mL)	2617.6 (29.2)	2509.0 (24.2)
C _{max} (ng/mL)	184.0 (30.2)	181.1 (25.2)
C _{tau} (ng/mL)	98.3 (34.6)	93.8 (27.1)
T _{max} (h) ^a	4.00 (4.00, 4.00)	4.00 (4.00, 4.00)
T _{last} (h) ^a	24.0 (24.0, 24.0)	24.0 (24.0, 24.0)
t _{1/2} (h) ^a	56.90 (35.25, 119.80)	39.22 (30.53, 49.45)
CL _{ss} /F (mL/h)	10,299.2 (28.3)	10,524.1 (24.1)

a Median (Q1, Q3)

Table 8: RAL Plasma Pharmacokinetic Parameters Following Administration of RAL Alone and in Combination with LDV (Cohort 4)

RAL PK Parameter	Mean (%CV)	
	RAL Alone (N = 29)	LDV+RAL (N = 28)
AUC _{tau} (ng•h/mL)	7909.1 (68.0)	6902.1 (72.5)
C _{max} (ng/mL)	2125.4 (77.8)	1921.9 (94.0)
C _{tau} (ng/mL)	218.0 (74.4)	264.7 (90.5)
T _{max} (h) ^a	6.00 (3.00, 8.00)	3.00 (2.00, 8.00)
T _{last} (h) ^a	12.0 (12.0, 12.0)	12.0 (12.0, 12.0)
t _{1/2} (h) ^a	1.91 (1.49, 6.54)	3.08 (1.82, 5.21)
CL _{ss} /F (mL/h)	94,954.9 (86.2)	97,102.7 (71.6)

a Median (Q1, Q3)

Table 9: EVG Plasma Pharmacokinetic Parameters Following Administration of EVG+COBI Alone and in Combination with SOF/LDV (Cohort 5)

EVG PK Parameter	Mean (%CV)	
	EVG+COBI Alone (N = 29)	SOF/LDV + EVG+COBI (N = 29)
AUC _{tau} (ng•h/mL)	28,289.8 (29.4)	28,656.1 (26.0)
C _{max} (ng/mL)	2394.8 (24.3)	2136.9 (30.1)
C _{tau} (ng/mL)	588.7 (52.4)	753.0 (33.8)
T _{max} (h) ^a	4.00 (4.00, 4.00)	4.00 (4.00, 4.00)
T _{last} (h) ^a	24.0 (24.0, 24.0)	24.0 (24.0, 24.0)
t _{1/2} (h) ^a	13.25 (9.80, 21.91)	20.66 (15.62, 25.69)
CL _{ss} /F (mL/h)	5704.2 (26.8)	5593.9 (27.1)

a Median (Q1, Q3)

Table 10: COBI Plasma Pharmacokinetic Parameters Following Administration of EVG+COBI Alone and in Combination with SOF/LDV (Cohort 5)

COBI PK Parameter	Mean (%CV)	
	EVG+COBI Alone (N = 29)	SOF/LDV + EVG+COBI (N = 29)
AUC _{tau} (ng•h/mL)	12,234.9 (50.6)	19,019.6 (44.4)
C _{max} (ng/mL)	1567.6 (28.8)	1958.3 (28.8)
C _{tau} (ng/mL)	56.5 (144.2)	200.8 (126.5)
T _{max} (h) ^a	3.00 (3.00, 4.00)	4.00 (3.00, 4.00)
T _{last} (h) ^a	24.0 (24.0, 24.0)	24.0 (24.0, 24.0)
t _{1/2} (h) ^a	3.58 (3.18, 4.38)	4.96 (4.06, 5.92)
CL _{ss} /F (mL/h)	14,645.3 (36.1)	9149.7 (36.9)

a Median (Q1, Q3)

Table 11: ATV Plasma Pharmacokinetic Parameters Following Administration of ATV/r Alone and in Combination with SOF/LDV (Cohort 6)

ATV PK Parameter	Mean (%CV)	
	ATV/r Alone (N = 30)	SOF/LDV+ATV/r (N = 30)
AUC _{tau} (ng•h/mL)	62,150.3 (34.3)	82,608.6 (34.2)
C _{max} (ng/mL)	6964.7 (28.4)	7395.0 (25.0)
C _{tau} (ng/mL)	1500.1 (57.4)	2549.7 (52.9)
T _{max} (h) ^a	3.00 (3.00, 4.00)	4.00 (3.00, 4.00)
T _{last} (h) ^a	24.0 (24.0, 24.0)	24.0 (24.0, 24.0)
t _{1/2} (h) ^a	27.84 (17.22, 40.82)	29.80 (20.28, 57.35)
CL _{ss} /F (mL/h)	5432.1 (35.7)	4087.3 (35.9)

a Median (Q1, Q3)

Table 12: RTV Plasma Pharmacokinetic Parameters Following Administration of ATV/r Alone and in Combination with SOF/LDV (Cohort 6)

RTV PK Parameter	Mean (%CV)	
	ATV/r Alone (N = 30)	SOF/LDV+ATV/r (N = 30)
AUC _{tau} (ng•h/mL)	10,696.9 (28.1)	11,393.0 (34.7)
C _{max} (ng/mL)	1818.4 (29.6)	1709.5 (33.7)
C _{tau} (ng/mL)	64.6 (62.7)	104.2 (74.6)
T _{max} (h) ^a	4.00 (4.00, 4.00)	4.00 (4.00, 4.00)
T _{last} (h) ^a	24.0 (24.0, 24.0)	24.0 (24.0, 24.0)
t _{1/2} (h) ^a	6.90 (5.74, 8.42)	7.69 (5.92, 8.97)
CL _{ss} /F (mL/h)	10,124.6 (29.8)	9861.6 (35.9)

a Median (Q1, Q3)

A summary of the effect of LDV and SOF/LDV on PK of HIV ARVs is presented in the Tables 13 and 14, respectively. “↔” presented in these tables indicate that the 90% CI of %GLSM ratio is within 80% to 125%.

Table 13: Summary of the Effect of LDV (% change (90% CI)) on PK of HIV ARVs

PK Parameter	EFV/FTC/TDF+LDV			DRV/r+LDV		RAL + LDV	RPV + LDV
	EFV	FTC	TFV	DRV	RTV	RAL	RPV
AUCtau (ng•h/mL)	↔	↔	↑38% (↑23%-↑55%)	↔	↑37% (↑22%-↑55%)	↓15% (↓30%-↑2%)	↔
Cmax (ng/mL)	↔	↔	↔	↔	↑33% (↑20%-↑47%)	↓18% (↓34%-↑2%)	↔
Ctau (ng/mL)	↔	↔	↑55% (↑35%-↑77%)	↔	↑33% (↑7%-↑66%)	↑15% (↓10%-↑46%)	↔

Note: “↔” indicates that the 90% CI of %GLSM ratio is within 80% to 125%

Table 14: Summary of the Effect of SOF/LDV (% change (90% CI)) on PK of HIV ARVs

PK Parameter	EVG+COBI+SOF/LDV		ATV/r+SOF/LDV	
	EVG	COBI	ATV	RTV
AUCtau (ng•h/mL)	↔	↑59% (↑49%-↑70%)	↑33% (↑25%-↑42%)	↔
Cmax (ng/mL)	↔	↔	↔	↔
Ctau (ng/mL)	↑36% (↑23%-↑49%)	↑325% (↑247%-↑422%)	↑75% (↑58%-93%)	↑56% (↑42%-71%)

Note: “↔” indicates that the 90% CI of %GLSM ratio is within 80% to 125%

Pharmacokinetic results from this study demonstrated that

- Coadministration of ARVs with LDV did not alter the PK of EFV or FTC (given as components of a FDC of EFV/FTC/TDF in Atripla), DRV or RPV
- Increases in TFV AUCtau and Ctau of approximately 38% and 55%, respectively, were observed following coadministration of EFV/FTC/TDF and LDV; TFV Cmax remained comparable in both treatments (administration separated by 2 hours)
- Increases in RTV PK (33% to 37%) were observed upon administration of DRV/r with LDV. The magnitude of an increase in RTV in conjunction with a lack of effect of LDV on DRV does not warrant dose adjustment.
- Raltegravir mean AUCtau and Cmax were 15% and 18% lower, respectively, upon administration in conjunction with LDV. The decrease in RAL exposure was comparable or less than that observed when RAL was coadministered with RTV-boosted tipranavir (18%, 24%, and 54% decreases in Cmax, AUC0-12, and Ctau, respectively) or EFV (36%, 36%, and 21% decrease in Cmax, AUCinf, and Ctau, respectively), which did not necessitate RAL dose adjustment and suggested that the decreases observed in AUCtau and Cmax when RAL was coadministered with LDV are not clinically significant.
- Administration of SOF/LDV with EVG plus COBI resulted in 59% and 325% higher COBI AUCtau and Ctau, respectively, and approximately 36% higher Ctau for EVG. No changes in any other PK parameters of EVG or COBI were observed. Because EVG/COBI is marketed as a part of Stribild® (EVG/COBI/FTC/TDF), potential additive drug interactions in combination with TDF should be considered, since COBI also

increases tenofovir exposures. As such, a dose recommendation regarding concomitant use with Stribild cannot be made at this time.

- Concomitant dosing of SOF/LDV with ATV/r resulted in approximately 56% higher RTV Ctau, 33% ATV AUCtau and 75% ATV Ctau. The magnitude of increase in ATV Ctau with SOF/LDV was similar to that observed with telaprevir; coadministration of ATV/r with telaprevir resulted in approximately 85% higher Cmin, which did not warrant dose adjustment. For protease inhibitors, adverse effects are generally correlated with Cmax thus, a 33% increase in ATV AUCtau and 75% increase in ATV Ctau without a change in ATV Cmax by coadministration of SOF/LDV would not warrant a dose adjustment.

Reviewer's Note: Drug interaction between ATR and LDV was conducted with 2 hours separation in administration, and thus may underestimate the magnitude of effect of LDV on TFV exposures if they are administered simultaneously.

SOF, GS-56650, and GS-331007:

Table 15 presents summary PK parameters of SOF, GS-56650, and GS-331007 following administration of SOF/LDV alone or in combination with EVG+COBI (Cohort 5).

Table 15: Summary PK parameters of SOF, GS-56650, and GS-331007 Following Administration of SOF/LDV Alone or in Combination with EVG+COBI (Cohort 5)

PK Parameter	SOF Mean (%CV)		GS-56650 Mean (%CV)		GS-331007 Mean (%CV)	
	SOF/LDV Alone (N = 30)	SOF/LDV + EVG+COBI (N = 29)	SOF/LDV Alone (N = 30)	SOF/LDV + EVG+COBI (N = 29)	SOF/LDV Alone (N = 30)	SOF/LDV + EVG+COBI (N = 29)
AUCtau (ng•h/mL)	2817.7 (40.4)	3716.6 (32.0)	3170.5 (20.3)	3811.6 (22.8)	11,306.8 (17.7)	16,394.1 (19.1)
Cmax	1518.2 (53.5)	1949.2 (42.1)	678.0 (19.3)	820.1 (58.5)	889.9 (18.0)	1231.0 (47.5)
Ctau	NA	NA	20.0	11.3 (54.5)	332.9 (20.8)	511.7 (22.6)
T _{max} (h) ^a	2.0 (1.0, 3.0)	2.0 (1.0, 3.0)	3.00 (2.00, 3.00)	3.00 (2.00, 4.00)	4.00 (4.00, 4.00)	4.00 (3.00, 4.00)
T _{last} (h) ^a	6.00 (4.00, 6.00)	6.00 (6.00, 6.00)	16.0 (16.0, 20.0)	24.0 (24.0, 24.0)	24.0 (24.0, 24.0)	24.0 (24.0, 24.0)
t _{1/2} (h) ^a	0.50 (0.44, 0.55)	0.53 (0.45, 0.62)	2.90 (2.62, 3.38)	5.35 (4.46, 6.32)	29.87 (27.13, 38.13)	32.14 (27.69, 38.95)
CL _{SS} /F (mL/h)	164,985.4 (42.4)	116,345.3 (26.2)	NA	NA	NA	NA

^a Median (Q1, Q3)
NA = not applicable

Table 16 presents summary PK parameters of SOF, GS-56650, and GS-331007 following administration of SOF/LDV alone or in combination with ATV/r (Cohort 6).

Table 16: Summary PK parameters of SOF, GS-56650, and GS-331007 Following Administration of SOF/LDV Alone or in Combination with ATV/r (Cohort 6)

PK Parameter	SOF Mean (%CV)		GS-56650 Mean (%CV)		GS-331007 Mean (%CV)	
	SOF/LDV Alone (N = 30)	SOF/LDV + EVG+COBI (N = 29)	SOF/LDV Alone (N = 30)	SOF/LDV + EVG+COBI (N = 29)	SOF/LDV Alone (N = 30)	SOF/LDV + EVG+COBI (N = 29)
AUC _{tau} (ng•h/mL)	2854.8 (27.3)	3072.6 (24.4)	3187.2 (23.1)	3595.0 (22.4)	11,185.1 (18.3)	13,979.8 (28.1)
C _{max} (ng/mL)	1709.7 (50.2)	1604.0 (47.0)	658.3 (29.3)	729.1 (25.6)	875.2 (21.0)	991.1 (21.2)
C _{tau} (ng/mL)	NA	NA	1.1 (305.7)	10.9 (56.0)	342.0 (22.2)	446.8 (32.3)
T _{max} (h) ^a	2.00 (1.00, 2.00)	2.00 (1.00, 2.00)	3.00 (2.00, 3.00)	3.00 (2.00, 3.00)	4.00 (3.00, 4.00)	4.00 (3.00, 4.00)
T _{last} (h) ^a	6.00 (4.00, 6.00)	6.00 (6.00, 6.00)	20.0 (16.0, 20.0)	24.0 (24.0, 24.0)	24.0 (24.0, 24.0)	24.0 (24.0, 24.0)
t _{1/2} (h) ^a	0.51 (0.45, 0.57)	0.55 (0.49, 0.66)	3.19 (2.87, 4.04)	5.53 (4.70, 7.64)	28.57 (22.65, 34.07)	29.56 (23.46, 35.99)
CL _{SS} /F (mL/h)	150,184.2 (26.4)	137,899.1 (24.5)	NA	NA	NA	NA

^a Median (Q1, Q3)
NA = not applicable

As shown in the Table 17, modest increases in SOF (33% to 36%) and GS-331007 (33% to 53%) were observed following administration of SOF/LDV with EVG+COBI. Coadministration of ATV/r with SOF/LDV did not affect the PK of SOF or GS-56650, but increased the AUC_{tau} and C_{tau} of GS-331007 by 23% and 28%. These changes are less than the effect of mild renal impairment on the PK parameters of SOF and its metabolites, and are not considered clinically significant.

Table 17: Summary of the Effect of HIV ARVs (% change (90% CI)) on PK of SOF and its Metabolites

PK Parameter	EVG+COBI+SOF/LDV			ATV/r+SOF/LDV		
	SOF	GS-56650	GS-331007	SO	GS-56650	GS-331007
AUC _{tau} (h•ng/mL)	↑ 36% (↑21%-↑52%)	↔	↑ 44% (↑41%-↑48%)	↔	↔	↑23% (↑18%-↑29%)
C _{max} (ng/mL)	↑ 33% (↑14% -↑56%)	↔	↑ 33% (↑22%-↑44%)	↔	↔	↔
C _{tau} (ng/mL)	NA	NA	↑ 53% (↑47%-↑59%)	NA	NA	↑28% (↑21%-↑36%)

NA = not applicable

Note: “<->” indicates that the 90% CI of %GLSM ratio is within 80% to 125

LDV: Tables 18 to 28 show the PK parameters of HIV Antiretroviral drugs with or without LDV or SOF/LDV.

Table 18: LDV Plasma Pharmacokinetic Parameters Following Administration of LDV Alone and in Combination with EFV/FTC/TDF (Cohort 1)

LDV PK Parameter	Mean (%CV)	
	LDV Alone (N = 15)	LDV+EFV/FTC/TDF (N = 14)
AUC _{tau} (ng•h/mL)	9271.2 (42.1)	7488.9 (59.7)
C _{max} (ng/mL)	541.9 (39.1)	444.6 (56.2)
C _{tau} (ng/mL)	315.1 (45.6)	251.5 (69.2)
T _{max} (h) ^a	4.00 (4.00, 6.00)	6.00 (4.00, 6.00)
T _{last} (h) ^a	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
t _{1/2} (h) ^a	58.23 (39.62, 76.32)	45.12 (31.20, 63.19)
CL _{ss} /F (mL/h)	11,174.0 (35.8)	15,611.7 (49.2)

^a Median (Q1, Q3)

Table 19: LDV Plasma Pharmacokinetic Parameters Following Administration of LDV Alone and in Combination with DRV/r (Cohort 2)

LDV PK Parameter	Mean (%CV)	
	LDV Alone (N = 23)	LDV+DRV/r (N = 23)
AUC _{tau} (ng•h/mL)	8395.2 (33.1)	11,637.3 (30.5)
C _{max} (ng/mL)	476.3 (28.4)	693.1 (28.2)
C _{tau} (ng/mL)	293.8 (38.9)	408.3 (35.0)
T _{max} (h) ^a	6.00 (4.00, 6.00)	4.00 (4.00, 6.00)
T _{last} (h) ^a	24.0 (24.0, 24.0)	24.0 (24.0, 24.0)
t _{1/2} (h) ^a	51.78 (35.72, 66.56)	63.14 (43.50, 88.58)
CL _{ss} /F (mL/h)	11,787.7 (31.1)	8420.4 (29.5)

^a Median (Q1, Q3)

Table 20: LDV Plasma Pharmacokinetic Parameters Following Administration of LDV Alone and in Combination with RPV (Cohort 3)

LDV PK Parameter	Mean (%CV)	
	LDV Alone (N = 23)	LDV+RPV (N = 23)
AUC _{tau} (ng•h/mL)	8883.8 (35.7)	8202.6 (24.3)
C _{max} (ng/mL)	500.8 (29.0)	469.1 (21.9)
C _{tau} (ng/mL)	312.3 (43.5)	289.2 (30.0)
T _{max} (h) ^a	4.00 (4.00, 6.00)	4.00 (4.00, 6.00)
T _{last} (h) ^a	24.0 (24.0, 24.0)	24.0 (24.0, 24.0)
t _{1/2} (h) ^a	56.52 (38.06, 75.77)	54.80 (43.53, 74.97)
CL _{ss} /F (mL/h)	11,207.3 (30.7)	11,638.0 (25.3)

^a Median (Q1, Q3)

Table 21: LDV Plasma Pharmacokinetic Parameters Following Administration of LDV Alone and in Combination with RAL (Cohort 4)

LDV PK Parameter	Mean (%CV)	
	LDV Alone (N = 29)	LDV+RAL (N = 28)
AUC _{tau} (ng•h/mL)	8939.3 (39.6)	8473.5 (50.3)
C _{max} (ng/mL)	504.3 (38.5)	474.7 (45.4)
C _{tau} (ng/mL)	305.1 (42.5)	283.7 (53.4)
T _{max} (h) ^a	6.00 (4.00, 6.00)	4.00 (4.00, 6.00)
T _{last} (h) ^a	24.0 (24.0, 24.0)	24.0 (24.0, 24.0)
t _{1/2} (h) ^a	39.34 (31.80, 44.81)	44.58 (33.10, 57.18)
CL _{ss} /F (mL/h)	11,950.7 (45.0)	13,746.5 (54.9)

^a Median (Q1, Q3)

Table 22: LDV Plasma Pharmacokinetic Parameters Following Administration of SOF/LDV Alone and in Combination with EVG+COBI (Cohort 5)

LDV PK Parameter	Mean (%CV)	
	SOF/LDV Alone (N = 30)	SOF/LDV + EVG+COBI (N = 29)
AUC _{tau} (ng•h/mL)	12,837.1 (54.8)	22,176.2 (39.4)
C _{max} (ng/mL)	716.7 (48.3)	1141.4 (37.1)
C _{tau} (ng/mL)	477.6 (64.3)	862.4 (44.4)
T _{max} (h) ^a	4.00 (4.00, 6.00)	6.00 (4.00, 6.00)
T _{last} (h) ^a	24.0 (24.0, 24.0)	24.0 (24.0, 24.0)
t _{1/2} (h) ^a	56.86 (37.09, 103.18)	96.40 (73.56, 160.88)
CL _{ss} /F (mL/h)	9128.9 (54.5)	5102.3 (64.4)

^a Median (Q1, Q3)

Table 23: LDV Plasma Pharmacokinetic Parameters Following Administration of SOF/LDV Alone and in Combination with ATV/r (Cohort 6)

LDV PK Parameter	Mean (%CV)	
	SOF/LDV Alone (N = 30)	SOF/LDV+ATV/r (N = 30)
AUC _{tau} (ng•h/mL)	11,856.5 (52.5)	24,261.0 (35.8)
C _{max} (ng/mL)	660.7 (43.5)	1273.8 (32.0)
C _{tau} (ng/mL)	429.1 (57.8)	960.8 (40.0)
T _{max} (h) ^a	4.00 (4.00, 6.00)	4.00 (4.00, 6.00)
T _{last} (h) ^a	24.0 (24.0, 24.0)	24.0 (24.0, 24.0)
t _{1/2} (h) ^a	63.45 (44.29, 100.28)	103.74 (63.66, 217.18)
CL _{ss} /F (mL/h)	9210.8 (40.3)	4254.4 (41.6)

^a Median (Q1, Q3)

As shown in the Table 24, LDV plasma exposure parameters were higher (39% to 45%) following administration of LDV with DRV/r, relative to LDV dosing alone. In contrast, the magnitude of an increase in LDV PK was higher following dosing of SOF/LDV with EVG+COBI or with ATV/r. Briefly, LDV exposures were approximately 63% to 91% higher in the presence of EVG+COBI and 98% to 136% higher with ATV/r.

Table 24: Summary of the Effect of HIV ARVs (% change (90% CI)) on PK of LDV

PK Parameter	EFV/FTC/TDF+ LDV	DRV/r + LDV	RPV+LDV	RAL+LDV	EVG+COBI+ SOF/LDV	ATV/r + SOF/LDV
	LDV	LDV	LDV	LDV	SOF/LDV	SOF/LDV
AUC _{tau} (ng•h/mL)	↓ 24% (↓15%-↓32%)	↑ 39% (↑28%-↑49%)	↔	↔	↑ 78% (↑64%-↑94%)	↑ 113% (↑89%-↑140%)
C _{max} (ng/mL)	↓ 23% (↓14%-↓30%)	↑ 45% (↑34%-↑56%)	↔	↔	↑ 63% (↑51%-↑75%)	↑ 98% (↑78%-↑120%)
C _{tau} (ng/mL)	↓ 28% (↓17%-↓37%)	↑ 39% (↑29%-↑51%)	↔	↔	↑ 91% (↑76%-↑108%)	↑ 136% (↑108%-↑167%)

Note: “<->” indicates that the 90% CI of %GLSM ratio is within 80% to 125

The use of LDV with DRV/r, EVG+COBI or ATV/r is acceptable based on the following:

- The use of P-gp inhibitors has been permitted in all HCV-infected subjects in the LDV/SOF **Phase 2/3 clinical program**. No serious AEs were reported despite 2.4-fold higher LDV exposure in females (N=7) on chronic P-gp inhibitors vs. males not on P-gp inhibitors (N=1252)
- In the **thorough QT study** (GS-US-344-0109, n =59, 30% females) 120 mg of LDV was given twice daily for 10 days, no Grade 3 or 4 or serious AEs occurred during the study despite 4-fold higher LDV exposures vs. LDV 90 mg QD and exposures were higher than in females on chronic P-gp inhibitors.

Conclusion:

- Dose modification of EFV, FTC, DRV/r, RPV, RAL or ATV/r is not warranted upon coadministration with SOF/LDV.
- LDV increased TFV systemic exposures. The recommendation for coadministration of these agents is pending further evaluation.
- Coadministration of EVG+COBI with SOF/LDV resulted in a 2-way drug PK interaction. The mechanism for this interaction is currently unknown; additional evaluations may be required prior to recommending administration of SOF/LDV with HIV ARV regimens that contain EVG+COBI.

4.2.4.2 GS-US-337-0127: A Phase 1 Study to Evaluate Pharmacokinetic Drug-Drug Interactions between Sofosbuvir/Ledipasvir (SOF/LDV) Fixed-Dose Combination (FDC) Tablet and Antiretroviral Regimens Efavirenz/Emtricitabine/Tenofovir Disoproxil Fumarate (EFV/FTC/TDF; Atripla®) or Emtricitabine/Rilpivirine/Tenofovir Disoproxil Fumarate (FTC/RPV/TDF; Complera®), and the Relative Bioavailability and Pharmacokinetics of SOF/LDV FDC upon Administration with a Representative H2-Receptor Antagonist or a Proton Pump Inhibitor

Objectives:

- To evaluate the pharmacokinetics (PK) of sofosbuvir (SOF) and ledipasvir (LDV) upon administration of SOF/LDV fixed-dose combination (FDC) with Atripla® (ATR);

efavirenz/emtricitabine/tenofovir disoproxil fumarate [EFV/FTC/TDF]) or Complera® (CPA; FTC/rilpivirine [RPV]/TDF)

- To evaluate the PK of EFV, RPV, FTC, and tenofovir (TFV) upon administration of ATR or CPA with SOF/LDV FDC
- To evaluate the relative bioavailability and PK of SOF and LDV upon SOF/LDV FDC administration with a representative H₂-receptor antagonist (H₂RA) or proton pump inhibitor (PPI)

Study Design: Healthy subjects (n=92) were enrolled into 1 of 2 cohorts, and into 1 of 2 groups within each cohort as follows:

Cohort 1, Group 1: Subjects (n=32) received 14 days of SOF/LDV 400 mg/90 mg (Days 1-14; Treatment A) or ATR (Days 1-14; Treatment B) once daily, followed by 14 days of SOF/LDV+ATR (Days 15-28; Treatment C) once daily.

Cohort 1, Group 2: Subjects (n=32) received 10 days of SOF/LDV 400 mg/90 mg (Days 1-10; Treatment D) or CPA (Days 1-10; Treatment E) once daily, followed by 10 days of SOF/LDV+CPA (Days 11-20; Treatment F) once daily.

Cohort 2, Group 1: Subjects (n=12) received a single dose of SOF/LDV 400 mg/90 mg (Day 1; Treatment G) followed by a 10-day washout period (Days 2-11), a single dose of SOF/LDV+famotidine (Day 12; Treatment H), followed by a 10-day washout period (Days 13-22), a single dose of famotidine (Day 23 in the evening), and a single dose of SOF/LDV (Day 24 in the morning; Treatment I).

Cohort 2, Group 2: Subjects (n=16) received a single dose of SOF/LDV 400 mg/90 mg (Day 1; Treatment J) followed by a 10-day washout period (Days 2-11), omeprazole 20 mg administered in the morning for 5 days (Days 12-16; Treatment K), followed by a single dose of SOF/LDV and omeprazole (Day 17; Treatment L).

For Cohort 1, Group 1 and Cohort 2, Group 2 all study drugs were administered in the morning under fasted conditions (no food or liquids except water for at least 10 hours prior to dosing and 4 hours after dosing).

For Cohort 1, Group 2 and Cohort 2, Group 1 all study drugs were administered in the morning within 5 minutes of completing a moderate-fat meal (approximately 600 calories, 25-30% fat). One exception is for Cohort 2, Group 1 subjects who received a single dose of famotidine in the evening of Day 23.

Formulation: SOF/LDV 400/90-mg tablets: Lot# DK1205B2;
EFV/FTC/TDF (ATR) 600/200/300-mg tablets: Lot# 00475; FTC/RPV/TDF (CPA) 200/25/300-mg tablets: Lot# HBKP;
Famotidine 40-mg tablets: Lot# LL12129; omeprazole 20-mg tablets: Lot# H013300

PK Sampling: For Cohort 1, serial blood samples were collected on Days 14 and 28 (Group 1) and Days 10 and 20 (Group 2). Samples for determination of SOF, GS-566500, GS-331007, LDV, EFV, RPV, FTC, and TFV plasma concentrations were collected at the following time points relative to dosing: predose (≤ 5 min) and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 4.5, 5, 6, 8, 10, 12, 16, 20, and 24 hours postdose.

For Cohort 2, serial blood samples were collected on Days 1, 12, and 24 (Group 1) and Days 1 and 17 (Group 2). Samples for determination of SOF, GS-566500, GS-331007, and LDV plasma concentrations were collected at the following time points relative to dosing: predose (≤ 5 min) and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 4.5, 5, 6, 8, 10, 12, 16, 20, 24, 48, 72, 96, 120, and 144 hours postdose.

Urine samples for SOF, GS-566500, GS-331007, and TFV concentrations were collected pre-dose and 0-4, 6-12, and 12-24 hours post-dose on Days 14 and 28 for Cohort 1, Group 1 and on Days 10 and 20 for Cohort 1, Group 2.

Analytical Methods: Concentrations of SOF, GS-566500, GS-331007, LDV, EFV, RPV, FTC, and TFV in plasma samples and SOF, GS-566500, GS-331007 and TFV in urine samples were determined using fully validated high-performance liquid chromatography/tandem mass spectroscopy (LC/MS/MS) bioanalytical methods. All samples were analyzed in the time frame supported by frozen stability storage data. The assays for SOF, GS-566500, GS-331007, LDV, EFV, FTC, RPV, and TFV were performed and validated (b) (4). Assay validation parameters are summarized in Table 1, Table 2, and Table 3.

Table 1: Bioanalytical Assay Validation for SOF, GS-566500, GS-331007, and LDV in Human Plasma

Parameter	SOF	GS-566500	GS-331007	LDV
Linear range (ng/mL)	5 to 5000	10 to 5000	10 to 5000	1 to 2000
LLOQ (ng/mL)	5	10	10	1
Interday precision range (%CV)	1.3 to 7.4 ^a	3.3 to 9.1 ^a	0.8 to 5.6 ^a	1.0 to 8.8
Interday accuracy range (%RE)	-3.3 to 8.2 ^a	-5.2 to 5.9 ^a	-0.1 to 7.5a	-7.1 to -3.6
Stability in frozen matrix (days)	485 at -70°C and 174 at -20°C	485 at -70°C and 174 at -20°C	485 at -70°C and 174 at -20°C	623 at -20°C and -70°C

^a Intraday ranges are reported.

Table 2: Bioanalytical Assay Validation for EFV, FTC, TFV and RPV in Human Plasma

Parameter	EFV	FTC	TFV	RPV
Linear range (ng/mL)	5 to 5000	5 to 3000	5 to 3000	1 to 500
LLOQ (ng/mL)	5	5	5	1
Interday precision range (%CV)	1.5 to 6.3	1.4 to 5.7	2.4 to 6.5	2.8 to 5.7
Interday accuracy range (%RE)	-5.2 to 7.5	-7.8 to 2.4	-4.7 to 2.0	-7.2 to 2.2
Stability in frozen matrix (days)	1301 at -70°C and -20°C	190 at -20°C and -70°C	190 at -20°C and -70°C	783 at -20°C and -70°C

Table 3: Bioanalytical Assay Validation for SOF, GS-566500, GS-331007, and TFV in Human Urine

Parameter	SOF	GS-566500	GS-331007	TFV
Linear range (ng/mL)	10 to 10,000	10 to 10,000	10 to 10,000	500 to 200,000
LLOQ (ng/mL)	10	10	10	500
Interday precision range (%CV)	2.2 to 7.7 ^a	3.2 to 9.6	2.1 to 15.5	1.1 to 2.1
Interday accuracy range (%RE)	-6.3 to 1.9 ^a	-4.7 to 3.8	-11.6 to -1.1	-3.7 to -0.7
Stability in frozen matrix (days)	178 at -70°C	133 at -70°C	133 at -70°C	225 at -20°C and -70°C

^a Intraday ranges are reported

The standard curve and QC data indicated that the plasma assay methods for SOF, GS-566500, GS-331007, LDV, EFV, RPV, FTC, and TFV; as well as the urine assay methods for SOF, GS-566500, GS-331007 and TFV were precise and accurate.

Pharmacokinetic Results:

Cohort 1:

Tables 4 and 5 present the PK parameters for SOF and its metabolites following administration of SOF/LDV or SOF/LDV+ATR under fasted conditions (Cohort 1, Group 1) and administration of SOF/LDV or SOF/LDV+CPA with a moderate-fat meal (Cohort 1, Group 2), respectively. Table 6 shows the PK parameters for LDV in Cohort 1. Table 7 and Table 8 show the PK parameters of the components in ATR and CPA.

Table 4: Pharmacokinetic Parameters for SOF and its Metabolites Following Administration of Multiple Doses of SOF/LDV or SOF/LDV+ATR (Cohort 1, Group 1)

PK Parameter	SOF Mean (%CV)		GS-566500 Mean (%CV)		GS-331007 Mean (%CV)	
	SOF/LDV (N = 14)	SOF/LDV+ATR (N = 14)	SOF/LDV (N = 14)	SOF/LDV+ATR (N = 14)	SOF/LDV (N = 14)	SOF/LDV+ATR (N = 14)
AUC _{tau} (h·ng/mL)	1673.4 (27.8)	1575.9 (30.9)	1411.6 (23.6)	1272.2 (16.3)	11,119.7 (15.0)	10,054.9 (21.0)
C _{max} (ng/mL)	1529.9 (32.2)	1529.0 (20.3)	341.1 (22.1)	293.0 (15.6)	1062.1 (20.6)	917.4 (25.4)
C _{last} (ng/mL)	10.04 (57.75)	8.69 (47.19)	14.29 (25.63)	16.33 (22.30)	232.02 (21.71)	251.60 (26.68)
T _{max} (h) ^a	0.50 (0.50, 0.75)	0.50 (0.50, 0.75)	1.50 (1.00, 1.50)	1.00 (1.00, 2.00)	3.00 (2.00, 3.00)	3.00 (2.00, 3.00)
T _{last} (h) ^a	4.75 (4.50, 5.00)	4.50 (4.00, 5.00)	12.00 (12.00, 12.00)	12.00 (12.00, 12.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
t _{1/2} (h) ^a	0.49 (0.43, 0.53)	0.49 (0.45, 0.56)	2.36 (2.18, 2.55)	2.89 (2.55, 3.02)	20.43 (13.17, 24.08)	26.40 (17.60, 33.01)
CL _{ss} /F (mL/h)	26,2046.0 (35.8)	274,341.9 (27.5)	NA	NA	NA	NA
CL _{renal} (L/min)	0.117 (32.3)	0.148 (19.0)	0.113 (29.1)	0.117 (17.2)	0.134 (30.4)	0.155 (15.3)

^a Median (Q1, Q3)
NA = not applicable

Table 5: Pharmacokinetic Parameters for SOF and its Metabolites Following Administration of Multiple Doses of SOF/LDV or SOF/LDV+CPA (Cohort 1, Group 2)

PK Parameter	SOF Mean (%CV)		GS-566500 Mean (%CV)		GS-331007 Mean (%CV)	
	SOF/LDV (N = 14)	SOF/LDV+CPA (N = 14)	SOF/LDV (N = 14)	SOF/LDV+CPA (N = 14)	SOF/LDV (N = 14)	SOF/LDV+CPA (N = 14)
AUC _{tau} (h·ng/mL)	3118.1 (33.0)	3506.2 (40.4)	2628.4 (20.9)	2478.4 (23.27)	11,247.3 (16.2)	12,860.1 (20.7)
C _{max} (ng/mL)	1524.9 (38.0)	1641.1 (37.7)	544.6 (22.3)	511.9 (29.15)	867.7 (15.32)	916.0 (16.1)
C _{last} (ng/mL)	13.61 (66.75)	14.75 (47.00)	21.28 (44.98)	14.29 (32.88)	340.23 (22.16)	394.49 (24.73)
T _{max} (h) ^a	2.00 (1.50, 2.00)	2.00 (2.00, 3.00)	3.00 (3.00, 4.00)	4.00 (3.00, 4.50)	4.50 (4.00, 4.50)	4.50 (4.00, 5.00)
T _{last} (h) ^a	6.00 (6.00, 6.00)	6.00 (6.00, 8.00)	16.00 (12.00, 16.00)	16.00 (16.00, 20.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
t _{1/2} (h) ^a	0.47 (0.43, 0.54)	0.53 (0.46, 0.66)	2.37 (2.27, 2.41)	2.59 (2.31, 2.89)	26.83 (24.12, 33.78)	26.90 (24.06, 38.92)
CL _{ss} /F (mL/h)	139,795.3 (29.0)	127,153.3 (32.2)	NA	NA	NA	NA
CL _{renal} (L/min)	0.171 (35.5)	0.152 (35.2)	0.122 (17.1)	0.109 (24.8)	0.158 (14.7)	0.131 (29.2)

^a Median (Q1, Q3)
NA = not applicable

Table 6: Pharmacokinetic Parameters for LDV Following Administration of Multiple Doses of SOF/LDV, SOF/LDV+ATR or SOF/LDV+CPA (Cohort 1, Groups 1 and 2)

LDV PK Parameter	Mean (%CV)			
	Cohort 1, Group 1		Cohort 1, Group 2	
	SOF/LDV (N = 14)	SOF/LDV+ATR (N = 14)	SOF/LDV (N = 17)	SOF/LDV+CPA (N = 15)
AUC _{tau} (h·ng/mL)	8073.2 (38.8)	5270.1 (35.8)	10,805.9 (26.4)	11,944.0 (30.2)
C _{max} (ng/mL)	480.5 (34.4)	314.5 (32.1)	640.0 (26.3)	659.5 (30.5)
C _{tau} (ng/mL)	265.18 (42.00)	173.40 (41.37)	389.47 (28.33)	462.10 (34.26)
T _{max} (h) ^a	4.50 (4.50, 4.50)	4.50 (4.50, 4.50)	4.50 (4.50, 5.00)	5.00 (4.50, 8.00)
T _{last} (h) ^a	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
t _{1/2} (h) ^a	33.38 (27.82, 40.81)	34.06 (24.37, 45.68)	56.90 (50.19, 72.57)	67.79 (50.26, 149.61)
CL _{ss} /F (mL/h)	13,163.6 (45.5)	19,309.5 (36.6)	8928.0 (27.9)	8327.9 (36.6)

^a Median (Q1, Q3)

Table 7: EFV, FTC and TFV Plasma Pharmacokinetic Parameters Following Administration of Multiple Doses of ATR or SOF/LDV+ATR (Cohort 1, Group 1)

PK Parameter	EFV Mean (%CV)		FTC Mean (%CV)		TFV Mean (%CV)	
	ATR (N = 17)	SOF/LDV+ATR (N = 15)	ATR (N = 17)	SOF/LDV+ATR (N = 15)	ATR (N = 17)	SOF/LDV+ATR (N = 15)
AUC _{tau} (h·ng/mL)	73,041.6 (55.0)	58,052.8 (55.8)	9763.5 (21.1)	9886.4 (17.3)	2271.1 (29.0)	4399.4 (27.1)
C _{max} (ng/mL)	4427.5 (46.4)	3500.5 (41.6)	1757.4 (31.2)	1798.3 (22.0)	307.3 (35.8)	527.3 (29.9)
C _{tau} (ng/mL)	2461.68 (65.48)	1893.36 (63.29)	73.93 (27.64)	76.70 (22.81)	43.58 (27.63)	113.24 (32.95)
T _{max} (h) ^a	4.50 (4.50, 4.50)	4.0 (3.00, 4.50)	2.00 (1.50, 2.00)	1.50 (1.50, 2.00)	1.00 (1.00, 1.50)	1.00 (1.00, 1.50)
T _{last} (h) ^a	24.0 (24.00, 24.00)	24.0 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
t _{1/2} (h) ^a	32.55 (21.91, 43.03)	32.99 (24.42, 40.56)	10.45 (9.32, 11.56)	10.46 (9.04, 11.54)	12.55 (12.01, 13.71)	20.38 (18.47, 25.33)
CL _{ss} /F (mL/h)	10,333.7 (42.5)	12,322.9 (33.0)	21,249.4 (18.7)	20,755.3 (15.9)	64,601.5 (26.3)	33,618.9 (31.0)
CL _{renal} (mL/min)	NA	NA	NA	NA	182 (30.7)	191 (21.9)

NA = not applicable

Table 8: FTC, RPV and TFV Plasma Pharmacokinetic Parameters Following Administration of Multiple Doses of CPA or SOF/LDV+CPA (Cohort 1, Group 2)

PK Parameter	FTC Mean (%CV)		RPV Mean (%CV)		TFV Mean (%CV)	
	CPA (N = 14)	SOF/LDV+CPA (N = 14)	CPA (N = 14)	SOF/LDV+CPA (N = 14)	CPA (N = 14)	SOF/LDV+CPA (N = 14)
AUC _{tau} (h·ng/mL)	10,453.7 (25.1)	10,913.6 (24.7)	2642.4 (41.6)	2644.9 (39.6)	3396.6 (27.5)	4782.3 (28.6)
C _{max} (ng/mL)	1774.7 (23.8)	1799.3 (19.4)	186.4 (43.6)	177.0 (40.0)	373.4 (25.7)	489.5 (24.1)
C _{tau} (ng/mL)	89.01 (27.86)	93.60 (25.70)	103.65 (45.17)	114.58 (44.02)	60.95 (22.37)	117.64 (26.37)
T _{max} (h) _a	2.50 (1.50, 3.00)	3.00 (2.00, 4.00)	4.50 (4.00, 4.50)	4.50 (4.00, 4.50)	2.00 (1.50, 3.00)	3.00 (2.00, 4.00)
T _{last} (h) _a	24.0 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
t _{1/2} (h) _a	10.27 (8.41, 12.23)	10.19 (8.90, 12.01)	58.76 (46.40, 79.44)	64.25 (53.62, 82.69)	10.01 (8.88, 10.92)	18.85 (15.86, 21.06)
CL _{ss} /F (mL/h)	20,153.7 (22.4)	19,241.3 (21.2)	12,348.3 (74.1)	11,628.5 (56.9)	43,044.0 (25.4)	30,867.5 (28.8)
CL _{renal} (mL/min)	NA	NA	NA	NA	227 (24.9)	193 (27.7)

NA = not applicable

Cohort 2:

Table 9, 10 and 11 shows the PK parameters for SOF and its metabolites following administration of SOF/LDV or SOF/LDV+famotidine (coadministered), SOF/LDV or SOF/LDV+famotidine (12 h staggered), and SOF/LDV or SOF/LDV+omeprazole, respectively.

Table 9: Pharmacokinetic Parameters for SOF and its Metabolites Following Administration of Single Dose of SOF/LDV or SOF/LDV+Famotidine (Coadministered) (Cohort 2, Group 1)

PK Parameter	SOF Mean (%CV)		GS-566500 Mean (%CV)		GS-331007 Mean (%CV)	
	SOF/LDV (N = 12)	SOF/LDV+ Famotidine (Coadministered) (N = 12)	SOF/LDV (N = 12)	SOF/LDV+ Famotidine (Coadministered) (N = 12)	SOF/LDV (N = 12)	SOF/LDV+ Famotidine (Coadministered) (N = 12)
AUC _{inf} (h·ng/mL)	2516.8 (43.4)	2722.7 (31.9)	2480.5 (19.9)	2323.2 (16.2)	13,469.5 (14.6)	14,299.2 (15.3)
AUC _{last} (h·ng/mL)	2505.7 (43.6)	2713.0 (31.9)	2412.4 (21.0)	2266.4 (17.0)	12,958.3 (15.2)	13,780.8 (15.8)
%AUC _{exp}	0.49 (80.91)	0.36 (40.05)	2.92 (43.77)	2.54 (48.97)	3.89 (26.05)	3.70 (25.96)
C _{max} (ng/mL)	1570.3 (40.5)	1749.3 (38.1)	577.0 (22.9)	534.9 (18.4)	654.7 (15.2)	702.8 (25.3)
C _{last} (ng/mL)	15.54 (59.87)	14.81 (55.76)	20.62 (40.92)	17.13 (44.04)	12.99 (19.48)	12.12 (20.49)
T _{max} (h) ^a	1.50 (1.00, 2.00)	1.50 (1.25, 2.00)	3.00 (1.75, 3.50)	2.00 (1.75, 3.00)	4.00 (3.00, 4.50)	4.50 (4.00, 4.50)
T _{last} (h) ^a	5.50 (4.75, 6.00)	5.50 (5.00, 6.00)	12.00 (12.00, 16.00)	14.00 (12.00, 16.00)	28.03 (24.70, 30.09)	144.00 (120.00, 144.00)
t _{1/2} (h) ^a	0.45 (0.41, 0.52)	0.46 (0.42, 0.51)	2.39 (2.15, 2.46)	2.29 (2.23, 2.31)	120.00 (120.00, 144.00)	30.41 (27.58, 30.81)
CL/F (mL/h)	180,764.6 (32.4)	159,661.8 (28.9)	NA	NA	NA	NA

^a Median (Q1, Q3)
NA = not applicable

Table 10: Pharmacokinetic Parameters for SOF and its Metabolites Following Administration of Single Dose of SOF/LDV or SOF/LDV+Famotidine (12 Hours Staggered) (Cohort 2, Group 1)

PK Parameter	SOF Mean (%CV)		GS-566500 Mean (%CV)		GS-331007 Mean (%CV)	
	SOF/LDV (N = 12)	SOF/LDV+ Famotidine (12 h stagger) (N = 12)	SOF/LDV (N = 12)	SOF/LDV+ Famotidine (12 h stagger) (N = 12)	SOF/LDV (N = 12)	SOF/LDV+ Famotidine (12 h stagger) (N = 12)
AUC _{inf} (h·ng/mL)	2516.8 (43.4)	2326.2 (31.2)	2480.5 (19.9)	2283.6 (19.8)	13,469.5 (14.6)	14,307.5 (16.1)
AUC _{last} (h·ng/mL)	2505.7 (43.6)	2317.6 (31.3)	2412.4 (21.0)	2220.5 (20.3)	12,958.3 (15.2)	13,754.7 (16.6)
%AUC _{exp}	0.49 (80.91)	0.39 (53.50)	2.92 (43.77)	2.80 (51.58)	3.89 (26.05)	3.93 (20.26)
C _{max} (ng/mL)	1570.3 (40.5)	1527.3 (34.6)	577.0 (22.9)	541.3 (21.0)	654.7 (15.2)	748.8 (20.6)
C _{last} (ng/mL)	15.54 (59.87)	14.48 (49.14)	20.62 (40.92)	19.64 (55.25)	12.99 (19.48)	13.31 (18.19)
T _{max} (h) ^a	1.50 (1.00, 2.00)	2.00 (1.00, 3.00)	3.00 (1.75, 3.50)	3.00 (1.75, 4.00)	4.00 (3.00, 4.50)	4.00 (4.00, 4.50)
T _{last} (h) ^a	5.50 (4.75, 6.00)	6.00 (5.00, 6.00)	12.00 (12.00, 16.00)	12.00 (12.00, 16.00)	28.03 (24.70, 30.09)	120.00 (120.00, 144.00)
t _{1/2} (h) ^a	0.45 (0.41, 0.52)	0.41 (0.39, 0.46)	2.39 (2.15, 2.46)	2.21 (2.05, 2.33)	20.00 (120.00, 144.00)	29.75 (26.52, 31.36)
CL/F (mL/h)	180,764.6 (32.4)	187,487.6 (30.7)	NA	NA	NA	NA

^a Median (Q1, Q3)
NA = not applicable

Table 11: Pharmacokinetic Parameters for SOF and its Metabolites Following Administration of Single Dose of SOF/LDV or SOF/LDV+Multiple Doses of Omeprazole (Cohort 2, Group 2)

PK Parameter	SOF Mean (%CV)		GS-566500 Mean (%CV)		GS-331007 Mean (%CV)	
	SOF/LDV (N = 16)	SOF/LDV+ Omeprazole (N = 16)	SOF/LDV (N =16)	SOF/LDV+ Omeprazole (N = 16)	SOF/LDV (N = 16)	SOF/LDV+ Omeprazole (N = 16)
AUC _{inf} (h·ng/mL)	1599.2 (73.6)	1455.9 (46.9)	1670.5 (44.0)	1356.4 (34.2)	12,312.4 (21.5)	12,840.3 (26.0)
AUC _{last} (h·ng/mL)	1592.9 (73.6)	1438.8 (47.5)	1622.8 (45.0)	1298.6 (36.2)	11,821.5 (22.1)	12,316.5 (26.7)
%AUC _{exp}	0.48 (54.25)	1.30 (169.13)	3.36 (45.89)	5.04 (79.22)	4.09 (27.81)	4.20 (33.74)
C _{max} (ng/mL)	1132.5 (56.5)	1329.3 (64.8)	389.0 (34.8)	315.3 (32.3)	850.4 (23.1)	970.1 (20.2)
C _{last} (ng/mL)	9.80 (52.34)	23.64 (158.53)	15.05 (29.63)	16.89 (36.25)	13.97 (25.57)	13.73 (24.59)
T _{max} (h) ^a	1.00 (0.63, 1.75)	0.75 (0.50, 1.75)	1.75 (1.50, 3.00)	1.75 (1.00, 2.50)	3.50 (2.00, 4.50)	4.00 (3.00, 4.50)
T _{last} (h) ^a	4.75 (4.25, 5.00)	5.00 (4.00, 5.50)	12.00 (12.00, 12.00)	12.00 (12.00, 12.00)	96.00 (96.00, 120.00)	96.00 (96.00, 120.00)
t _{1/2} (h) ^a	0.44 (0.38, 0.49)	0.48 (0.38, 0.53)	2.17 (2.10, 2.35)	2.22 (2.08, 2.41)	23.23 (21.46, 27.92)	25.57 (22.73, 28.78)
CL/F (mL/h)	365,968.1 (62.1)	341,419.6 (48.2)	NA	NA	NA	NA

^a Median (Q1, Q3)
NA = not applicable

Table 12 shows the PK parameters for LDV in Cohort 2.

Table 12 Pharmacokinetic Parameters for LDV Following Administration of Single Dose of SOF/LDV or SOF/LDV+Famotidine or Omeprazole (Cohort 2, Groups 1 and 2)

LDV PK Parameter	Mean (%CV)				
	Cohort 2, Group 1			Cohort 2, Group 2	
	SOF/LDV (N = 12)	SOF/LDV+ Famotidine (Coadministered) (N = 12)	SOF/LDV+ Famotidine (12-Hour Stagger) (N = 12)	SOF/LDV (N = 16)	SOF/LDV+ Famotidine (N = 16)
AUC _{inf} (h·ng/mL)	7354.9 (26.1)	7106.2 (44.7)	8000.6 (51.2)	7993.4 (66.2)	6661.1 (51.8)
AUC _{last} (h·ng/mL)	6521.2 (23.8)	6091.8 (41.5)	6405.4 (42.7)	7163.2 (65.8)	5696.0 (51.8)
%AUC _{exp}	10.63 (49.43)	12.63 (50.56)	16.55 (56.28)	10.35 (41.28)	13.56 (41.15)
C _{max}	218.5 (25.5)	179.8 (31.8)	186.9 (33.5)	241.5 (68.6)	176.3 (51.1)
C _{last}	11.21 (47.39)	12.16 (61.22)	14.68 (69.16)	11.82 (74.54)	11.34 (57.51)
T _{max} (h) ^a	4.75 (4.50, 5.50)	4.75 (4.50, 6.00)	4.75 (4.50, 5.00)	4.50 (4.50, 5.50)	5.00 (4.50, 6.00)
T _{last} (h) ^a	144.00 (144.00, 144.00)	144.00 (144.00, 144.00)	144.00 (144.00, 144.00)	144.00 (144.00, 144.00)	144.00 (144.00, 144.00)
t _{1/2} (h) ^a	45.54 (40.37, 56.12)	48.37 (42.90, 59.63)	58.95 (47.96, 66.57)	46.32 (40.25, 51.99)	52.97 (47.33, 57.95)
CL/F (mL/h)	13,071.0 (26.9)	16,094.2 (55.2)	15,023.4 (61.3)	22,905.4 (118.2)	17,521.4 (50.8)

^a Median (Q1, Q3)

Statistic Summary:

Tables 13 and 14 summarize the differences in PK parameters of the evaluated ARVs, SOF, its metabolite GS-331007, and LDV when SOF/LDV or the ARVs were administered alone compared with when SOF/LDV was administered with each of the ARVs (Cohort 1) and when SOF/LDV was administered alone or with acid-reducing agents (Cohort 2), respectively. GS-566500 is not shown here because the pattern of exposures for GS-566500 and SOF are similar.

The data show that

- The PK of LDV was not altered by CPA. However, a reduction (approximately 34%) in LDV systemic exposures was observed following administration of SOF/LDV+ATR. Additional efficacy data from an NIAID trial (GS-US-337-0116) are available in 11 male and 2 female HIV/HCV infected subjects receiving ATR and LDV/SOF for 12 weeks. All 13 subjects achieved SVR₄, suggesting no impact on efficacy of LDV/SOF when administered with ATR.
- The observed reduction in LDV plasma exposures following administration of SOF/LDV+ATR did not result in significantly lower systemic exposures of SOF or its metabolites. The PK of SOF or its metabolites was not altered by CPA.

- The PK of EFV, RPV, and FTC were not significantly altered following coadministration of ATR or CPA with SOF/LDV.
- Increases in TFV AUC_{tau}, C_{max}, and C_{tau} of approximately 2.0-, 1.8-, and 2.6-fold, respectively, were observed following coadministration of ATR and SOF/LDV. Similarly, increases in TFV AUC_{tau}, C_{max}, and C_{tau} of approximately 1.4-, 1.3-, and 1.9-fold, respectively, were observed following coadministration of CPA and SOF/LDV.
- Administration of SOF/LDV with famotidine 40 mg (coadministered or with a 12-hour stagger under fed conditions) did not significantly alter the systemic exposure (AUC and C_{max}) of SOF, GS-566500, or GS-331007.
- The PK parameters of SOF and GS-331007 were not altered following administration of SOF/LDV+omeprazole (fasted conditions).
- Administration of SOF/LDV+omeprazole also resulted in approximately 4% to 8% lower LDV AUC and approximately 11% lower LDV C_{max} compared with SOF/LDV alone. As the magnitude of the reduction in LDV AUC and C_{max} was small, SOF/LDV may be simultaneously administered with omeprazole 20 mg or equivalent.
- Since the onset of the antisecretory effect of H₂RA and PPIs, such as famotidine and omeprazole, occurs between 1 to 2 hours, staggered administration of SOF/LDV with H₂RA or PPIs within 2 hours may result in lower LDV concentrations. As shown in Study GS-US-256-0110, a substantial decrease in LDV plasma exposure (~ 42% to 48% lower AUC and C_{max}) was observed upon administration of omeprazole 2 hours earlier. Therefore, H₂RA should be administered simultaneously or 12 hours apart with SOF/LDV and PPI should be administered simultaneously with SOF/LDV.

Table 13: Drug-Drug Interaction Evaluations between Sofosbuvir/Ledipasvir and Antiretroviral Agents (Cohort 1)

Analyte	Ratio of SOF/LDV+ARV to ARV											
	ARV PK Parameters			SOF PK Parameters		GS-331007 PK Parameters			LDV PK Parameters			
	AUC _{tau}	C _{max}	C _{tau}	AUC _{tau}	C _{max}	AUC _{tau}	AUC _{tau}	C _{max}	C _{tau}	AUC _{tau}	C _{max}	C _{tau}
ATR			↔	↔	↓8% (↓21%-↑6%)	↔	↔	↓15% (↓24%-↓4%)	↔	↓34% (↓25%-↓41%)	↓34% (↓25%-↓41%)	↓34% (↓24%-↓43%)
EFV	↔	↓13% (↓3%-↓21%)	↔									
FTC	↔	↔	↔									
TFV	↑98% (↑77%-↑123%)	↑79% (↑56%-↑104%)	↑163% (↑137%-197%)									
CPA			↔	↔	↔	↔	↔	↔	↔	↔	↔	↔
RPV	↔	↔	↔									
FTC	↔	↔	↔									
TFV	↑40% (↑31%-↑50%)	↑32% (↑25%-↑39%)	↑91% (↑74%-↑110%)									

Table 14: Effect of Famotidine and Omeprazole on the Pharmacokinetics of Sofosbuvir, GS-566500, GS-331007, and Ledipasvir

Acid-Reducing Agents	SOF PK Parameters			GS-331007 PK Parameters			LDV PK Parameters		
	AUC _{inf}	AUC _{last}	C _{max}	AUC _{inf}	AUC _{last}	C _{max}	AUC _{inf}	AUC _{last}	C _{max}
Famotidine									
Coadministered	↔	↔	↑15% (↓14%-↑50%)	↔	↔	↔	↓11% (↓24%-↑6%)	↓13% (↓26%-↑3%)	↓20% (↓31%-↓7%)
Staggered	↔	↔	0% (↓24%-↑32%)	↔	↔	↔	↔	↓9% (↓24%-↑9%)	↓17% (↓31%-0%)
Omeprazole	↔	↔	↑12% (↓12%-↑42%)	↔	↔	↔	↓4% (↓34%-↑39%)	↓7% (↓35%-↑32%)	↓11% (↓39%-↑30%)

Note: 90% CIs of the %GSLM ratios were within (↔), extended above (↑), or extended below (↓) the equivalence boundaries of 80% to 125%.

Conclusion:

- Based on the safety and PK data, SOF/LDV may be coadministered with CPA without dose adjustment to any of the agents. However, coadministration of SOF/LDV with ATR should be evaluated further due to the magnitude of interaction on tenofovir. Furthermore, the efficacy of LDV when coadministered with ATR is confirmed with the interim efficacy data from trial GS-US-337-0116, where SVR4 was attained in 13 out of 13 subjects.
- SOF/LDV may be administered with famotidine 40 mg or lower simultaneously or 12 hours apart.
- SOF/LDV may be simultaneously administered with omeprazole 20 mg equivalent or lower. Since the onset of the antisecretory effect of PPIs, such as omeprazole, occurs within 1 hour and the maximum effect occurs within 2 hours, staggered administration of SOF/LDV with PPIs may result in lower LDV concentrations, and thus, is not recommended.

4.2.4.3 GS-US-337-0128: A Phase 1 Study to Evaluate Pharmacokinetic Drug-Drug Interaction between Sofosbuvir/Ledipasvir (SOF/LDV) Fixed Dose Combination Tablet (FDC) and Abacavir/Lamivudine (Epzicom®)

Objectives:

- To evaluate the pharmacokinetics (PK) of sofosbuvir (SOF) and ledipasvir (LDV) upon administration of SOF/LDV fixed dose combination (FDC) with Epzicom® (Abacavir/Lamivudine; ABC/3TC)
- To evaluate the pharmacokinetics of ABC and 3TC upon administration of Epzicom® with SOF/LDV FDC

Study Design: This was a phase I, randomized, open-label, single cohort, multiple-dose study in healthy male and female volunteers. Thirty-five subjects were enrolled in a single cohort. Eligible subjects were randomized to 1 of 2 treatment sequences and received 2 treatments. A minimum of 17 subjects were enrolled to target 15 evaluable subjects in each sequence. The treatments were as follows:

Treatment A: SOF/LDV FDC (400 mg/90 mg) tablet administered orally once-daily in the morning with a moderate fat meal

Treatment B: ABC/3TC (600 mg/300 mg) tablet administered orally once-daily in the morning with a moderate fat meal

Treatment C: SOF/LDV FDC (400 mg/90 mg) tablet plus ABC/3TC (600 mg/300 mg) tablet administered once-daily in the morning with a moderate fat meal

The treatment sequences were as follows:

Sequence 1 (AC): Subjects received Treatment A on Days 1 through 10 followed by Treatment C on Days 11 through 20

Sequence 2 (BC): Subjects received Treatment B on Days 1 through 10 followed by Treatment C on Days 11 through 20

Formulation: SOF/LDV FDC (400 mg/90 mg) tablets (Lot #: DK1205B2) and ABC/3TC (600 mg/300 mg) tablets (Lot #: 3ZP6400)

PK Sampling: Serial blood samples were collected to determine plasma concentrations of SOF, SOF metabolites (GS-331007 and GS-566500), LDV, ABC, and 3TC on Days 10 and 20 relative to study drug administration in the morning: predose (≤ 5 minutes) and at 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 18, and 24 hours postdose.

Analytical methods: Concentrations of SOF, GS-566500, GS-331007, LDV, ABC, and 3TC in plasma samples were determined using fully validated high-performance liquid chromatography-tandem mass spectroscopy (LC/MS/MS) bioanalytical methods. All samples were analyzed in the timeframe supported by frozen stability storage data. The assays for SOF, GS-566500, GS-331007, LDV, ABC, and 3TC were all performed and validated [REDACTED] (b) (4)

Table 1: Bioanalytical Assay Validation for Sofosbuvir, Sofosbuvir Metabolites GS-566500 and GS-331007, and Ledipasvir in Human Plasma

Parameter	SOF	GS-566500	GS-331007	LDV
Linear range (ng/mL)	5 - 2500	10 - 5000	10 - 5000	1 - 2000
Lower limit of quantitation (ng/mL)	5	10	10	1
Interday precision range (%CV)	2.4 to 9.7	5.1 to 7.7	2.5 to 7.2	1.0 to 8.8
Interday accuracy range (%RE)	-5.1 to 3.4	-2.2 to 2.9	-1.0 to 2.5	-7.1 to -3.6
Stability in frozen matrix (days)	485 Days at -70°C and 174 Days at -20°C	485 Days at -70°C and 174 Days at -20°C	485 Days at -70°C and 174 Days at -20°C	623 Days at -20°C and -70°C

Table 2: Bioanalytical Assay Validation for Abacavir and Lamivudine in Human Plasma

Parameter	ABC	3TC
Linear range (ng/mL)	5 – 5000	2 – 2000
Lower limit of quantitation (ng/mL)	5	2
Interday precision range (%CV)	4.1 to 7.7	1.3 to 7.5 ^a
Interday accuracy range (%RE)	-7.7 to 1.5	-8.1 to 1.9 ^a
Stability in frozen matrix (days)	80 days at -70°C	310 days at -70°C

^a Intraday Ranges reported

The standard curve and QC data indicated that the plasma assay methods for SOF, GS-566500, GS-331007, LDV, ABC, and 3TC were precise and accurate.

Pharmacokinetic Results:

Table 3 presents the PK parameters for SOF and its metabolites, and Table 4 presents the PK parameters for LDV, ABC and 3TC, following administration of SOF/LDV or SOF/LDV+ABC/3TC once-daily with a moderate fat meal.

Table 5 shows the statistical summary of the interaction between SOF/LDV and ABC/3TC. Following coadministration of SOF/LDV and ABC/3TC compared to SOF/LDV or ABC/3TC alone, no clinically significant interactions were observed in the PK of SOF, GS-566500, GS-331007, LDV, ABC, or 3TC.

Table 3: Pharmacokinetic Parameters for SOF and its Metabolites Following Administration of Multiple Doses of SOF/LDV or SOF/LDV+ABC/3TC

PK Parameter	SOF Mean (%CV)		GS-566500 Mean (%CV)		GS-331007 Mean (%CV)	
	SOF/LDV (N = 16)	SOF/LDV+ABC/3TC (N = 13)	SOF/LDV (N = 16)	SOF/LDV+ABC/3TC (N = 13)	SOF/LDV (N = 16)	SOF/LDV+ABC/3TC (N = 13)
AUCtau (h·ng/mL)	2306.7 (42.3)	2747.3 (41.0)	2426.1 (20.9)	2574.2 (23.7)	11,530.3 (20.4)	12,398.6 (15.9)
Cmax (ng/mL)	1246.7 (46.1)	1321.6 (42.9)	534.0 (20.6)	519.3 (20.7)	943.8 (21.8)	978.5 (15.5)
Ctau (ng/mL)	NA	NA	NA	11.61 (0.33) ^a	333.9 (24.1)	360.2 (18.5)
Tmax (h) ^a	2.51 (2.00, 3.00)	2.00 (2.00, 3.00)	3.00 (3.00, 3.00)	3.00 (3.00, 4.00)	4.00 (3.00, 4.00)	4.00 (3.00, 5.00)
Tlast (h) ^a	6.00 (5.50, 6.00)	6.00 (5.00, 8.00)	12.01 (12.00, 18.00)	18.00 (12.00, 18.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
t1/2 (h) ^a	0.44 (0.40, 0.49)	0.44 (0.42, 0.51)	2.41 (2.14, 2.57)	2.55 (2.47, 3.16)	24.06 (19.22, 27.47)	23.16 (21.34, 26.44)
CLss/F (mL/h)	20,6161.1 (43.0)	169,249.3 (40.1)	NA	NA	NA	NA

^a Median (Q1, Q3)
NA = not applicable

Table 4: Pharmacokinetic Parameters for LDV, ABC, and 3TC Following Administration of Multiple Doses of SOF/LDV or SOF/LDV+ABC/3TC

PK Parameter	LDV Mean (%CV)		ABC Mean (%CV)		3TC Mean (%CV)	
	SOF/LDV (N = 16)	SOF/LDV+ABC/3TC (N = 13)	SOF/LDV (N = 16)	SOF/LDV+ABC/3TC (N = 13)	SOF/LDV (N = 16)	SOF/LDV+ABC/3TC (N = 13)
AUCtau (h·ng/mL)	10,152.4 (35.9)	12,812.3 (41.0)	14,642.0 (22.5)	12,908.3 (21.8)	14,180.8 (18.9)	13,099.3 (13.0)
Cmax (ng/mL)	624.4 (33.3)	721.6 (37.3)	4134.0 (21.8)	3765.9 (19.0)	2815.8 (28.7)	2537.2 (18.3)
Ctau (ng/mL)	356.5 (41.1)	480.9 (49.3)	10.1 (49.5) ^b	8.17 (-) ^c	68.4 (18.2)	75.6 (14.9)
Tmax (h) ^a	5.00 (5.00, 5.00)	5.00 (5.00, 6.00)	3.00 (2.00, 3.00)	3.00 (3.00, 3.00)	3.00 (3.00, 3.00)	3.00 (3.00, 4.00)
Tlast (h) ^a	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	15.00 (12.00, 18.00)	12.00 (12.00, 18.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
t1/2 (h) ^a	35.15 (27.27, 42.01)	35.78 (27.88, 48.14)	1.91 (1.71, 2.65)	1.79 (1.60, 2.07)	6.88 (6.58, 7.30)	7.07 (6.11, 8.38)
CLss/F (mL/h)	10,275.5 (44.9)	8221.0 (43.1)	42,605.4 (18.9)	48,422.8 (20.1)	21,821.1 (17.6)	23,277.5 (13.3)

^a Median (Q1, Q3)

^b N = 3

^c N = 1

Table 5: Statistical Summary of Drug Interaction between SOF/LDV and ABC/3TC

Analyte	SOF/LDV or ABC/3TC alone / SOF/LDV+ABC/3TC %GLSM ratio (90% CI)		
	AUC _{tau}	C _{max}	C _{tau}
SOF	121.08 (108.89, 134.63)	107.54 (85.47, 135.31)	–
GS-566500	105.12 (100.05, 110.45)	98.29 (90.71, 106.51)	–
GS-331007	104.85 (100.75, 109.12)	100.13 (93.55, 107.18)	107.66 (101.33, 114.38)
LDV	118.48 (109.91, 127.72)	109.82 (101.27, 119.09)	125.96 (116.86, 135.78)
ABC	89.57 (85.32, 94.03)	92.01 (87.32, 96.94)	–
3TC	94.06 (90.24, 98.04)	93.30 (86.85, 100.24)	111.95 (104.86, 119.53)

Conclusion: No dose adjustment is required when SOF/LDV is coadministered with ABC/3TC.

4.2.4.4 GS-US-248-0125: A Phase 1 Study to Evaluate Transporter-Mediated Drug-Drug-Interactions between Oral Antiviral (OAV) Combinations GS-9451/GS-5885/Tegobuvir and Probe Drugs

Note: Only the results of the effect of rifampin, verapamil and cyclosporine on GS-5885 (LDV) were reviewed.

Objectives:

- To evaluate the effect and drug interaction potential of LDV+ GS-9451 (VDV)+ tegobuvir (TGV) on organic anion transporting polypeptide (OATP)/breast cancer resistance protein (BCRP), and Pgp substrates using phenotypic probes
- To evaluate the drug interaction potential of LDV+VDV+TGV with:
 - Pgp inducers (rifampin)
 - Pgp inhibitors (verapamil)
 - Mixed OATP/Pgp/multidrug resistance-associated protein (MRP) 2 inhibitors (cyclosporine)

Study Design: Healthy subjects were assigned to 1 of 5 cohorts and then randomized to 1 of 2 treatment sequences within their respective cohort.

Cohort 1 (OATP substrate pravastatin and OATP/BCRP substrate rosuvastatin): Subjects received single dose of pravastatin 40 mg, followed by a 3-day washout and a single dose of rosuvastatin 10 mg (Treatment A), followed by a 10-day washout for Sequence AB, and VDV 200 mg (2 x 100 mg tablets) and LDV 90 mg (9 x 10 mg tablets) administered once a day plus TGV 30 mg administered twice a day (BID) for 16 days, with a single dose of pravastatin 40 mg administered on the 10th day and a single dose of rosuvastatin 10 mg administered on the 14th day (Treatment B). Subjects were randomized 1:1 to receive either Treatment A→B or Treatment B→A, with at least a 9-day washout period between treatment dosing periods.

Cohort 2 (Pgp substrate digoxin 0.25 mg and LDV+VDV+TGV): Subjects received a single dose of digoxin 0.25 mg (1 x 0.25 mg tablet) (Treatment C) followed by a 14-day washout for Sequence CD, and VDV 200 mg (2 x 100 mg tablet) and LDV 90 mg (9 x 10 mg tablet)

administered once a day (QD) plus TGV 30 mg (1 x 30 mg capsule) administered BID for 13 days, with a single dose of digoxin 0.25 mg (1 x 0.25 mg tablet) administered on the 10th day (Treatment D). Subjects were randomized 1:1 to receive either Treatment C→D or Treatment D→C, with at least a 9-day washout period between treatment dosing periods.

Cohort 3: (Pgp inducer rifampin 600 mg and LDV+VDV+TGV): Subjects received single doses of GS-9451 200 mg (2 x 100 mg tablet) plus GS-5885 90 mg (9 x 10 mg tablet) plus TGV 30 mg (1 x 30 mg capsule) as Treatment E. Subjects received rifampin 600 mg (2 x 300 mg capsule) QD for 7 days, followed by single doses of VDV 200 mg (2 x 100 mg tablet) plus LDV 90 mg (9 x 10 mg tablet) plus TGV 30 mg (1 x 30 mg capsule) on the 8th day as Treatment F. Subjects were randomized 1:1 to receive either Treatment E→F or Treatment F→E, with 10-day washout period between treatment dosing periods.

Cohort 4: (Pgp inhibitor verapamil sustained release (SR) 240mg and LDV+VDV+TGV): Subjects received single doses of VDV 200 mg (2 x 100 mg tablet) plus LDV 90 mg (9 x 10 mg tablet) plus TGV 30 mg (1 x 30 mg capsule) as Treatment G. Subjects received verapamil SR 240mg (1 x 240 mg tablet) QD for 11 days with single doses of VDV 200 mg (2 x 100 mg tablet) plus LDV 90 mg (9 x 10 mg tablet) plus TGV 30 mg (1 x 30 mg capsule) on the 8th day as Treatment H. Subjects were randomized 1:1 to receive either Treatment G→H or Treatment H→G, with 10-day washout period between treatment dosing periods.

Cohort 5: (OATP/MRP2/Pgp inhibitor cyclosporine 300 mg and LDV+VDV+TGV): Subjects received a single dose of cyclosporine 300 mg (3 x 100 mg capsule) as Treatment I. Subjects received VDV 200 mg (2 x 100 mg tablet) and LDV 90 mg (9 x 10 mg tablet) administered QD plus TGV 30 mg (1 x 30 mg capsule) administered BID for 12 days, with a single dose of cyclosporine 300 mg (3 x 100 mg capsule) administered on the 10th day as Treatment J. Subjects were randomized 1:1 to receive either Treatment I→J or Treatment J→I, with a 10-day washout period between treatment dosing periods.

Formulation: LDV 10-mg tablets (Lot # CF1102B1)

PK Sampling: Intensive PK samplings for LDV were collected as follow:

Cohort 3, Sequence EF; Cohort 4, Sequence GH: on Days 1 and 19 at the following time points: 0 (predose), 0.5, 1, 1.5, 2, 3, 4, 4.5, 5, 6, 8, 10, 12, 18, 24, 36, 48, 72, and 96 hours postdose.

Cohort 3, Sequence FE: on Days 8 and 19 at the following time points: 0 (predose), 0.5, 1, 1.5, 2, 3, 4, 4.5, 5, 6, 8, 10, 12, 18, 24, 36, 48, 72, and 96 hours postdose.

Cohort 4, Sequence HG: on Days 8 and 22 at the following time points: 0 (predose), 0.5, 1, 1.5, 2, 3, 4, 4.5, 5, 6, 8, 10, 12, 18, 24, 36, 48, 72, and 96 hours postdose.

Cohort 5: Sequence IF: on Days 1 and 21 at the following time points: 0 (predose), 0.5, 1, 1.5, 2, 3, 4, 4.5, 5, 6, 8, 10, 12, 18, 24, 36, 48, and 72 hours postdose and on Day 20 at the following time points: 0 (predose), 0.5, 1, 1.5, 2, 3, 4, 4.5, 5, 6, 8, 10, 12, 18, and 24 hours postdose.

Cohort 5: Sequence FI: on Days 10 and 23 at the following time points: 0 (predose), 0.5, 1, 1.5, 2, 3, 4, 4.5, 5, 6, 8, 10, 12, 18, 24, 36, 48, and 72 hours postdose and on Day 9 at the following time points: 0 (predose), 0.5, 1, 1.5, 2, 3, 4, 4.5, 5, 6, 8, 10, 12, 18, and 24 hours postdose.

Analytical methods: Concentrations of LDV in plasma samples were determined using a validated high-performance liquid chromatography-tandem mass spectroscopy (LC-MS/MS) bioanalytical method. All samples were analyzed in the timeframe supported by frozen stability

storage data. The assay for LDV was performed using validated methods (b) (4)

Analyte	Linear range (ng/mL)	LLOQ (ng/mL)	Interassay precision range (%CV)	Interassay accuracy range (%RE)	Stability in frozen matrix (days)
GS-5885	1–2,000	1	1.0 to 8.8	-7.1 to -3.6	69 days at -80°C

The standard curve and QC data indicated that the plasma assay method for LDV was precise and accurate.

Pharmacokinetic Results: Mean and median single dose pharmacokinetic parameters of LDV with or without rifampin are listed in Table 1. LDV mean exposure parameters AUC and C_{max} were substantially lower after administration of rifampin 600 mg, as compared to LDV+VDV+TGV administration alone. Median T_{max} was unchanged upon coadministration with rifampin; median t_{1/2} of LDV was shorter and CL/F (absolute and weight-adjusted) was higher in the presence of rifampin.

Table 1: Summary of LDV Single-Dose PK Parameters with or without Rifampin

LDV PK Parameter	LDV+VDV+TGV (N = 32)	Rifampin 600 mg QD plus LDV+VDV+TGV (N = 31)
AUC _{last} (ng•h/mL), Mean (%CV)	4634.2 (50.6)	1987.8 (46.8)
AUC _{inf} (ng•h/mL), Mean (%CV)	5897.1 (54.3)	2353.2 (48.2)
C _{max} (ng/mL), Mean (%CV)	193.8 (40.5)	132.8 (56.4)
C _{last} (ng/mL), Mean (%CV)	17.65 (68.27)	5.78 (69.67)
T _{max} (h), Median (Q1, Q3)	4.50 (4.50, 5.00)	4.50 (4.50, 5.00)
T _{last} (h), Median (Q1, Q3)	96.00 (96.00, 96.00)	96.00 (96.00, 96.00)
t _{1/2} (h), Median (Q1, Q3)	46.25 (37.55, 53.61)	38.49 (31.49, 47.68)
CL/F (mL/h), Mean (%CV)	20179.8 (58.4)	47565.4 (45.5)
Weight-adjusted CL/F (mL/h/kg), Mean (%CV)	262.2 (52.1)	621.4 (39.2)

Statistical summaries of LDV primary pharmacokinetic parameters when given as a DAA combination alone and after 7 days of rifampin dosing are shown in the Table 2. Addition of rifampin to DAAs resulted in ~ 56% lower AUC_{last}, 59% lower AUC_{inf} and 35% lower C_{max} of LDV, as compared to DAA administration alone.

Reviewer note: Concomitant administration of GS-5885 and GS-9451 results in ~ 2-fold higher plasma exposure of GS-5885 (GS-US-248-0102, not reviewed); substantial reduction in GS-9451 exposure by rifampin (not shown in the review) could have also contributed to the decrease in GS-5885 exposure.

Table 2: Statistical Comparison of LDV PK Parameter Estimates with or without Rifampin

LDV PK	Geometric Least Squares Means		

Parameter	Test Treatment Rifampin +DAAs (N=31)	Reference Treatment DAAs (N=31)	Geometric Least Squares Mean Ratio (%)	90% Confidence Interval
AUC _{inf} (ng•h/mL)	2105.90	5109.43	41.22	35.60, 47.71
AUC _{last} (ng•h/mL)	1792.41	4087.03	43.86	38.33, 50.17
C _{max} (ng/mL)	114.50	175.98	65.06	55.60, 76.14

Mean and median single dose pharmacokinetic parameters of LDV with or without verapamil are listed in Table 3. LDV mean exposure parameters AUC and C_{max} were higher after administration of verapamil SR 240 mg QD, as compared to DAAs administration alone. Median T_{max} was comparable in the two treatments; median t_{1/2} of GS-5885 was slightly prolonged and CL/F (absolute and weight-adjusted) was lower in the presence of verapamil.

Table 3: Summary of LDV Single-Dose PK Parameters with or without Verapamil

LDV PK Parameter	VDV +LDV + TGV (N = 31)	Verapamil SR 240 mg QD + VDV +LDV + TGV (N = 30)
AUC _{last} (ng•h/mL), Mean (%CV)	5963.6 (47.2)	8426.8 (34.7)
AUC _{inf} (ng•h/mL), Mean (%CV)	8001.2 (53.9)	13304.5 (48.8)
C _{max} (ng/mL), Mean (%CV)	227.4 (44.0)	274.6 (38.6)
C _{last} (ng/mL), Mean (%CV)	25.16 (62.91)	43.47 (47.60)
T _{max} (h), Median (Q1, Q3)	4.50 (4.50, 5.00)	4.75 (4.50, 5.00)
T _{last} (h), Median (Q1, Q3)	96.00 (96.00, 96.00)	96.00 (96.00, 96.00)
t _{1/2} (h), Median (Q1, Q3)	47.67 (35.14, 63.45)	59.21 (43.44, 81.85)
CL/F (mL/h), Mean (%CV)	15390.2 (57.3)	8695.8 (54.9)
Weight-adjusted CL/F (mL/h/kg), Mean (%CV)	206.8 (56.4)	116.1 (53.3)

Statistical summaries of LDV primary pharmacokinetic parameters when given as a triple DAA combination alone and after 7 days of verapamil SR 240 mg QD are shown in the Table 4. LDV AUC was about 50% to 70% higher and C_{max} was about 22% higher upon administration of DAAs with verapamil, as compared to DAA administration alone.

Table 4: Statistical Comparison of LDV PK Parameter Estimates with or without Verapamil

LDV PK Parameter	Geometric Least Squares Means		Geometric Least Squares Mean Ratio (%)	90% Confidence Interval
	Test Treatment Verapamil +DAAs (N=30)	Reference Treatment DAAs (N=30)		
AUC _{inf} (ng•h/mL)	11789.54	7066.49	166.84	151.96, 183.17
AUC _{last} (ng•h/mL)	7884.70	5413.66	145.64	132.45, 160.15
C _{max} (ng/mL)	254.36	208.75	121.85	111.98, 132.58

Mean and median steady-state pharmacokinetic parameters of LDV with or without single dose of cyclosporine are listed in Table 5. LDV mean exposure parameters AUC and C_{max} were similar upon coadministration with a single dose of cyclosporine 300 mg, as compared to DAA administration alone.

Table 5: Summary of LDV Multiple-Dose PK Parameters with or without Cyclosporine

LDV PK Parameter	VDV +LDV + TGV (N = 32)	Cyclosporine 300 mg SD + VDV +LDV + TGV (N = 32)
AUC _{tau} (ng•h/mL), Mean (%CV)	9678.9 (37.3)	11031.1 (33.0)
C _{max} (ng/mL), Mean (%CV)	576.8 (32.6)	647.2 (29.5)
C _{tau} (ng/mL), Mean (%CV)	341.6 (42.2)	393.1 (35.3)
T _{max} (h), Median (Q1, Q3)	4.50 (4.50, 4.50)	4.50 (4.50, 5.00)
T _{last} (h), Median (Q1, Q3)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
t _{1/2} (h), Median (Q1, Q3)	35.61 (26.94, 61.42)*	34.01 (28.73, 43.01)
CL _{ss} /F (mL/h), Mean (%CV)	10438.8 (32.1)	8958.9 (29.5)
Weight-adjusted CL _{ss} /F (mL/h/kg), Mean (%CV)	141.5 (34.8)	120.9 (29.4)

* N=31

Statistical summaries of LDV primary pharmacokinetic parameters at steady-state administered as LDV+VDV+TGV alone and with a single dose of cyclosporine 300 mg are shown in the Table 6. LDV primary exposure parameters AUC_{tau}, C_{max}, and C_{tau} were comparable in both treatments.

Table 6: Statistical Comparison of GS-5885 PK Parameter Estimates with or without Cyclosporine

LDV PK Parameter	Geometric Least Squares Means		Geometric Least Squares Mean Ratio (%)	90% Confidence Interval
	Test Treatment Cyclosporine +DAAs (N=32)	Reference Treatment DAAs (N=32)		
AUC _{tau} (ng•h/mL)	10508.17	9107.79	115.38	111.26, 119.64
C _{tau} (ng/mL)	371.40	316.10	117.49	112.29, 122.94

C _{max} (ng/mL)	622.42	549.58	113.25	108.52, 118.19
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Reviewer's note: verapamil and cyclosporine are both potent P-gp inhibitors. Coadministration of verapamil with the 3 DAAs resulted in a 50% to 70% increase in LDV AUC, while coadministration of cyclosporine had little effect on LDV AUC. Both verapamil and cyclosporine increase VDV AUC by about 2 fold, while VDV can increase LDV concentration. The differential effect of verapamil and cyclosporine on LDV exposures could be partially due to multiple doses of verapamil vs. single dose of cyclosporine. The magnitude of increase of LDV exposure due to P-gp inhibitors does not warrant dose adjustments when coadministering LDV with P-gp inhibitors. This is further supported by the permitted use of P-gp inhibitors in all HCV-infected subjects in the LDV/SOF Phase 2/3 clinical program. No serious AEs were reported despite 2.4-fold higher LDV exposure in females (N=7) on chronic P-gp inhibitors vs. males not on P-gp inhibitors (N=1252).

Conclusion: LDV exposure is increased with coadministration of P-gp inhibitors but no dose adjustment is recommended.

4.2.4.5 GS-US-256-0129 (Leslie): A Phase 1, Randomized, Open-label, Pharmacokinetic Drug-drug Interaction Study of TMC435 and GS-5885

Trial Period and Site

27 Dec 2010 to 16 Feb 2011 (b) (4)

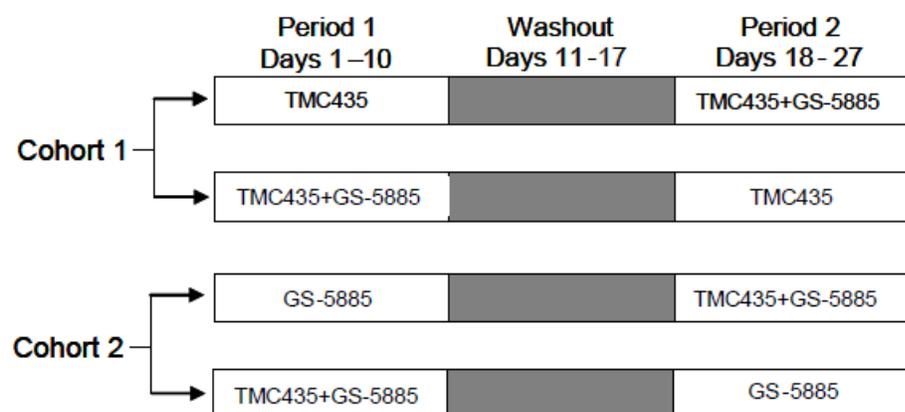
Trial Rationale

In this study, the potential for a pharmacokinetic drug-drug interaction between GS-5885 (ledipasvir, LDV) and TMC435 (simeprevir) was evaluated. The safety and tolerability of the combination of GS-5885 and TMC435 was also evaluated.

Trial Design

This was a randomized, crossover, open-label, two cohort, two period, multiple-dose study with four treatments. Cohort 1 received Treatments A or B and Cohort 2 received Treatments C or D (Figure 1).

Figure 1: Treatment schema (source: Study Report Figure 7-1)



Treatment A

TMC435 150 mg QD with food for 10 days

Treatments B and C TMC435 150 mg plus GS-5885 30 mg QD with food for 10 days
Treatment D GS-5885 30 mg QD with food for 10 days

Rationale for Dose Selection

The dose of GS-5885 30 mg (the highest dose evaluated in HCV-infected patients at the time the study was conducted) was selected based on preliminary data on safety and antiviral activity (GS-US-256-0102) and PK (GS-US-256-0101). The dose of TMC435 150 mg is the dose planned for evaluation in Phase 3 trials.

Investigational Product

GS-5885 10 mg film-coated oral tablets (b) (4) contained the inactive ingredients lactose monohydrate, microcrystalline cellulose, croscarmellose sodium, magnesium stearate, polyethylene glycol, hypromellose, and titanium dioxide (Gilead Sciences, Foster City, California; Lot CF100 4B 1A). TMC435 150 mg hard gelatin oral capsules (b) (4) contained TMC435 (b) (4) and the inactive ingredients sodium lauryl sulphate, magnesium stearate, silica colloidal anhydrous, croscarmellose sodium, and lactose monohydrate (Janssen Pharmaceuticals NV, Beerse, Belgium; Lot 10F01/G007).

Key Inclusion and Exclusion Criteria

Subjects were healthy and females males between the ages of 18 and 45 years, inclusive, with a BMI between 19 and 30 kg/m² and creatinine clearance ≥80 mL/min. Subjects had to agree to use a highly effective method of contraception during the study and for 30 or 90 days after the last dose of study drug for females and males, respectively. Females who were pregnant or lactating were excluded. Exclusion criteria also included history of nicotine use (within 90 days), any serious or active medical or psychiatric illness, positive test result for HIV-1 antibody, hepatitis C antibody, or hepatitis B surface antigen, or a positive urine screen for drugs of abuse.

Concomitant Medications

The following medications and substances were disallowed while subjects were participating in the study:

- All prescription and over-the-counter medications (with the exception of acetaminophen, ibuprofen, vitamins, and oral contraceptive) from within 28 days prior to and during dosing
- systemic steroids, immunosuppressant therapies, or chemotherapeutic agents from within 3 months of screening and during dosing

Sample Collection

Blood samples were collected to assess concentrations of GS-5885 and TMC435 at the following timepoints:

Days 10 and 27 – predose and 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h postdose

Analytical Methods

Concentrations of TMC435 in plasma samples were measured by LC-MS/MS (b) (4) (b) (4) validation report BA1513; study report JNJ-38733214/035). Sample receipt commenced on 31 Jan 2011 (storage temperature: -20°C) and analysis was completed on 3 Mar 2011. The first day of sample collection was 22 Jan 2011, so the maximum storage sample time was 40 days, which is within the validated long-term frozen stability

duration of 1184 days. The calibration curve concentrations ranged from 2-2000 ng/mL and the QC concentrations were 6.00, 100, and 1600 ng/mL. All inter-assay accuracy and precision estimates were within the acceptable range (Table 1).

Concentrations of GS-5885 in plasma samples were measured by LC-MS/MS (b) (4) (b) (4) validation report (b) (4) 60-1028; study report (b) (4) 60-1054). Sample receipt commenced on 25 Jan 2011 (storage temperature: -70°C) and analysis was completed on 5 Mar 2011. The first day of sample collection was 22 Jan 2011, so the maximum storage sample time was 42 days, which is within the validated long-term frozen stability duration of 69 days. The calibration curve concentrations ranged from 1-2000 ng/mL and the QC concentrations were 1, 3, 75, 750, and 1600 ng/mL. All inter-assay accuracy and precision estimates were within the acceptable range (Table 1).

Table 1: Bioanalytical assay validation (source: Study Report Table 7-3)

Parameter	GS-5885	TMC435
Linear range (ng/mL)	1–2000	2–2000
LLQ (ng/mL)	1	2
Interassay precision range (%CV)	1.0 to 8.8	0.0 to 5.9
Interassay accuracy range (%RE)	-7.1 to -3.6	-1.7 to 12.1
Stability in frozen matrix (days)	69 days at -80°C	1184 days at -20°C

Source: Appendix 16.1.10

Results

Trial Population

Fifty healthy subjects between the ages of 19 and 45 were enrolled in and completed the study (28 subjects received TMC435 with and without GS-5885; 22 subjects received GS-5885 with and without TMC435). The majority of subjects were male (68%). Most of the subjects were white (78%) and the remainder were black or African American; 92% of subjects reported that they were of Hispanic or Latino ethnicity.

Pharmacokinetics of TMC435

Upon coadministration with GS-5885, TMC435 plasma concentrations were higher compared to when TMC435 is administered alone (Figure 2). TMC435 AUC and C_{max} increased by approximately 2.6-fold following coadministration with GS-5885, although half-life was unchanged (Table 2), suggesting that GS-5885 impacts the bioavailability of TMC435 rather than its clearance. The increases in TMC435 exposures are statistically significant (Table 3).

Figure 2: Mean ± SD TMC435 concentration-time profile following administration alone (closed circles) or in combination with GS-5885 (open circles) (semi-log scale; source: Study Report Figure 10-1)

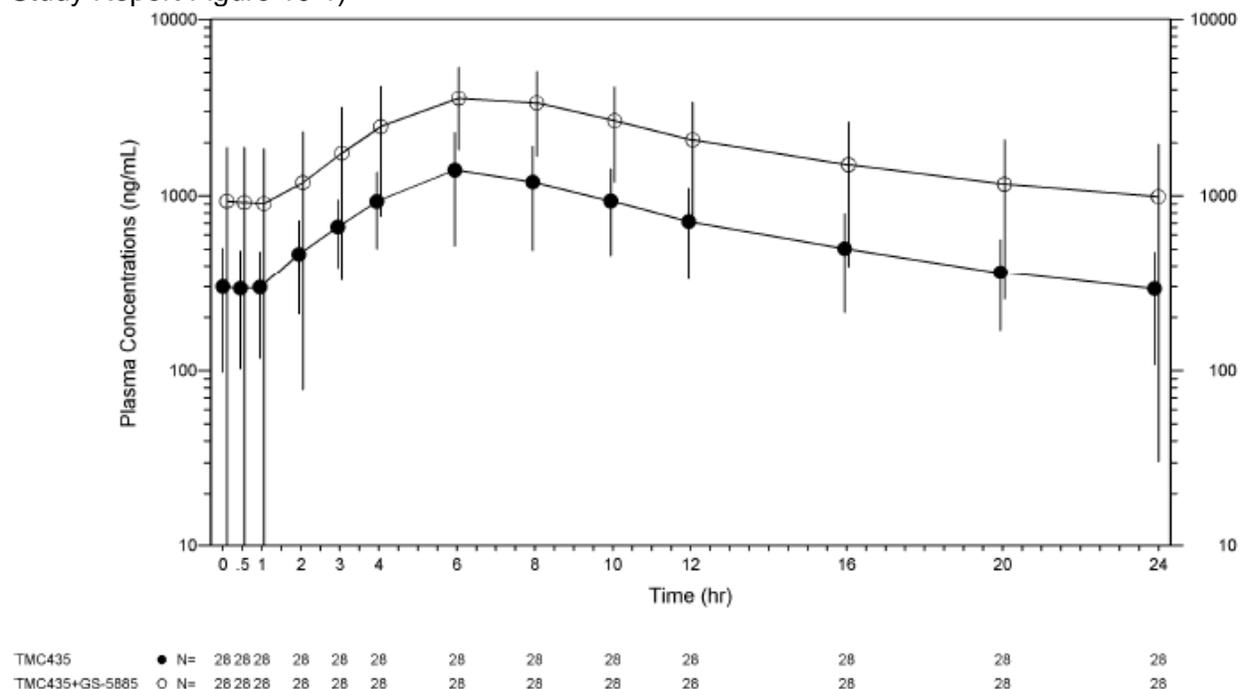


Table 2: TMC435 plasma pharmacokinetic parameters following administration alone or in combination with GS-5885 (source: Study Report Table 10-1)

TMC435 PK Parameter	Mean (%CV)		
	TMC435 (Cohort 1) (N = 28)	TMC435+GS-5885 (Cohort 1) (N = 28)	TMC435+GS-5885 (Cohort 2) (N = 22)
C_{max} (ng/mL)	1447.9 (60.1)	3712.1 (49.6)	4073.6 (51.3)
T_{max} (h) ^a	6.00 (6.00, 6.00)	6.00 (6.00, 7.00)	6.00 (6.00, 6.00)
C_{tau} (ng/ml)	292.7 (63.0)	989.5 (96.9)	1100.5 (89.7)
T_{last} (h) ^a	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
AUC_{tau} (h·ng/mL)	16,251.3 (51.5)	46,139.5 (63.1)	50,925.0 (65.3)
$t_{1/2}$ (h) ^a	8.87 (8.21, 9.88)	9.09 (7.78, 11.04)	8.81 (6.96, 9.91)

a Median (Q1, Q3)

Source: Section 15.1, Tables 5.1.1, 5.1.2, 5.1.3

Table 3: Statistical comparisons of TMC435 plasma pharmacokinetic parameter estimates following administration of TMC435 alone or in combination with GS-5885
(source: Study Report Table 10-2)

TMC435 PK Parameter	GLSM		%GLSM Ratio (TMC435+GS-5885/ TMC435)	90% Confidence Interval
	TMC435+GS-5885 (N = 28)	TMC435 (N = 28)		
C_{max} (ng/mL)	3350.93	1283.17	261.15	238.63, 285.79
AUC_{tau} (ng-h/mL)	39,347.49	14,630.94	268.93	244.36, 295.97

Note: GLSMs calculated using mixed model covariance parameter estimates.

Source: Section 15.1, Table 6.1

Pharmacokinetics of GS-5885

Steady-state GS-5885 plasma concentrations were higher following coadministration with TMC435 compared to administration of GS-5885 alone (Figure 3). GS-5885 AUC and C_{max} increased by 1.9- and 1.8-fold following coadministration with TMC435 (Table 4). Half-life was largely unchanged, suggesting that TMC435 primarily impacts the bioavailability of GS-5885 rather than its clearance. The increases in GS-5885 exposures are statistically significant (Table 3).

Reviewer Comments:

Mean GS-5885 exposures upon coadministration with TMC435 were approximately 30% higher in Cohort 1 compared to Cohort 2 (the difference between mean TMC435 exposures in the presence of GS-5885 between cohorts was approximately 10%, despite the high interindividual variability observed in TMC435 exposures throughout the TMC435 development program), which may reflect the influence of gender on GS-5885 exposures (10.7% and 59.1% female in Cohorts 1 and 2, respectively).

Figure 3: Mean \pm SD GS-5885 concentration-time profile following administration of GS-5885 alone (closed circles) or in combination with SMV (open circles) (semi-log scale; source: Study Report Figure 10-2)

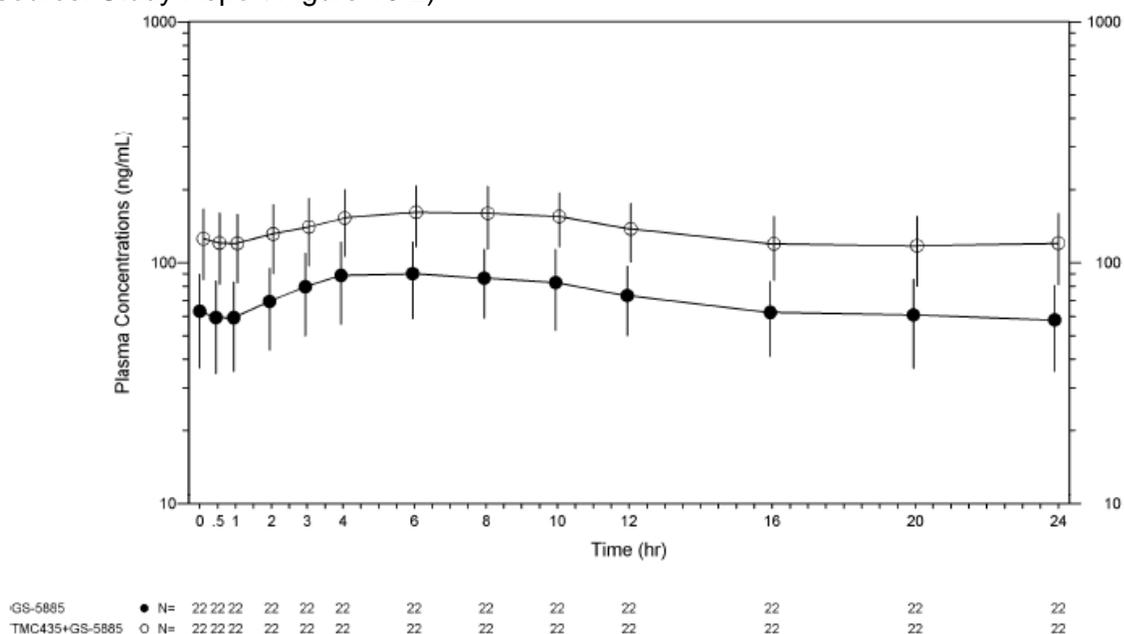


Table 4: GS-5885 plasma pharmacokinetic parameters following administration of GS-5885 alone or in combination with TMC435 (source: Study Report Table 10-3)

GS-5885 PK Parameter	Mean (%CV)		
	GS-5885 (Cohort 2) (N = 22)	TMC435+GS-5885 (Cohort 1) (N = 28)	TMC435+GS-5885 (Cohort 2) (N = 22)
C_{max} (ng/mL)	96.5 (32.3)	134.6 (32.4)	171.3 (25.6)
T_{max} (h) ^a	6.00 (4.00, 8.00)	6.00 (6.00, 8.00)	7.00 (6.00, 8.00)
C_{tau} (ng/mL)	58.2 (38.6)	91.1 (38.2)	120.5 (32.5)
T_{last} (h) ^a	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
AUC_{tau} (h·ng/mL)	1721.3 (34.1)	2549.8 (33.9)	3243.3 (28.3)
$t_{1/2}$ (h) ^a	28.60 (22.58, 37.36)	35.34 (31.66, 39.78)	39.15 (30.22, 43.06)

a Median (Q1, Q3)

Source: Section 15.1, Tables 5.2.1, 5.2.2, 5.2.3

Table 5: Statistical comparisons of GS-5885 plasma pharmacokinetic parameter estimates following administration of GS-5885 alone or in combination with TMC435 (source: Study Report Table 10-4)

GS-5885 PK Parameter	GLSM		% GLSMs Ratio (TMC435+GS-5885/ GS-5885)	90% Confidence Interval
	TMC435+GS-5885 (N = 22)	GS-5885 (N = 22)		
C_{max} (ng/mL)	165.99	91.66	181.09	169.01, 294.03
AUC_{tau} (ng·h/mL)	3115.20	1626.22	191.56	177.08, 207.22

Note: GLSMs calculated using mixed-model covariance parameter estimates.

Source: Section 15.1, Table 6.2

Conclusion

In the study, the steady-state pharmacokinetics of TMC435 (simeprevir) and GS-5885 (ledipasvir) in plasma were evaluated following administration of TMC435 and GS-5885 alone and in combination. Following coadministration, exposures (AUC_{tau}) of TMC435 and GS-5885 increased by approximately 2.4-fold and 1.8-fold, respectively. These increases are likely due to a two-way presystemic interaction (i.e. inhibition of P-gp in the gut) as half-life was not substantially affected.

A 30 mg dose of GS-5885 was used in this study rather than the proposed marketed dose of 90 mg; however, as intestinal efflux transport does not appear to be saturated at a dose of 90 mg, it is expected that the magnitude of the pharmacokinetic interaction will be similar to that observed in this study. Based on the safety profile of ledipasvir, no reduction in ledipasvir dose is necessary when coadministered with simeprevir. However, evidence of an exposure-safety relationship for simeprevir in Phase 3 trials (in which higher simeprevir exposures were associated with a higher incidence of rash) and the large degree of interindividual variability in simeprevir pharmacokinetics preclude the concomitant use of simeprevir with ledipasvir.

4.2.4.6 GS-US-334-0101 (Leslie): A Phase 1 Study to Evaluate the Effect of GS-5885 and GS-9669 on the Pharmacokinetics of GS-7977 and its Metabolites

Trial Period and Site

20 Feb to 22 May 2012

(b) (4)

Trial Rationale

In this study, the effects of GS-5885 (ledipasvir, LDV) and GS-9669 on the pharmacokinetics (PK) of sofosbuvir (SOF, GS-7977) and its metabolites were evaluated. This review will focus on the PK interaction between LDV and SOF (and its metabolites).

Trial Design

This was an open-label, three cohort study.

Cohort 1 – Seventeen healthy subjects received a single dose of SOF 400 mg followed by a three-day washout period (Days 2-4), ten days of GS-5885 90 mg QD (Days 5-14), and a single dose of GS-5885 90 mg + SOF 400 mg (Day 15). Study drugs were administered in the morning under fasted conditions. Subjects were confined to the clinic from Day 0 until Day 19.

Cohort 2 – The pharmacokinetics of various combinations of GS-9669, GS-5885, and SOF were evaluated.

Cohort 3 – The safety and tolerability of single and multiple suprathreshold doses of GS-9669 (2000 mg QD and 1500 mg BID) were evaluated in Phase 3 trials to support the current NDA.

For the remainder of this review, only Cohort 1 will be discussed.

Rationale for Dose Selection

The dose of GS-5885 90 mg was selected based on antiviral activity and a favorable safety profile in patients infected with HCV genotype 1a in study GS-US-256-0102. The dose of SOF 400 mg is the highest dose being evaluated in subjects with HCV infection. Single- and multiple-dose pharmacokinetics of SOF are similar, although GS-331007 exposures are increased approximately 20% upon multiple dosing. These doses were evaluated as a fixed-dose combination in Phase 3 studies.

Investigational Product

GS-5885 30 mg film-coated oral tablets (b) (4) contained the inactive ingredients copovidone, lactose monohydrate, microcrystalline cellulose, croscarmellose sodium, magnesium stearate, polyvinyl alcohol, titanium dioxide, polyethylene glycol, and talc

(b) (4) SOF 400 mg film-coated oral tablets were (b) (4) capsule-shaped and contained the inactive ingredients mannitol, microcrystalline cellulose, croscarmellose sodium, colloidal silicon dioxide, magnesium stearate, polyvinyl alcohol, titanium dioxide, (b) (4) talc (b) (4)

Key Inclusion and Exclusion Criteria

Subjects were healthy and females males between the ages of 18 and 45 years, inclusive, with a BMI between 19 and 30 kg/m² and creatinine clearance ≥80 mL/min. Subjects had to agree to use a highly effective method of contraception. Females who were pregnant or lactating were excluded. Exclusion criteria also included history of nicotine use (within 90 days), any serious or active medical or psychiatric illness, positive test result for HIV-1 antibody, hepatitis C antibody, or hepatitis B surface antigen, or a positive urine screen for drugs of abuse.

Concomitant Medications

The following medications and substances were disallowed while subjects were participating in the study:

- all prescription and over-the-counter medications (with the exception of acetaminophen, ibuprofen, or vitamins) from within 28 days prior to and during dosing
- systemic steroids, immunosuppressant therapies, or chemotherapeutic agents from within 3 months of screening and during dosing

Sample Collection

Blood samples were collected to assess concentrations of GS-5885 and SOF and its metabolites at the following timepoints:

Day 14 – predose and 0.5, 1, 1.5, 2, 3, 4, 4.5, 5, 6, 8, 10, 12, and 24 h postdose

Days 1 and 15 – the timepoints listed for Day 14 and 36, 48, 72, and 96 h postdose

Analytical Methods

Concentrations of SOF and the SOF metabolites GS-566500 and GS-331007 in plasma samples were measured by LC-MS/MS b (b) (4) (b) (4) validation report (b) (4) 86-0938; study report (b) (4) 60-1219A). Sample receipt commenced on 20 Mar 2012 (storage temperature: -70°C) and analysis was completed on 11 May 2012. The first day of sample collection was 13 Mar 2012, so the maximum storage sample time was 58 days, which is within the validated long-term frozen stability duration for SOF, GS-566500, and GS-331007 in plasma. For SOF, GS-566500, and GS-331007, the quality control (QC) concentrations ranged from 5-4000 ng/mL, 10-4000 ng/mL, and 10-4000 ng/mL, respectively. All inter-assay accuracy and precision estimates were within the acceptable range (Table 1).

Concentrations of GS-5885 in plasma samples were measured by LC-MS/MS (b) (4) (b) (4) validation report (b) (4) 60-1028; study report (b) (4) 60-1219B). Sample receipt commenced on 3 Apr 2012 (storage temperature: -70°C) and analysis was completed on 16 May 2012. The first day of sample collection was 26 Mar 2012, so the maximum storage sample time was 49 days, which is within the validated long-term frozen stability duration of 351 days for plasma. The QC concentrations ranged from 1-1600 ng/mL. All inter-assay accuracy and precision estimates were within the acceptable range (Table 1).

Table 1: Bioanalytical assay validation (source: Study Report Table 7-6)

Parameter	SOF	GS-566500	GS-331007	GS-5885	GS-9669
Linear range (ng/mL)	5 to 5000	10 to 5000	10 to 5000	1 to 2000	10 to 10,000
LLOQ (ng/mL)	5	10	10	1	10
Interassay precision range (%CV)	1.3 to 7.4 ^a	3.3 to 9.1 ^a	0.8 to 5.6 ^a	1.0 to 8.8	2.6 to 5.7
Interassay accuracy range (%RE)	-3.3 to 8.2 ^a	-5.2 to 5.9 ^a	-0.1 to 7.5 ^a	-7.1 to -3.6	-1.3 to 4.6
Stability in frozen matrix (days)	377 at -70°C and 36 at -20°C	308 at -70°C and 25 at -20°C	308 at -70°C and 25 at -20°C	351 at -20°C and -70°C	217 at -10°C to -30°C and at -60°C to -80°C

a Intraday ranges are reported.

Source: Appendix 16.1.10

Results

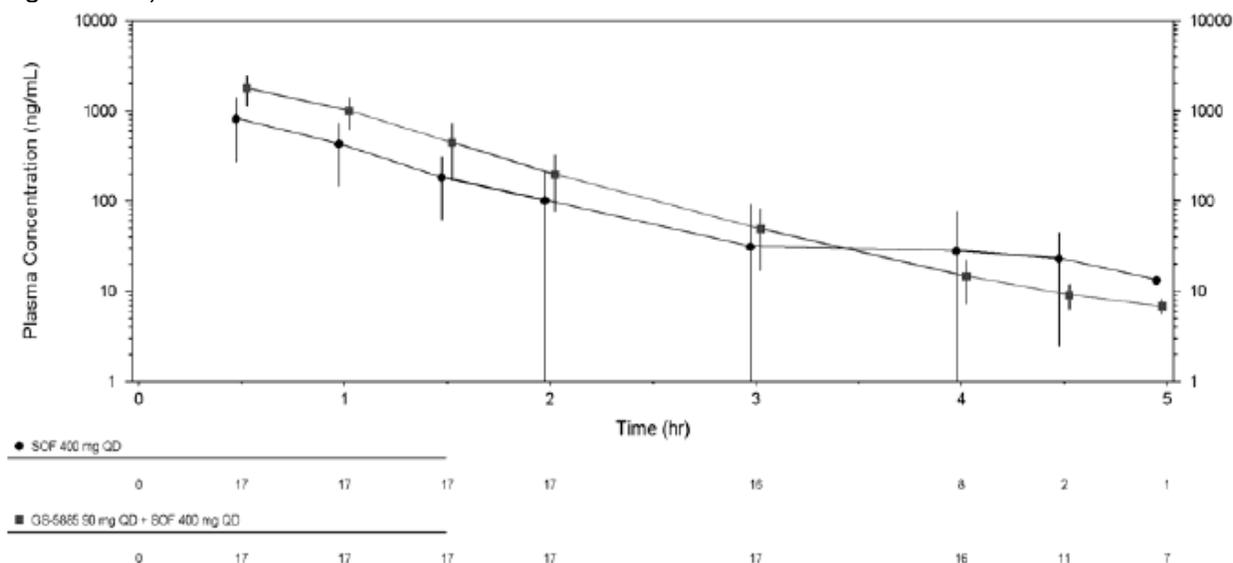
Trial Population

Seventeen healthy subjects between the ages of 19 and 45 were enrolled in and completed the study. The majority of subjects were male (64.7%). Most of the subjects were white (52.9%) and the remainder were black or African American; 47.1% of subjects reported that they were of Hispanic or Latino ethnicity.

Pharmacokinetics of Sofosbuvir

Upon coadministration with GS-5885, SOF plasma concentrations were initially higher compared to when SOF is administered alone; between 3 and 4 h postdose, SOF plasma concentrations were lower in the presence of GS-5885 compared to SOF alone (Figure 1). SOF AUC and C_{max} increased slightly more than two-fold following coadministration with GS-5885 and half-life was slightly longer (Table 2). SOF concentrations were BLQ after 5 h postdose, consistent with its short half-life (~0.5 hours).

Figure 1: Mean ± SD SOF concentration-time profile following administration alone (circles) or in combination with GS-5885 (squares) (semi-log scale; source: Study Report Figure 10-1)



Source: Section 15.1, Figure 2.1

Table 2: SOF plasma pharmacokinetic parameters following administration alone or in combination with GS-5885 (source: Study Report Table 10-1)

SOF PK Parameter	Mean (%CV)	
	SOF (N = 17)	SOF+GS-5885 (N = 17)
AUC _{last} (ng•h/mL)	787.7 (36.6)	1744.5 (27.8)
AUC _{inf} (ng•h/mL)	793.6 (36.4)	1751.2 (27.8)
C _{max} (ng/mL)	928.7 (52.3)	1873.3 (27.9)
C _{last} (ng/mL)	8.83 (27.9)	8.36 (44.7)
T _{max} (h) ^a	0.50 (0.50, 0.50)	0.50 (0.50, 0.50)
T _{last} (h) ^a	3.02 (3.00, 4.00)	4.50 (4.00, 5.00)
t _{1/2} (h) ^a	0.41 (0.40, 0.52)	0.61 (0.50, 0.66)
CL/F (mL/h)	603,805.1 (55.0)	249,538.0 (36.0)

^a Median (Q1, Q3)

Source: Section 15.1, Table 4.1

Table 3: Statistical comparisons of SOF plasma pharmacokinetic parameter estimates following administration of SOF alone or in combination with GS-5885 (source: Study Report Table 10-2)

SOF PK Parameter	GLSM		%GLSM Ratio (90% CI) SOF+GS-5885 / SOF
	SOF+GS-5885 (N = 17)	SOF (N = 17)	
AUC _{inf} (ng•h/mL)	1681.93	733.91	229.17 (190.57, 275.60)
AUC _{last} (ng•h/mL)	1675.04	727.27	230.32 (191.28, 277.33)
C _{max} (ng/mL)	1802.15	814.73	221.20 (176.21, 277.66)

Source: Section 15.1, [Table 5.1](#)

Pharmacokinetics of GS-566500

Upon coadministration of SOF and GS-5885, GS-566500 exposures (AUC and C_{max}) were higher compared to following administration of SOF alone (Table 4). These increases were statistically significant (outside the predefined equivalence boundaries of 70-143%) but are unlikely to be clinically relevant as GS-566500 is a minor circulating metabolite with a short half-life (approximately 2 h, Study GS-US-334-0131) that accounts for approximately 7% of drug-related material AUC (Study P7977-0312).

Table 4: Statistical comparisons of GS-566500 plasma pharmacokinetic parameter estimates following administration of SOF alone or in combination with GS-5885 (source: Study Report Table 10-4)

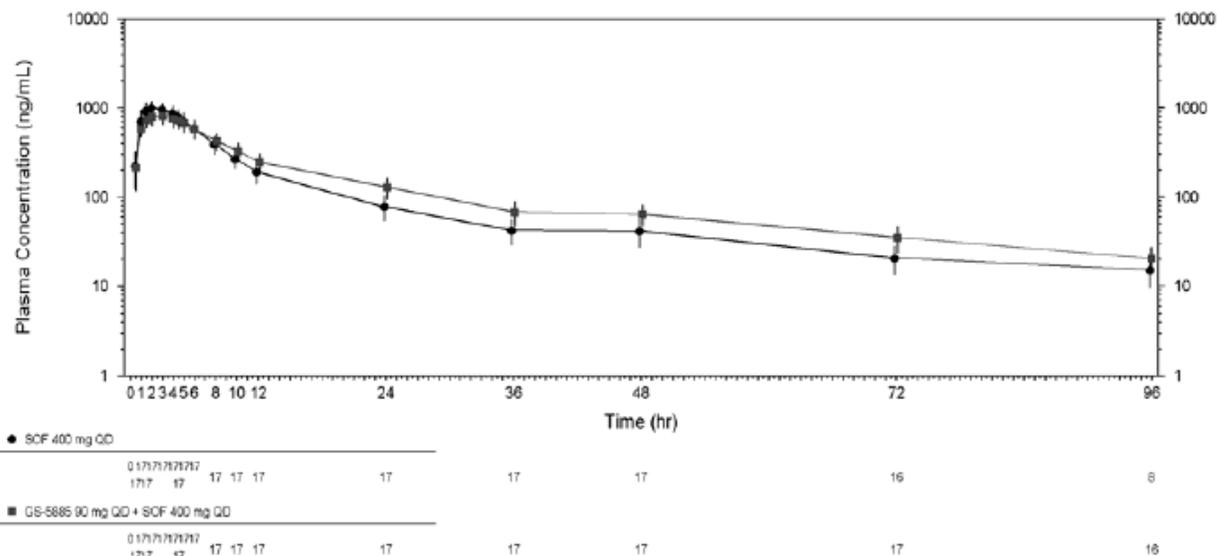
GS-566500 PK Parameter	GLSM		%GLSM (90% CI) SOF+GS-5885 / SOF
	SOF+GS-5885 (N = 17)	SOF (N = 17)	
AUC _{inf} (ng•h/mL)	1892.32	1057.30	178.98 (155.03, 206.63)
AUC _{last} (ng•h/mL)	1834.88	1010.72	181.54 (156.71, 210.30)
C _{max} (ng/mL)	531.28	291.21	182.44 (154.28, 215.74)

Source: Section 15.1, [Table 5.2](#)

Pharmacokinetics of GS-331007

Upon coadministration of SOF and GS-5885, plasma concentrations of the predominant circulating SOF-related compound GS-331007 paralleled those of SOF and were initially slightly higher before decreasing (6-8 h postdose) to slightly lower plasma concentrations than those observed after administration of SOF alone (Figure 2). GS-331007 AUC increased approximately 20% and C_{max} decreased approximately 20% following coadministration of SOF and GS-5885, with no change in half-life (Table 5). These differences are not considered clinically relevant.

Figure 2: Mean ± SD GS-331007 concentration-time profile following administration of SOF alone (circles) or in combination with GS-5885 (squares) (semi-log scale; source: Study Report Figure 10-3)



Source: Section 15.1, Figure 2.3

Table 5: GS-331007 plasma pharmacokinetic parameters following administration of SOF alone or in combination with GS-5885 (source: Study Report Table 10-5)

GS-331007 PK Parameter	Mean (%CV)	
	SOF (N = 17)	SOF+GS-5885 (N = 17)
AUC _{last} (ng•h/mL)	10,228.8 (17.9)	12,100.5 (15.5)
AUC _{inf} (ng•h/mL)	10,908.0 (17.5)	13,019.1 (16.7)
C _{max} (ng/mL)	1058.5 (17.3)	864.2 (20.1)
C _{last} (ng/mL)	16.15 (30.30)	20.89 (31.12)
T _{max} (h) ^a	2.00 (1.50, 3.00)	3.00 (2.00, 3.00)
T _{last} (h) ^a	72.00 (72.00, 95.93)	96.00 (96.00, 96.00)
t _{1/2} (h) ^a	28.14 (24.03, 32.45)	28.00 (27.00, 31.95)

a Median (Q1, Q3)

Source: Section 15.1, Table 4.3

Table 6: Statistical comparisons of GS-331007 plasma pharmacokinetic parameter estimates following administration of SOF alone or in combination with GS-5885 (source: Study Report Table 10-6)

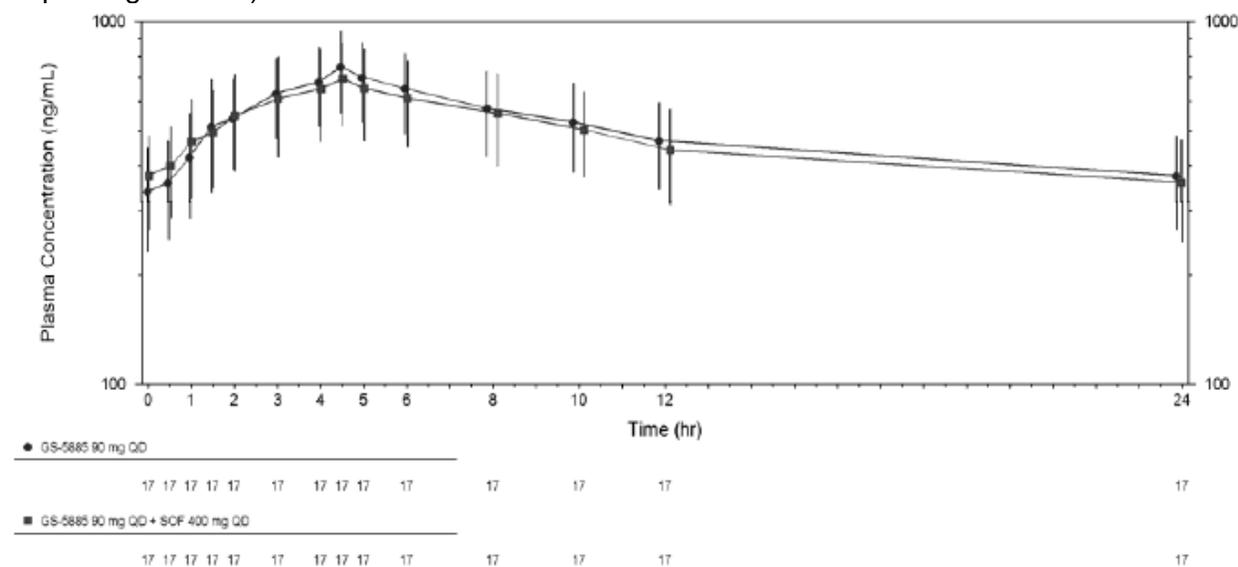
GS-331007 PK Parameter	GLSM		%GLSM (90% CI) SOF+GS-5885 / SOF
	SOF+GS-5885 (N=17)	SOF (N=17)	
AUC _{inf} (ng•h/mL)	12,850.84	10,758.19	119.45 (113.39, 125.84)
AUC _{last} (ng•h/mL)	11,962.94	10,078.89	118.69 (113.12, 124.54)
C _{max} (ng/mL)	847.84	1043.93	81.22 (76.89, 85.79)

Source: Section 15.1, Table 5.3

Pharmacokinetics of GS-5885

Steady-state GS-5885 plasma concentrations were slightly lower following coadministration with SOF compared to administration of GS-5885 alone (Figure 3). These minor differences in GS-5885 plasma concentrations in the presence or absence of SOF were reflected in the estimated pharmacokinetic parameters (Table 7), although the 90% confidence intervals for AUC_{tau}, C_{max}, and C_{tau} were within traditional equivalence boundaries (80-125%), suggesting that SOF coadministration does not significantly influence GS-5885 PK (Table 8).

Figure 3: Mean ± SD GS-5885 concentration-time profile following administration of GS-5885 alone (circles) or in combination with SOF (squares) (semi-log scale; source: Study Report Figure 10-4)



Source: Section 15.1, Figure 2.5

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Table 6: GS-5885 plasma pharmacokinetic parameters following administration of GS-5885 alone or in combination with SOF (source: Study Report Table 10-7)

GS-5885 PK Parameter	Mean (%CV)	
	GS-5885 (N = 17)	SOF+GS-5885 (N = 17)
AUC _{tau} (ng•h/mL)	11,866.9 (26.2)	11,387.3 (27.1)
C _{max} (ng/mL)	755.5 (24.7)	734.5 (27.0)
C _{tau} (ng/mL)	374.9 (28.8)	359.7 (31.2)
T _{max} (h) ^a	4.50 (4.50, 4.50)	4.50 (4.50, 4.52)
T _{last} (h) ^a	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
t _{1/2} (h) ^a	37.03 (27.70, 45.33)	40.99 (36.63, 67.96)
CL _{ss} /F (mL/h)	8029.7 (23.6)	8405.2 (24.7)

a Median (Q1, Q3)

Source: Section 15.1, Table 4.5

Table 7: Statistical comparisons of GS-5885 plasma pharmacokinetic parameter estimates following administration of GS-5885 alone or in combination with SOF (source: Study Report Table 10-8)

GS-5885 PK Parameter	GLSM		%GLSM (90% CI) SOF+GS-5885 / GS-5885
	SOF+GS-5885 (N = 17)	GS-5885 (N = 17)	
AUC _{tau} (ng•h/mL)	11,032.00	11,521.40	95.75 (92.11, 99.53)
C _{tau} (ng/mL)	345.56	362.01	95.46 (91.92, 99.13)
C _{max} (ng/mL)	709.29	735.01	96.50 (89.91, 103.58)

Source: Section 15.1, Table 5.5

Conclusion

In the study, the single-dose pharmacokinetics of sofosbuvir and the sofosbuvir metabolites GS-566500 and GS-331007 and the multiple-dose pharmacokinetics of GS-5885 in plasma were evaluated following administration of SOF and GS-5885 alone and in combination. Following coadministration of SOF and GS-5885, GS-331007 and GS-5885 exposures were not substantially affected, but exposures of SOF and GS-566500 increased by approximately 2-fold and 1.8-fold, respectively. The increases in SOF and GS-566500 AUC and C_{max} are likely due at least in part to inhibition of P-gp and BCRP by GS-5885 (SOF is a substrate of P-gp and BCRP; GS-331007 is not). The increases in SOF and GS-566500 exposure are not expected to be clinically relevant given the favorable safety profile for SOF and the short half-lives for both SOF and GS-566500 (approximately 0.5 and 2 h, respectively). Additionally, they are relatively minor components of total SOF-related material exposures compared to the predominant circulating metabolite, GS-331007.

4.2.4.7 GS-US-334-0146 (Leslie): A Phase 1, Open Label Drug Interaction Study Evaluating the Effect of Sofosbuvir or GS-5885 on the Pharmacokinetics of a Representative Hormonal Contraceptive Medication, Norgestimate/Ethinyl Estradiol

Trial Period and Site

3 Nov 2012 to 13 Mar 2013 (b) (4)

Trial Rationale

In this study, the effects of ledipasvir (LDV) and sofosbuvir (SOF) on the pharmacokinetics (PK) of ethinyl estradiol and norgestimate (Ortho Tri-Cyclen® Lo, an oral contraceptive [OC]) as well as the effects of Ortho Tri-Cyclen® Lo on the PK of LDV and SOF were evaluated.

Trial Design

This was an open-label, fixed-sequence, multiple-dose study with a 28-day Lead-in period (Day L1 to Day L28) for eligible subjects who had not been taking OC for at least one menstrual cycle or to synchronize menstrual cycles followed by an 84-day dosing period (Day 1 to Day 84) covering three menstrual cycles (Days 1-28, 29-56, and 57-84).

Cycle 1, Weeks 1-4: OC once daily

Cycle 2, Weeks 1, 3-4: OC once daily; Week 2 (Days 36-42): OC plus SOF once daily

Cycle 3, Weeks 1-2: OC plus LDV once daily (Days 57-70); Weeks 3-4: OC once daily

On days of serial blood sample collection, study drugs were administered in the morning within 5 minutes of consuming a moderate-fat meal following an overnight 8-hour fast.

Rationale for Dose Selection

The dose of GS-5885 90 mg was selected based on antiviral activity and a favorable safety profile in patients infected with HCV genotype 1a in study GS-US-256-0102. The dose of SOF 400 mg was selected based on antiviral activity and a favorable safety profile in patients infected with HCV genotype 1 (Study P7977-0422, PROTON). These doses were evaluated as a fixed-dose combination in Phase 3 studies. The doses of ethinyl estradiol 25 ug and norgestimate 180/215/250 ug are the approved doses of Ortho Tri-Cyclen® Lo for oral contraception.

Investigational Product

LDV 90 mg film-coated oral tablets (b) (4) contained the inactive ingredients copovidone, lactose monohydrate, microcrystalline cellulose, croscarmellose sodium, magnesium stearate, polyvinyl alcohol, titanium dioxide, polyethylene glycol, and talc

(b) (4) SOF 400 mg film-coated oral tablets were yellow and capsule-shaped and contained the inactive ingredients mannitol, microcrystalline cellulose, croscarmellose sodium, colloidal silicon dioxide, magnesium stearate, polyvinyl alcohol, titanium dioxide, (b) (4) talc, and yellow iron oxide (b) (4)

Ortho Tri-Cyclen Lo white tablets contained norgestimate/ethinyl estradiol 180/25 ug, light blue tablets contained norgestimate/ethinyl estradiol 215/25 ug, dark blue tablets contained norgestimate/ethinyl estradiol 250/25 ug, and dark green tablets contained only inactive ingredients (refer to package insert, Janssen Pharmaceuticals, Inc.; Lot 2BM413).

Key Inclusion and Exclusion Criteria

Subjects were healthy, nonpregnant, nonlactating, nonsmoking, premenopausal females between the ages of 18 and 45 years, inclusive, with a BMI between 19 and 30 kg/m² and creatinine clearance ≥80 mL/min. Subjects had to agree to be abstinent or use an additional nonhormonal contraceptive during the study and for at least 30 days after the last dose of LDV.

Exclusion criteria also included history of nicotine use (within 28 days), any serious or active medical or psychiatric illness, positive test result for HIV-1 antibody, hepatitis C antibody, or hepatitis B surface antigen, a history of hysterectomy, bilateral oophorectomy, or ovarian failure, or a positive urine screen for drugs of abuse.

Concomitant Medications

The following medications and substances were disallowed while subjects were participating in the study:

- medroxyprogesterone acetate (Depo-Provera) injection within 3 months of screening
- current use of a progesterone-releasing IUD
- all prescription and over-the-counter medications (with the exception of acetaminophen, ibuprofen, or vitamins)
- systemic steroids, immunosuppressant therapies, or chemotherapeutic agents from within 3 months of screening

Sample Collection

Blood samples were collected to assess concentrations of norgestimate, norelgestromin (NGMN, the major active metabolite of norgestimate), norgestrel, and ethinyl estradiol (EE) at the following timepoints on Days 14, 42, and 70: predose and 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 14, 16, 20, and 24 h postdose. Blood samples were collected to assess concentrations of SOF and metabolites at the same timepoints on Day 42 and LDV on Day 70.

Blood samples for the pharmacodynamic markers luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were collected predose on Days 14, 42, and 70 and blood samples for progesterone were collected predose on Days 21, 49, and 77.

Analytical Methods

Concentrations of SOF and the SOF metabolites GS-566500 and GS-331007 in plasma samples were measured by LC-MS/MS (b) (4) (b) (4) validation report (b) (4) 86-0938; study report (b) (4) 60-1219A). Sample receipt commenced on 20 Mar 2012 (storage temperature: -70°C) and analysis was completed on 11 May 2012. The first day of sample collection was 13 Mar 2012, so the maximum storage sample time was 58 days, which is within the validated long-term frozen stability duration for SOF, GS-566500, and GS-331007 in plasma. For SOF, GS-566500, and GS-331007, the quality control (QC) concentrations ranged from 5-4000 ng/mL, 10-4000 ng/mL, and 10-4000 ng/mL, respectively. All inter-assay accuracy and precision estimates were within the acceptable range (Table 1).

Concentrations of GS-5885 in plasma samples were measured by LC-MS/MS (b) (4) (b) (4) validation report (b) (4) 60-1028; study report (b) (4) 60-1219B). Sample receipt commenced on 3 Apr 2012 (storage temperature: -70°C) and analysis was completed on 16 May 2012. The first day of sample collection was 26 Mar 2012, so the maximum storage sample time was 49 days, which is within the validated long-term frozen stability duration of 351 days for plasma. The QC concentrations ranged from 1-1600 ng/mL. All inter-assay accuracy and precision estimates were within the acceptable range (Table 1).

Table 1: Bioanalytical assay validation for LDV and SOF and its metabolites (source: Study Report Table 7-6)

Parameter	Sofosbuvir (PSI-7977)	GS-566500 (PSI-352707)	GS-331007 (PSI-6206)	Ledipasvir
Linear range (ng/mL)	5 to 5000	10 to 5000	10 to 5000	1 to 2000
Lower limit of quantitation (ng/mL)	5	10	10	1
Interday precision range (%CV)	1.3 to 7.4 ^a	3.3 to 9.1 ^a	0.8 to 5.6 ^a	1.0 to 8.8
Interday accuracy range (%RE)	-3.3 to 8.2 ^a	-5.2 to 5.9 ^a	-0.1 to 7.5 ^a	-7.1 to -3.6
Stability in frozen matrix (days)	377 days at -70°C and 36 days at -20°C	308 days at -70°C and 25 days at -20°C	308 days at -70°C and 25 days at -20°C	623 days at -20°C and -70°C

a Intraday data reported

Source: Appendix 16.1.10

Concentrations of norgestrel and norgestimate in plasma samples were measured by LC-MS/MS (b) (4) (b) (4) validation report (b) (4) 42-1226; study report (b) (4) 60-12106C). Sample receipt commenced on 22 Jan 2013 (storage temperature: -70°C) and analysis was completed on 3 Apr 2013. The maximum storage sample time was 102 days, which is within the validated long-term frozen stability duration of 129 days for plasma. The QC concentrations ranged from 20-20000 pg/mL for norgestrel and 50-50000 pg/mL for norgestimate. All inter-assay accuracy and precision estimates were within the acceptable range (Table 2).

Concentrations of ethinyl estradiol in plasma samples were measured by LC-MS/MS (b) (4) (b) (4) validation report (b) (4) 42-0931; study report (b) (4) 60-12106D). Sample receipt commenced on 29 Jan 2013 (storage temperature: -70°C) and analysis was completed on 14 Mar 2013. The maximum storage sample time was 102 days, which is within the validated long-term frozen stability duration of 182 days for plasma. The QC concentrations ranged from 2.5-500 pg/mL. All inter-assay accuracy and precision estimates were within the acceptable range (Table 2).

Concentrations of 17-desacetyl norgestimate in plasma samples were measured by LC-MS/MS (b) (4) (b) (4) validation report (b) (4) 42-0728; study report (b) (4) 60-12106E). Sample receipt commenced on 29 Jan 2013 (storage temperature: -70°C) and analysis was completed on 9 Mar 2013. The maximum storage sample time was 67 days, which is within the validated long-term frozen stability duration of 153 days for plasma. The QC concentrations ranged from 20-10000 pg/mL. All inter-assay accuracy and precision estimates were within the acceptable range (Table 2).

Table 2: Bioanalytical assay validation for norgestrel, norgestimate, ethinyl estradiol, and 17-desacetyl norgestimate (NGMN) (source: Study Report Table 7-7)

Parameter	Norgestrel	Norgestimate	Ethinyl Estradiol	17-Desacetyl Norgestimate
Linear range (ng/mL)	0.02 to 20	0.05 to 50	0.0025 to 0.5	0.02 to 10
Lower limit of quantitation (ng/mL)	0.02	0.05	0.0025	0.02
Interday precision range (%CV)	1.3 to 5.6	1.8 to 3.8	3.8 to 7.9	2.3 to 8.0
Interday accuracy range (%RE)	-2.3 to 4.3	-10.8 to 5.8	-4.1 to 1.2	-5.2 to 3.1
Stability in frozen matrix (days)	129 days at -20°C and -70°C	129 days at -20°C and -70°C	182 days at -20°C and -70°C	153 days at -20°C and -70°C

Source: Appendix 16.1.10

Results

Trial Population

Twenty-one healthy female subjects between the ages of 26 and 45 were enrolled in the study; fifteen were dosed with study drugs and completed the study (the other six were alternates and only completed the lead-in period). All subjects were white and were of Hispanic or Latino ethnicity.

Pharmacokinetics of NGMN

The NGMN mean concentration-time profiles were similar following administration of OC alone or in combination with SOF or LDV (data not shown). Accordingly, NGMN pharmacokinetic parameters were similar regardless of treatment (Table 3). The 90% CIs the GLSM ratios of AUC_{tau} , C_{max} , and C_{tau} were within the predefined no-effect boundaries, indicating that SOF or LDV did not significantly impact NGMN PK (Table 4).

Table 3: NGMN plasma pharmacokinetic parameters following administration of OC alone or in combination with LDV or SOF (source: Study Report Table 10-1)

Norelgestromin PK Parameter	Mean (%CV)		
	OC (N = 15)	OC+SOF (N = 15)	OC+LDV (N = 15)
AUC_{tau} (hr·pg/mL)	17353.8 (26.5)	18147.8 (21.9)	17691.2 (21.3)
C_{max} (pg/mL)	1669.6 (21.9)	1795.6 (24.6)	1707.6 (23.3)
C_{tau} (pg/mL)	428.2 (35.1)	451.6 (31.9)	462.3 (31.5)
T_{max} (hr) ^a	3.00 (2.00, 4.00)	2.50 (2.00, 4.00)	2.00 (1.50, 4.00)
T_{last} (hr) ^a	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
$t_{1/2}$ (hr) ^a	20.48 (18.06, 29.58)	23.75 (21.93, 26.62)	28.58 (21.92, 33.22)

a Median (Q1, Q3)

Source: Section 15.1, Table 4.1

Table 4: Statistical comparisons of NGMN plasma pharmacokinetic parameter estimates following administration of OC alone or in combination with LDV or SOF (source: Study Report Table 10-2)

Norelgestromin PK Parameter	GLSM		%GLSM Ratio (90%CI)
	OC+SOE (N = 15)	OC (N = 15)	OC+SOE versus OC (N = 15)
AUC _{tau} (hr·pg/mL)	17776.96	16840.95	105.56 (92.26, 120.77)
C _{max} (pg/mL)	1750.53	1637.06	106.93 (93.58, 122.18)
C _{tau} (pg/mL)	433.85	406.80	106.65 (88.98, 127.83)
Norelgestromin PK Parameter	GLSM		%GLSM Ratio (90%CI)
	OC+LDV (N = 15)	OC (N = 15)	OC+LDV versus OC (N = 15)
AUC _{tau} (hr·pg/mL)	17350.65	16840.95	103.03 (90.05, 117.87)
C _{max} (pg/mL)	1667.54	1637.06	101.86 (89.15, 116.39)
C _{tau} (pg/mL)	444.55	406.80	109.28 (91.17, 130.98)

Source: Section 15.1, Table 5.1

Pharmacokinetics of norgestrel

The norgestrel mean concentration-time profiles were similar following administration of OC alone or in combination with LDV but were higher in combination with SOF (Figure 1). Norgestrel median $t_{1/2}$ was slightly longer following coadministration of OC and SOF or LDV compared to OC alone (Table 5). The point estimates for the GLSM ratios of norgestrel AUC_{tau}, C_{max}, and C_{tau} were approximately 20% higher following coadministration of OC and SOF compared to administration of OC alone (Table 6).

Table 6: Statistical comparisons of norgestrel plasma pharmacokinetic parameter estimates following administration of OC alone or in combination with SOF or LDV
(source: Study Report Table 10-4)

Norgestrel PK Parameter	GLSM		%GLSM Ratio (90%CI)
	OC+SOF (N = 15)	OC (N = 15)	OC+SOF versus OC (N = 15)
AUC _{tau} (hr·pg/mL)	49559.27	41493.73	119.44 (98.39, 144.98)
C _{max} (pg/mL)	2548.68	2155.36	118.25 (99.15, 141.03)
C _{tau} (pg/mL)	1872.12	1523.27	122.90 (99.84, 151.28)
Norgestrel PK Parameter	GLSM		%GLSM Ratio (90%CI)
	OC+LDV (N = 15)	OC (N = 15)	OC+LDV vs OC (N = 15)
AUC _{tau} (hr·pg/mL)	41056.24	41493.73	98.95 (81.51, 120.11)
C _{max} (pg/mL)	2224.43	2155.36	103.20 (86.53, 123.09)
C _{tau} (pg/mL)	1527.55	1523.27	100.28 (81.47, 123.44)

Source: Section 15.1, Table 5.2

Pharmacokinetics of EE

The EE mean concentration-time profile was slightly higher following administration of OC plus LDV compared to OC alone, with OC plus SOF falling in between (Figure 2). Slight elevations in EE exposure parameters were also observed in the presence of SOF and LDV (Table 7), with increases in EE AUC_{tau} and C_{max} of approximately 10-15% and 20-40% in presence of SOF and LDV, respectively, although only C_{max} was statistically significantly increased following combination treatment with OC and LDV (Table 8).

Table 6: Statistical comparisons of EE plasma pharmacokinetic parameter estimates following administration of OC alone or in combination with SOF or LDV (source: Study Report Table 10-6)

Ethinyl Estradiol PK Parameter	GLSM		%GLSM Ratio (90%CI)
	OC+SOF (N = 15)	OC (N = 15)	OC+SOF versus OC (N = 15)
AUC _{tau} (hr·pg/mL)	700.24	644.19	108.70 (93.95, 125.77)
C _{max} (pg/mL)	71.90	62.68	114.72 (96.60, 136.24)
C _{tau} (pg/mL)	12.53	12.68	98.85 (79.66, 122.66)
Ethinyl Estradiol PK Parameter	GLSM		%GLSM Ratio (90%CI)
	OC+LDV (N = 15)	OC (N = 15)	OC+LDV versus OC (N = 15)
AUC _{tau} (hr·pg/mL)	775.79	644.19	120.43 (104.09, 139.33)
C _{max} (pg/mL)	87.70	62.68	139.92 (117.81, 166.17)
C _{tau} (pg/mL)	12.47	12.68	98.32 (79.24, 122.00)

Source: Section 15.1, Table 5.3

Pharmacokinetics of SOF and its metabolites GS-566500 and GS-331007

Following coadministration of SOF and OC, the mean SOF, GS-566500, and GS-331007 AUC and C_{max} values were comparable historical values (Table 7). The median SOF and GS-331007 half-lives are similar to the SOF and GS-331007 half-lives described in the current sofosbuvir (Sovaldi®) label (0.4 and 27 h, respectively).

Table 7: Plasma pharmacokinetic parameters of SOF, GS-566500, and GS-331007 following administration of SOF alone (historical data; GS-US-334-0101) or in combination with OC (source: Study Report Table 10-7 and GS-US-334-0101)

PK Parameter	SOF mean (%CV)		GS-566500 mean (%CV)		GS-331007 mean (%CV)	
	SOF+OC	Historical	SOF+OC	Historical	SOF+OC	Historical
AUC (h·ng/mL)	829.4 (61.3)	787.7 (36.6)	1002.2 (26.3)	1063.1 (32.7)	9906.7 (18.5)	10228.8 (17.9)
C _{max} (ng/mL)	671.1 (93.1)	928.7 (52.3)	212.1 (27.0)	311.5 (38.7)	1417.9 (31.7)	1058.5 (17.3)
C _{last} (ng/mL)	28.3 (124.8)	8.83 (27.9)	13.4 (20.0)	13.10 (17.38)	141.3 (19.8)	16.15 (30.30)
T _{max} ^a (h)	2.00 (1.50, 3.00)	0.50 (0.50, 0.50)	2.50 (2.00, 4.00)	1.00 (1.00, 1.50)	4.00 (3.00, 4.02)	2.00 (1.50, 3.00)
T _{last} ^a (h)	4.00 (4.00, 5.00)	3.02 (3.00, 4.00)	12.00 (12.00, 14.00)	10.00 (10.00, 12.00)	24.00 (24.00, 24.00)	72.00 (72.00, 95.93)
t _{1/2} ^a (h)	0.53 (0.38, 0.70)	0.41 (0.40, 0.52)	2.46 (2.31, 2.73)	2.38 (2.17, 2.56)	27.98 (19.82, 38.10)	28.14 (24.03, 32.45)
CL/F (mL/h)	621202.6 (52.2)	603805.1 (55)	NA	NA	NA	NA

^a median (Q1, Q3)

Pharmacokinetics of LDV

Following coadministration of LDV and OC, the mean LDV AUC_{tau} and C_{max} were comparable historical values (Table 8).

Table 8: Plasma pharmacokinetic parameters of LDV following administration of LDV alone (historical data; GS-US-334-0101) or in combination with OC (source: Study Report Table 10-8 and GS-US-334-0101)

PK Parameter	LDV mean (%CV)	
	LDV+OC	Historical
AUC _{tau} (h.ng/mL)	13278.4 (35.5)	11866.9 (26.2)
C _{max} (ng/mL)	698.5 (33.9)	755.5 (24.7)
C _{tau} (ng/mL)	513.0 (38.0)	374.9 (28.8)
T _{max} ^a (h)	5.00 (5.00, 5.00)	4.50 (4.50, 4.50)
T _{last} ^a (h)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
t _{1/2} ^a (h)	73.13 (57.38, 84.01)	37.03 (27.70, 45.33)
CL/F (mL/h)	7746.9 (40.1)	8029.7 (23.6)

^a median (Q1, Q3)

Pharmacodynamic markers (LH, FSH, and progesterone)

Serum concentrations of LH and FSH were assessed predose on Day 14 of each cycle and serum concentrations of progesterone was assessed predose on Day 21 of each cycle (Table 9).

Table 9. Serum concentrations of LH, FSH, and progesterone following administration of OC alone or in combination with SOF or LDV (source: Study Report Table 10-9)

PD Analyte Median (Q1; Q3)	OC (N = 15)	OC+SOF (N = 15)	OC+LDV (N = 15)
LH (mIU/mL)	5.5 (3.4, 9.0)	4.6 (0.9, 8.7)	5.2 (1.6, 9.3)
FSH (mIU/mL)	3.7 (2.2, 5.8)	2.4 (1.4, 5.0)	3.6 (1.7, 5.6)
Progesterone (ng/mL)	0.31 (0.24, 0.45)	0.24 (0.20, 0.35)	0.16 (0.08, 0.21)

Source: Section 15.1, Tables 6.1, 6.2, and 6.3

Conclusion

In the study, the steady-state pharmacokinetics of ethinyl estradiol (EE), norelgestromin (NGMN), and norgestrel were evaluated following administration of norgestimate and ethinyl estradiol alone (Ortho TriCyclen® Lo [OC]) or in combination with sofosbuvir (SOF) or ledipasvir (LDV). (Norgestimate plasma concentrations were below the limit of quantitation.) The steady-state pharmacokinetics of SOF and LDV in the presence of OC were compared to historical pharmacokinetic data.

Statistically significant increases were observed for norgestrel AUC_{tau} and C_{tau} (approximately 20%) following coadministration of OC. It should be noted that the magnitude of the increases may be greater in the presence of SOF and LDV, as LDV increases exposures of SOF and GS-566500 (GS-US-334-0101). However, as SOF and GS-566500 have short systemic half-lives (approximately 2 h or less), it appears more likely that GS-331007 is the perpetrator of this pharmacokinetic drug interaction. In addition, the increases in norgestrel exposures are unlikely

to be clinically relevant because the biologic activity of norgestrel is limited due to its high affinity binding to sex hormone-binding globulin (SHBG).

A statistically significant increase in EE C_{max} (approximately 40%, accompanied by a statistically non-significant 20% increase in AUC) was observed following coadministration of OC and LDV. The magnitude of the increase in EE C_{max} is comparable to the difference in mean EE C_{max} values between Ortho TriCyclen® Lo (containing 25 ug EE) and Ortho TriCyclen® (containing 35 ug EE) listed in the respective package inserts. (The increase in EE AUC in the presence of LDV is less than the difference in mean EE AUC values between Ortho TriCyclen® Lo and Ortho TriCyclen®).

The pharmacokinetics of SOF and LDV were comparable to historical data (GS-US-334-0101).

The results of this study indicate that Ortho TriCyclen® Lo (ethinyl estradiol 25 ug and norgestimate 180/215/250 ug) can be coadministered with SOF 400 mg and LDV 90 mg (as individual components or in combination) without dose adjustment. While increases in norgestrel and EE exposures were observed in combination with SOF and LDV, respectively, these increases are not expected to be clinically relevant, especially as the duration of LDV and SOF administration will be limited to 24 weeks of treatment. Following consultation with the OCP Reproductive and Urologic Products team, and in light of the relatively short duration of the LDV/SOF regimen, no dose adjustments in ethinyl estradiol, norgestimate, SOF, or LDV are needed when these products are used concomitantly.

4.2.5 In vitro Studies (Leslie)

1. Absorption

- AD-256-2108: Permeability of GS-5885 across Caco-2 monolayers
- AD-256-2144: Effect of P-gp expression on LDV accumulation
- AD-256-2150: Effect of BCRP expression on LDV cellular accumulation

Summary: LDV had moderate to high permeability across Caco-2 monolayers; however, in overexpressing MDCKII cells, LDV was transported by P-gp and BCRP and this transport was shown to be specific through the use of probe inhibitors. In the absence of human mass balance or absolute bioavailability data characterizing the extent of absorption, the in vitro data indicating LDV efflux by P-gp and BCRP, as well as in vivo data from drug-drug interaction trials evaluating the PK of LDV in combination with P-gp and BCRP inhibitors such as ritonavir (Bierman et al. J Antimicrob Chemother 2010) and cobicistat (Lepist et al. Antimicrob Agents Chemother 2012), suggest that LDV should not be definitively classified as a high permeability drug (extent of absorption in humans >90% of dose administered; FDA Guidance for Industry: Waiver of In Vivo Bioavailability and Bioequivalence Studies for IR Solid Oral Dosage Forms Based on BCS).

2. Distribution

- AD-256-2094: GS-5885 protein binding

Summary: LDV was highly protein-bound (>99.8%) in plasma from all species evaluated (mice, rats, dogs, monkeys, and humans). LDV was not a substrate for the hepatic uptake transporters OCT1, OATP1B1, or OATP1B3 (studies listed under Section 4).

3. Metabolism and elimination

- AD-256-2098: CYP phenotyping of GS-5885

Summary: Rates of LDV metabolism were low for all CYP isoforms evaluated, suggesting that there will not be substantial metabolism of GS-5885 by CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4 in vivo.

4. Drug interaction potential

- AD-256-2139: LDV as a substrate for human OATP1B1 and 1B3
- AD-256-2096: CYP inhibition potential of GS-5885
- AD-256-2133: Human microsomal CYP2B6 inhibition potential of GS-5885
- AD-256-2097: Induction of metabolizing enzymes by GS-5885
- AD-256-2146: Induction potential of GS-5885 in cultured human hepatocytes
- AD-256-2109: Effect of GS-5885 on the accumulation of model substrates in P-gp, MRP2, and BCRP overexpressing cells
- AD-256-2132: Human UGT1A1 inhibition potential of GS-5885
- AD-256-2134: GS-5885 inhibition of human OATP1B1 and 1B3
- AD-256-2140: Inhibition studies of LDV with human MRP4, BSEP, OAT1, OAT3, OCT2, and MATE1 transporters
- AD-256-2143: Inhibition and substrate studies of LDV with human OCT1 transporter
- AD-337-2001: Effect of SOF and LDV on the bidirectional permeability of tenofovir DF through Caco-2 monolayers

Summary: LDV did not inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or CYP2D6 at concentrations up to 25 μM and was a weak inhibitor of CYP3A (IC_{50} : 9.9 μM).

LDV inhibited P-gp and BCRP efflux by 46.3% and 38.1%, respectively, at the anticipated LDV C_{max} of 1 μM . LDV (1-25 μM) and SOF (1000 μM) independently decreased the transcellular efflux of tenofovir disoproxil fumarate in Caco-2 cells, suggesting that coadministration of LDV and/or SOF with drugs that are substrates for P-gp and/or BCRP may lead to increased absorption and systemic exposures of the coadministered drug.

LDV inhibited UGT1A1, with a $C_{\text{max}}/\text{IC}_{50}$ ratio of 0.126. LDV inhibited OATP1B1 and OATP1B3 with IC_{50} values of 3.5 and 6.5 μM , respectively, and $C_{\text{max}}/\text{IC}_{50}$ values for OATP1B1 and OATP1B3 of 0.29 and 0.15, respectively. LDV inhibited BSEP with an IC_{50} of approximately 6 μM , giving a $C_{\text{max}}/\text{IC}_{50}$ ratio of 0.17. Although the LDV $C_{\text{max}}/\text{IC}_{50}$ ratios for UGT1A1, OATP1B1, OATP1B3, and BSEP were above 0.1 (suggesting the potential for drug interactions), the R-value is <1.25 . According to the draft Guidance for Industry (Drug Interaction Studies — Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations), this indicates that the potential for LDV drug interactions with OATP1B1, OATP1B3, UGT1A1, and BSEP substrates is low. Furthermore, the high degree of LDV plasma protein binding makes it unlikely that these interactions will be clinically relevant; thus, no additional in vivo studies are being requested at this time.

LDV (6 μM) was a weak inhibitor of OCT2 (13% inhibition) and appeared to activate MATE1 (18% stimulation). The clinical relevance of these relatively minor interactions is likely limited.

LDV (up to 10 μM) did not activate AhR and weakly activated PXR; activation of CAR was not evaluated in this study. At a LDV concentration of 1 μM (expected C_{max}), LDV increased mean CYP2B6 and CYP3A activity by approximately 50% and 30%, respectively, over vehicle control. These increases in enzyme activity may be clinically significant for concomitantly administered drugs that are a substrate of one or both of these enzymes and have a narrow therapeutic range. LDV did not induce CYP1A2, CYP2C9, P-gp, or UGT1A1 at concentrations up to 10 μM .

AD-256-2108: Permeability of GS-5885 across Caco-2 cell monolayers

Introduction

In this study, the bidirectional permeability of GS-5885 was determined using human colon carcinoma (Caco-2) cells.

Materials and Methods

Caco-2 cells between passages 62 and 70 were grown to confluence for at least 21 days on 24-well PET plates (BD Biosciences). Immediately prior to assay initiation, TEER values were assessed to evaluate membrane integrity. Forward (apical to basolateral, A to B) and reverse (basolateral to apical, B to A) permeability of GS-5885 was determined over 120 minutes. The efflux ratio was calculated as the average reverse apparent permeability (P_{app}) divided by the average forward P_{app} ($P_{\text{app,B:A}}/P_{\text{app,A:B}}$). Propranol and vinblastine served as the transporter negative and positive controls, respectively. Experimental conditions were conducted in duplicate and samples were analyzed by LC-MS/MS.

Results

At a concentration of 1 μM , GS-5885 had high permeability in the forward direction and medium permeability in the reverse direction (average P_{app} values of 1.76 and 0.68, respectively) and an efflux ratio of 0.38, indicating no significant efflux transport (Table 1).

Table 1: Bidirectional permeability of GS-5885 across Caco-2 monolayers (source: Study Report Table 1)

Direction	Target Conc. (μM)	Initial Conc. (μM)	Recovery (%)	P_{app} (10^{-6} cm/s)			Efflux Ratio
				R1	R2	Average	
Cell-Free	1	0.63	210	14.9	–	14.9	–
Forward		0.63	90	1.62	1.89	1.76	0.38
Reverse		0.76	84	0.59	0.76	0.68	

Conclusion

GS-5885 had medium to high permeability through Caco-2 cell monolayers, suggesting that intestinal absorption of the soluble dose should be high after oral administration. Active efflux appeared to be minimal.

AD-256-2144: Effect of P-glycoprotein expression on ledipasvir accumulation in vitro

Introduction

In this study, P-glycoprotein (P-gp) transport of ledipasvir (LDV) was determined using overexpressing cells (MDCKII-MDR1) in the presence and absence of the P-gp inhibitors verapamil and cyclosporine A (CsA).

Materials and Methods

MDCKII-WT and MDCKII-MDR1 cells were grown to 70-90% confluence in 48-well plates. Intracellular uptake of a mixture of [^3H]LDV (Moravek Biochemicals and Radiochemicals) and LDV (final concentration: 0.5 μM) was determined in a cell suspension after a 10 minute incubation at 37°C. Verapamil (100 μM) and CsA (10 μM) were added to the reaction mixture as positive controls. Experimental conditions were conducted in triplicate and the assay was conducted in duplicate. Radioactivity in the cell lysate was determined by scintillation counting.

Results

Approximately 70% less LDV accumulated in P-gp overexpressing cells compared to wild-type cells (Table 1). In the presence of the P-gp inhibitors CsA and verapamil, LDV accumulation in overexpressing and wild-type cells was comparable (Table 1). As expected, the P-gp inhibitors had no effect on LDV accumulation in wild-type cells.

Table 1: Accumulation of LDV in MDCKII-WT and MDCKII-MDR1 cells (source: Study Report Table 1)

0.5µM Ledipasvir	LDV Amount (pmol/106 cells) ^a	
	WT	MDR1
+ no inhibitor	107 ± 10	32 ± 3
+ 100 µM Verapamil	104 ± 14	102 ± 9
+ 10 µM Cyclosporin A	100 ± 17	79 ± 18

^a Average and standard deviation calculated from triplicate measurements from two independent assays

Conclusion

LDV is transported by P-gp in overexpressing MDCKII-MDR1 cells. Transport was demonstrated to be P-gp specific as it was inhibited by verapamil and CsA.

AD-256-2150: Effect of BCRP expression on ledipasvir cellular accumulation in vitro

Introduction

In this study, Breast Cancer Resistance Protein (BCRP) transport of ledipasvir (LDV) was determined using overexpressing cells (MDCKII-BCRP) in the presence and absence of the BCRP inhibitor cyclosporine A (CsA).

Materials and Methods

MDCKII-WT and MDCKII-MDR1 cells were grown to 70-90% confluence in 48-well plates. Intracellular uptake of a mixture of [³H]LDV (Moravek Biochemicals and Radiochemicals) and LDV (final concentration: 0.5 µM) was determined in a cell suspension after a 60 minute incubation at 37°C. The BCRP inhibitor CsA (10 µM) was added to the reaction mixture to evaluate transport specificity. Uptake of prazosin (10 µM) was also assessed as a positive control. Radioactivity in the cell lysate was determined by scintillation counting.

Results

Approximately 60% less LDV accumulated in BCRP overexpressing cells compared to wild-type cells (Table 1). In the presence of the BCRP inhibitor CsA, LDV accumulation in overexpressing and wild-type cells was comparable (Table 1). As expected, CsA had no substantial effect on LDV accumulation in wild-type cells.

Table 1: Accumulation of LDV and the positive control prazosin in MDCKII-WT and MDCKII-BCRP cells (source: Appendix Tables 1-4)

	Uptake as % of WT (mean ± SD)			
	WT		BCRP	
10 µM CsA	-	+	-	+
0.5 µM LDV	100 ± 0	130 ± 90	38 ± 9.2	135 ± 21
10 µM Prazosin	100	162	6	93

Conclusion

LDV was transported by BCRP in overexpressing MDCKII-BCRP cells and transport was inhibited by CsA.

AD-256-2094: In vitro determination of GS-5885 protein binding by equilibrium dialysis

Introduction

In this study, the protein binding of GS-5885 in CD-1 mouse, Sprague-Dawley rat, Beagle dog, Cynomolgus monkey, and human plasma was evaluated using equilibrium dialysis.

Materials and Methods

GS-5885 was added to pooled plasma from at least three males and three females from each species (rat, dog, mouse, monkey, and human) to final concentrations of 2 and 10 uM. Equilibrium dialysis with phosphate buffer was conducted at 37°C for 30 hours. Competitive equilibrium dialysis was also conducted with cell culture medium (10% fetal bovine serum) in place of phosphate buffer. Experimental conditions were conducted in triplicate and samples were analyzed using LC-MS/MS.

Results

Less than 1% of GS-5885 was unbound in plasma from all species evaluated (Table 1).

Table 1: GS-5885 protein binding in plasma (source: Study Report Table 1)

Matrix	Free Fraction (%)	
	2 µM	10 µM
Mouse	0.03 ± 0.01	0.04 ± 0.00
Rat	0.06 ± 0.02	0.11 ± 0.01
Dog	0.03 ± 0.00	0.04 ± 0.01
Monkey	0.05 ± 0.01	0.06 ± 0.04
Human	0.05 ± 0.03	0.06 ± 0.04

Conclusion

GS-5885 was highly bound to plasma proteins in all species tested (>99%) at both GS-5885 concentrations that were evaluated.

AD-256-2098: Cytochrome P450 phenotyping of GS-5885

Introduction

In this study, the cytochrome P450 (CYP) isoforms involved in GS-5885 metabolism were identified in human CYP enzyme preparations.

Materials and Methods

GS-5885 or positive control probe substrates (final concentration: 5 uM) were incubated with bacterially-expressed human CYP enzyme preparations coexpressed with human NADPH cytochrome P450 reductase (Bactosomes™, Cypex Ltd.) for 0, 5, 15, 30, or 45 min at 37°C in the presence of NADPH. Drug concentrations were assessed by LC-MS/MS and were used to determine the rate of metabolism. Assays were conducted (b) (4)

Results

Rates of GS-5885 metabolism were low for all CYP isoforms evaluated (range: 0.12-0.47 min⁻¹, Table 1).

Table 1: Rates of GS-5885 and probe substrate metabolism by human CYP isoforms (source: Study Report Table 1)

Compound	Metabolism Rate (min ⁻¹)					
	CYP1A2	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP3A4
GS-5885 (% Positive Control)	< 0.12 (< 0.9%)	< 0.23 (< 1.1%)	< 0.47 (< 1.2%)	< 0.12 (< 17.7%)	< 0.23 (< 0.6%)	< 0.47 (< 6.4%)
Ethoxycoumarin	12.7	—	—	—	—	—
Amodiaquine	—	22.2	—	—	—	—
Diclofenac	—	—	39.6	—	—	—
Diazepam	—	—	—	0.66 ^a	—	—
Dextromethorphan	—	—	—	—	36.7	—
Testosterone	—	—	—	—	—	7.3

^a Diazepam is a selective substrate for CYP2C19 but is metabolized slowly

Conclusion

Metabolism rates of GS-5885 were low for all CYP isoforms evaluated, suggesting that there will not be substantial metabolism of GS-5885 by CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4 in vivo.

AD-256-2139: In vitro assessment of ledipasvir as a substrate for human OATP1B1 and OATP1B3

Introduction

In this study, OATP1B1 and OATP1B3 transport of ledipasvir (LDV) was evaluated using transfected Chinese hamster ovary (CHO) cells in the presence and absence of the OATP inhibitor rifampicin.

Materials and Methods

CHO cells (wild-type or transfected with human OATP1B1 or OATP1B3) were stimulated with sodium butyrate and grown to confluence in 48-well plates. Cells were trypsinized and resuspended in assay buffer containing GS-5885 (final concentration: 0.4 μM) in the presence or absence of rifampicin (final concentration: 40 μM) at 37°C for 1 min. The OATP substrate atorvastatin (0.1 μM) served as a positive control and antipyrin (10 μM), a compound with high passive permeability, was a negative control. Drug concentrations in cell lysates were determined by scintillation counting or LC-MS/MS.

Results

The LDV uptake rate was approximately 60% lower in cells transfected with OATP1B1 or OATP1B3 compared to wild-type cells (Table 1), indicating negligible OATP-mediated uptake. The OATP inhibitor rifampicin did not substantially influence LDV uptake rate in OATP1B1 or OATP1B3 transfected cells, while the uptake rate decreased by approximately 9- and 15-fold in OATP1B1 and OATP1B3 transfected cells, respectively, in the presence of rifampicin (Table 1). As expected, antipyrin uptake was not affected by OATP1B1 or OATP1B3 expression or by rifampicin.

Table 1: Uptake rate of LDV and control compounds in transfected and wild-type CHO cells (source: Study Report Tables 2 and 3)

Uptake Rate (pmol/min/1x10 ⁶ cells)	0.1 uM LDV		0.1 uM atorvastatin		10 uM antipyrin	
	-	+	-	+	-	+
Rifampicin	-	+	-	+	-	+
CHO-WT	3.4	1.4	0.4	0.6	16	16
CHO-OATP1B1	1.5	1.0	6.9	0.8	16	17
CHO-OATP1B3	1.3	0.7	7.4	0.8	17	17
OATP1B1/WT ratio	0.4		16		1.0	
OATP1B3/WT ratio	0.4		17		1.1	

Conclusion

LDV is not a substrate of OATP1B1 or OATP1B3 in transfected CHO cells.

AD-256-2096: In vitro assessment of human liver cytochrome P450 inhibition potential of GS-5885

Introduction

In this study, the potential for GS-5885 to inhibit human cytochrome P450 (CYP) isoforms was assessed using isoform-specific probe substrates in human hepatic microsomal fractions.

Materials and Methods

Probe substrates were incubated with human liver microsomes and NADPH at 37°C in the presence of GS-5885 (up to 25 uM) or control inhibitors (Table 1). Drug concentrations were assessed by LC-MS/MS and were used to calculate IC₅₀ values. Assays were conducted (b) (4)

Table 1. CYP isoform-specific probe substrates and control inhibitors

CYP isoform	Probe substrate	Conc. (uM)	Inc. time (min)	Control inhibitor	Conc. range (uM)
CYP1A2	phenacetin	30	5	□-naphthoflavone	0-3
CYP2C8	paclitaxel	7.5	30	montelukast	0-3
CYP2C9	tolbutamide	120	60	sulfaphenazole	0-10
CYP2C19	S-mephenytoin	25	60	tranylcypromine	0-50
CYP2D6	dextromethorphan	5	30	quinidine	0-3
CYP3A	midazolam	2.5	5	ketoconazole	0-3
CYP3A	testosterone	50	5	ketoconazole	0-3

Results

GS-5885 IC₅₀ values were high for all CYP isoforms evaluated (range: 9.9 to >25 uM, Table 2). In contrast, control inhibitors had low IC₅₀ values, indicating potent inhibition.

Table 1: GS-5885 and control inhibitor IC₅₀ values on CYP isoform activity (source: Study Report Table 1)

Enzyme	Activity	Calculated IC ₅₀ (μM)	
		Control Inhibitor ^a	GS-5885
CYP1A2	Phenacetin O-deethylase	0.07	>25
CYP2C8	Paclitaxel 6α-hydroxylase	0.35	>25
CYP2C9	Tolbutamide 4-hydroxylase	0.83	>25
CYP2C19	S Mephenytoin 4'-hydroxylase	7.68	>25
CYP2D6	Dextromethorphan O-demethylase	0.03	>25
CYP3A	Midazolam 1'-hydroxylase	0.07	>25
	Testosterone 6β-hydroxylase	0.10	9.9

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

Conclusion

GS-5885 did not inhibit CYP1A2, CYP2C8, CYP2C9, CYP2C19, or CYP2D6 at concentrations up to 25 μM and was a weak inhibitor of CYP3A (IC₅₀: 9.9 μM). Although this result in a ratio of total C_{max} to IC₅₀ for CYP3A is approximately 0.1, the extent of plasma protein binding (>99%) makes it unlikely that CYP3A inhibition by GS-5885 will be clinically relevant.

AD-256-2133: In vitro assessment of human CYP2B6 inhibition potential of GS-5885

Introduction

In this study, the potential for GS-5885 to inhibit human cytochrome P450 isoform 2B6 (CYP2B6) was assessed using the probe substrate bupropion in human hepatic microsomal fractions.

Materials and Methods

Bupropion (110 μM) was incubated with human liver microsomes and NADPH at 37°C for 5 min in the presence of GS-5885 (0.1-25 μM) or the control inhibitor ticlopidine (0-25 μM). Drug concentrations were assessed by LC-MS/MS and were used to calculate IC₅₀ values. Assays were conducted (b) (4)

Results

The GS-5885 IC₅₀ value for CYP2B6 was greater than 25 μM, compared to an IC₅₀ of 1.27 for the CYP2B6 inhibitor ticlopidine (Table 1).

Table 1: GS-5885 and control inhibitor IC₅₀ values on CYP2B6 activity (source: Study Report Table 1)

Enzyme	Activity	Calculated IC ₅₀ (μM)	
		Control Inhibitor ^a	GS-5885
CYP2B6	Bupropion 4-hydroxylase	1.27	> 25

a Control Inhibitor: ticlopidine (0–25 μM)

Conclusion

GS-5885 did not inhibit CYP2B6 at concentrations up to 25 μM.

AD-256-2097: Induction of metabolizing enzymes by GS-5885

Introduction

In this study, the potential of GS-5885 to activate the aryl hydrocarbon receptor (AhR) and the pregnane X receptor (PXR) and potentiate induction of drug metabolizing enzymes was evaluated using the hematoma-derived cell lines DRE12.6 (expressing AhR and a luciferase reporter linked to enhancer regions of CYP3A4) and DPX2 (expressing PXR and a luciferase reporter linked to enhancer regions of CYP1A2).

Materials and Methods

DPX2 and DRE12.6 cells were plated in 96-well plates and allowed to recover for 24 h. Cells were incubated in 150 uL/well containing 0.5-20 uM b-naphthoflavone (AhR activator, DRE12.6 cells) or 10 uM rifampicin, mifepristone, or androstanol (PXR activators, DPX2 cells) for 24 h. Medium was replaced with 50 uL Bright-Glo™ luciferase substrate and fluorescence was measured after 5 min to measure reporter gene expression. Well conditions were assessed in triplicate and assays were conducted (b) (4)

Results

At concentrations up to 10 uM, GS-5885 did not activate AhR, while the positive control naphthoflavone activated AhR at higher concentrations (Table 1). At the highest concentration tested, GS-5885 increased CYP1A2 expression by about 71% of the increase observed after treatment with androstanol, a weak AhR activator (Table 1).

Table 1: Activation of PXR and AhR by GS-5885 and control compounds (source: Study Report Tables 2 and 3)

Fold induction over 0.1% DMSO ctrl	PXR (CYP1A2)				AhR (CYP3A4)	
	GS-5885	rifampicin	mifepristone	androstanol	GS-5885	<input type="checkbox"/> naphthoflavone
0.5						1.08
1	2.85				1.06	1.71
3	2.72				0.93	
5						5.33
10	4.08	27.32	15.55	5.76	0.79	10.35
20						7.67

Conclusion

At concentrations up to 10 uM, GS-5885 does not activate AhR and may be a weak activator of PXR. Induction of metabolizing enzymes regulated by PXR (including CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP3A, and UGT1A1) is possible at pharmacological concentrations of GS-5885 (1 uM or less), though unlikely due to the high degree of plasma protein binding. Note that activation of the constitutive androstane receptor (CAR, implicated in regulation of CYP2B6, CYP2C9, CYP2C19, CYP3A4, and UGT1A1) by GS-5885 was not evaluated in this study.

References

Sinz, Wallace, Sahi AAPS J. Jun 2008; 10(2): 391-400

AD-256-2146: Evaluation of induction potential of GS-5885 in cultured human hepatocytes

Introduction

In this study, the potential of GS-5885 to potentiate induction of drug transporters (P-gp) and metabolizing enzymes (CYP1A2, CYP2B6, CYP3A, CYP2C9, and UGT1A1) was evaluated using primary cultured human hepatocytes from three donors.

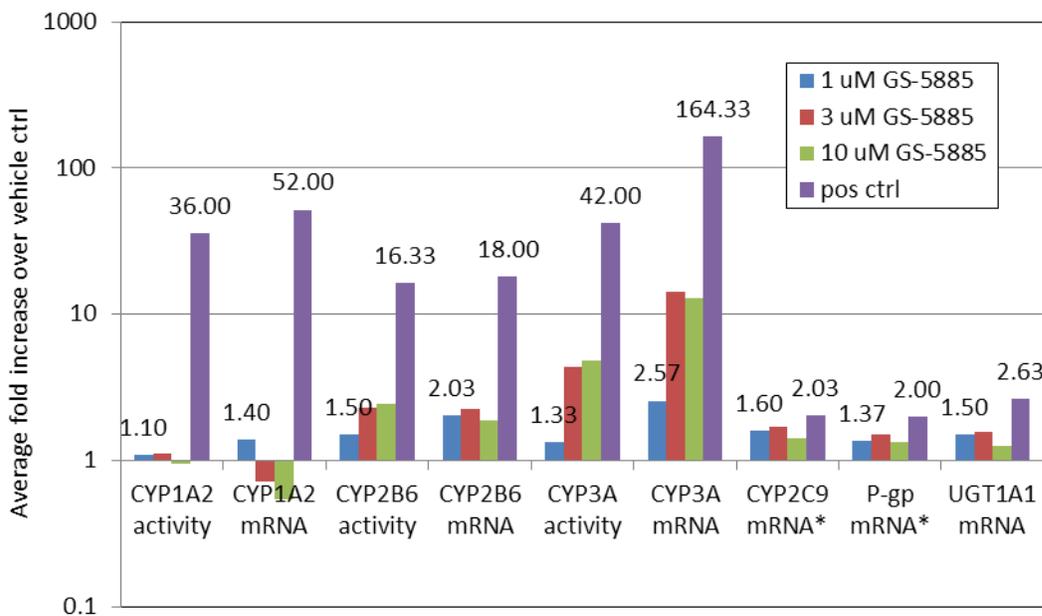
Materials and Methods

Cultured human hepatocytes were plated in collagen I-coated 24-well plates and incubated in 150 μ L/well containing 1, 3, or 10 μ M GS-5885 or positive control inducers (CYP1A2: 50 μ M omeprazole, CYP2B6 and P-gp: 1 mM phenobarbital, CYP2C9 and CYP3A4: 10 μ M rifampicin, UGT1A1: 20 μ M β -naphthoflavone) for three days. Induction of CYP1A2, CYP2B6, and CYP3A activity were measured using catalytic activity assays (probe substrates: 100 μ M phenacetin, 250 μ M bupropion, and 200 μ M testosterone, respectively) and LC-MS/MS quantitation and induction of CYP1A2, CYP2B6, CYP3A4, CYP2C9, P-gp, and UGT1A1 mRNA expression were assessed using real-time RT-PCR. Well conditions were assessed in triplicate and assays were conducted (b) (4)

Results

At concentrations up to 10 μ M, GS-5885 did not activate AhR, while the positive control β -naphthoflavone activated AhR at higher concentrations (Table 1). At the highest concentration tested, GS-5885 increased CYP1A2 expression by about 71% of the increase observed after treatment with androstanol, a weak AhR activator (Table 1).

Figure 1: Induction of CYP1A2, CYP2B6, CYP3A, CYP2C9, UGT1A1, and P-gp by GS-5885 and control compounds (mean values are displayed for 1 μ M GS-5885 and positive controls; asterisk indicates that data from one lot of hepatocytes was removed due to positive control induction <2-fold; source: Study Report Tables 6-14)



Conclusion

At concentrations up to 10 μM , GS-5885 was a weak inducer of CYP2B6 and CYP3A. At the pharmacologically relevant concentration (expected C_{max}) of 1 μM , GS-5885 increased mean CYP2B6 and CYP3A mRNA expression by 2.0- and 2.6-fold, respectively, compared to vehicle control. The increases in CYP2B6 and CYP3A mRNA expression are below the conservative cut-off of 4-fold induction (Fahmi et al. DMD 2010) and are not expected to result in clinically significant induction.

AD-256-2109: Effect of GS-5885 on the accumulation of model substrates in P-glycoprotein (Pgp), multidrug resistance associated protein 2 (MRP2) and breast cancer resistance protein (BCRP) overexpressing cells

Introduction

In this study, inhibition of efflux transport via P-gp, MRP2, and BCRP by GS-5885 was determined using overexpressing MDCKII cells.

Materials and Methods

MDCKII-WT, MDCKII-MDR1, MDCKII-MRP2, and MDCKII-BCRP cells were grown to confluence in black 96-well plates with clear bottoms. Intracellular accumulation of 10 μM calcein AM (P-gp and MRP2 substrate) or 10 μM Hoechst 33342 (BCRP substrate) with or without GS-5885 (concentration range: 1-1000 nM) was determined after 1, 2, or 3 h incubations (for MDCKII-MDR1, MDCKII-MRP2, and MDCKII-BCRP cells, respectively) at 37°C. Fluorescence of the cell lysate was determined. The percent inhibition was calculated by calculated the ratio of total fluorescence of transfected to wild-type cells in the presence (Ratio_i) or absence (Ratio_{Ni}) of GS-5885: $[(\text{Ratio}_i - \text{Ratio}_{Ni}) / (1 - \text{Ratio}_{Ni})] \times 100$.

Results

Dose-dependent inhibition of BCRP and P-gp transport was observed, with IC_{50} values <1 μM and approximately 1 μM , respectively (Tables 1 and 2). No dose-dependent inhibition of MRP2 efflux was observed (Table 3).

Table 1: GS-5885 inhibition of BCRP efflux of Hoechst 33342 in MDCKII- BCRP cells (source: Study Report Table 1)

Dosing (μM)	% Viability	% Inhibition
0.001	78.2	0.20
0.004	80.5	0.30
0.012	81.9	2.00
0.037	85.6	5.60
0.111	85.4	16.4
0.333	86.5	31.5
1.00	89.3	38.1

Values are the average of duplicate wells from one of two independent studies used to calculate the IC_{50} of > 1 μM .

Table 2: GS-5885 inhibition of P-gp efflux of calcein in MDCKII- MDR1 cells (source: Study Report Table 3)

Dosing (μM)	% Viability	% Inhibition
0.001	87.7	1.53
0.004	72.5	1.76
0.012	64.5	3.86
0.037	66.7	6.64
0.111	68.8	14.3
0.333	66.7	30.2
1.00	69.2	46.3

Values are the average of duplicate wells from one of two independent studies used to calculate the IC_{50} of $\geq 1 \mu\text{M}$.

Table 3: GS-5885 inhibition of MRP2 efflux of calcein in MDCKII- MRP2 cells (source: Study Report Table 2)

Dosing (μM)	% Viability	% Inhibition
0.001	110	1.50
0.004	95.6	4.80
0.012	83.8	4.60
0.037	91.8	5.20
0.111	103	4.00
0.333	106	6.30
1.00	100	2.30

Values are the average of duplicate wells from one of two independent studies used to calculate the IC_{50} of $\geq 1 \mu\text{M}$.

Conclusion

GS-5885 inhibited P-gp and BCRP efflux by 46.3% and 38.1%, respectively, at the anticipated GS-5885 C_{max} of 1 μM . Coadministration of GS-5885 with drugs that are substrates for P-gp and/or BCRP may lead to increased absorption of the coadministered drug.

AD-256-2132: In vitro assessment of human UGT1A1 inhibition potential of GS-5885

Introduction

In this study, inhibition of UGT1A1 catalytic activity by GS-5885 was determined using microsomal fractions from baculovirus-expressed human UGT1A1 insect cells (Supersomes™).

Materials and Methods

The UGT1A1 substrate estradiol (10 μM) was incubated with Supersomes™ (0.25 mg/mL protein), UDP-glucuronic acid (5 mM), and alamethicin (25 $\mu\text{g/mL}$) in the presence or absence of GS-5885 (concentration range: 0.4-100 μM) or the positive control inhibitor silybin (concentration range: 0-100 μM) for 30 min at 37°C. Concentrations of the UGT1A1-specific metabolite estradiol 3-glucuronide were assessed by LC-MS/MS and were used to determine the rate of metabolism. Assays were conducted (b) (4)

Results

The GS-5885 IC_{50} value for UGT1A1 was 7.95 μM , compared to an IC_{50} of 2.99 μM for the UGT1A1 inhibitor silybin (Table 1).

Table 1: GS-5885 and control inhibitor IC₅₀ values on UGT1A1 activity (source: Study Report Table 1)

Enzyme	Activity	Calculated IC ₅₀ (μM)	
		Control Inhibitor ^a	GS-5885
CYP1A1	Estradiol Glucuronidation	2.99	7.95

a Control Inhibitors: silybin (0–100 μM)

Conclusion

Although the ratio of the total C_{max} to IC₅₀ is greater than 0.1 (0.126), UGT1A1 inhibition by GS-5885 is not likely to be clinically relevant due to the extent of GS-5885 plasma protein binding (>99%).

AD-256-2134: In vitro assessment of GS-5885 inhibition of human OATP1B1 and OATP1B3

Introduction

In this study, inhibition of uptake transport via OATP1B1 or OATP1B3 by GS-5885 was determined using overexpressing CHO cells.

Materials and Methods

Wild-type CHO cells or CHO cells transfected with OATP1B1 or OATP1B3 were seeded in black 96-well plates with clear bottoms. Sodium butyrate (10 mM) was added to increase protein expression and the cells were grown to confluence. Intracellular accumulation of 2 μM Fluo-3 with or without GS-5885 (concentration range: 0.14 to 100 μM) was determined after a 1 h incubation at 37°C. Fluorescence of the cell lysate was determined. The percent inhibition was calculated using the following equation: $1 - [\text{OATP}_I - \text{WT}_{NI}] / (\text{OATP}_{NI} - \text{WT}_{NI}) * 100$, where OATP_I is the fluorescence in OATP-transfected cells in the presence of GS-5885 and OATP_{NI} and WT_{NI} are the fluorescence values of OATP-transfected and untransfected cells, respectively, in the absence of GS-5885.

Results

Dose-dependent inhibition of OATP1B1 and OATP1B3 transport was observed, with IC₅₀ values 3.5 μM and 6.5 μM, respectively (Table 1). The positive control rifampicin inhibited OATP1B1 and OATP1B3 with IC₅₀ values of 1.4 and 2.7 μM, respectively.

Table 1: GS-5885 and rifampicin inhibition of OATP1B1 and OATP1B3 uptake of Fluo-3 in CHO-transfected cells (source: Study Report Table 1)

Transporters	Uptake Transporters IC ₅₀ (μM)	
	OATP1B1	OATP1B3
GS-5885	3.5 ± 1.0	6.5 ± 2.8
Rifampicin	1.4 ± 0.3	2.7 ± 0.8

Conclusion

GS-5885 inhibited OATP1B1 and OATP1B3 efflux with IC_{50} values of 3.5 and 6.5 μ M, respectively. The ratios of the GS-5885 C_{max} to IC_{50} values for OATP1B1 and OATP1B3 are 0.29 and 0.15, respectively, suggesting that inhibition of these transporters is possible at pharmacological concentrations, though the high degree of plasma protein binding (>99%) makes inhibition unlikely.

AD-256-2140: In vitro inhibition studies of ledipasvir with human MRP4, BSEP, OAT1, OAT3, OCT2 and MAT1 transporters

Introduction

In this study, inhibition of transport via MRP4 (ABCC4), BSEP (ABCB11), OAT1 (SLC22A6), OAT3 (SLC22A8), OCT2 (SLC22A2), and MATE1 (SLC47A1) by GS-5885 was determined using overexpressing OAT1, OAT3, OCT2, or MATE1 cells or MRP4- or BSEP-containing membrane vesicles.

Materials and Methods

Vesicular transport inhibition assays – Membrane vesicles (50 μ g protein/well) made from Sf9 cells transfected with BSEP or HEK293 cells transfected with MRP4 were incubated with the probe substrates 2 μ M taurocholate or 0.2 μ M DHEAS, respectively, in the presence or absence of ATP. Ledipasvir (concentration range: 0.01-6 μ M) or positive control inhibitors 20 μ M cyclosporine A or 150 μ M MK571 were added. Reactions were started by the addition of 12 mM ATP and stopped after 5 min. The amount of substrate inside vesicles was determined by liquid scintillation counting. All assays were performed in duplicate.

Cellular transport inhibition assays – CHO cells transfected with OCT2, OAT1, or MATE1 or 293 FlpIn cells transfected with OAT3 were incubated with the probe substrates triethylamine (TEA), PAH, TEA, or estrone-3-sulfate, respectively, in the presence or absence of ledipasvir (concentration range: 0.01-6 μ M) or the positive control inhibitors verapamil, benzbromarone, quinidine, or probenecid, respectively. The amount of substrate in cell lysate was determined by liquid scintillation counting or fluorescence reading.

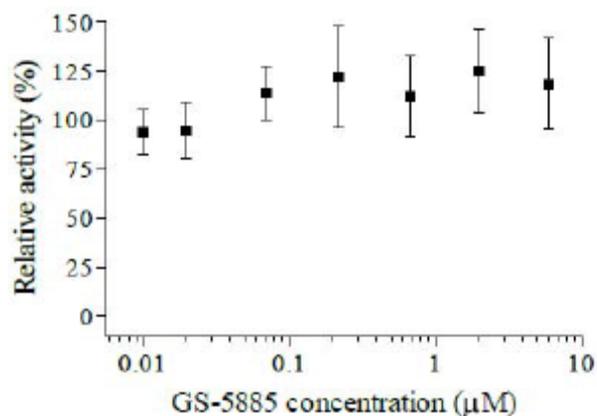
Results

Ledipasvir inhibited taurocholate transport by BSEP in membrane vesicles, with an IC_{50} value of approximately 6 μ M. Ledipasvir also inhibited TEA uptake by OCT2-transfected CHO cells, although inhibition was weak (13% inhibition at the highest ledipasvir concentration evaluated). Ledipasvir did not inhibit transport by MRP4, OAT1, OAT3, or MATE1. Ledipasvir appeared to increase MATE1 transport by approximately 20% incubation (Figure 1). The mechanism for the apparent activation is unknown. In the transport assay, cells were incubated with ledipasvir and TEA for 20 minutes, making it highly unlikely that induction (e.g. via nuclear receptor such as PXR) occurred in such a short time period. There are no reports of MATE1 activation by drugs in the literature at present.

Table 1: Effect of ledipasvir on BSEP, MRP4, OCT2, OAT1, OAT3, and MATE1 transport activity (source: Study Report Table 2)

Vesicular transport inhibition		
Transporter	Maximum inhibition at highest concentration tested, 6 μ M (% of control)	IC ₅₀ (μ M)
BSEP	51%	~ 6
MRP4	no inhibition	> 6
Uptake transporter inhibition		
Transporter	Maximum inhibition at highest concentration tested, 6 μ M (% of control)	IC ₅₀ (μ M)
OCT2	13%	> 6
OAT1	no inhibition	> 6
OAT3	no inhibition	> 6
MATE1	118% stimulation	> 6

Figure 1: Ledipasvir activation of MATE1 transport activity (source: Study Report Figure 4)



Conclusion

Ledipasvir inhibited BSEP transport activity with an IC₅₀ of approximately 6 μ M, giving a C_{max}/IC₅₀ ratio of 0.17. The potential for presystemic drug interactions mediated by BSEP exists; the high degree of plasma protein binding (>99%) makes drug interactions less likely when ledipasvir is in the systemic circulation. Ledipasvir was a weak inhibitor of OCT2 (13% inhibition in the presence of 6 μ M ledipasvir) and appeared to activate MATE1 (18% stimulation in the presence of 6 μ M ledipasvir). The clinical relevance of these relatively minor interactions is likely limited.

AD-256-2143: In vitro inhibition and substrate studies of ledipasvir with human OCT1 transporter

Introduction

In this study, interactions between ledipasvir and the uptake transporter OCT1 (SLC22A1) were evaluated using overexpressing OCT1 cells and the probe substrate triethylamine (TEA).

Materials and Methods

For the inhibition assay, CHO cells transfected with OCT1 were incubated with in the presence or absence of ledipasvir (concentration range: 0.01-6 μM) or the positive control inhibitor verapamil (100 μM). For the substrate assay, CHO cells transfected with OCT1 or empty vector were incubated with ledipasvir (1 and 5 μM) for 2 and 20 min at 37°C. The amount of substrate in cell lysate was determined by liquid scintillation counting or fluorescence reading.

Results

Ledipasvir did not inhibit TEA uptake by OCT1 at concentrations up to 6 μM (Figure 1). Ledipasvir was not a substrate of OCT1 as TEA accumulation in OCT1-transfected cells was less than two-fold that observed in control cells (Table 1).

Figure 1: Ledipasvir activation of MATE1 transport activity (source: Study Report Figure 4)

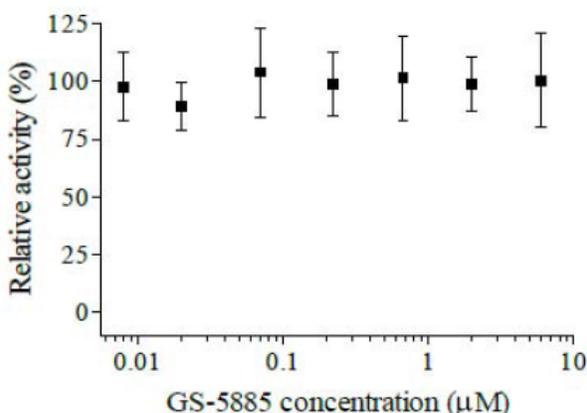


Table 1: OCT1-mediated transport of ledipasvir (source: Study Report Table 4)

Compound	Concentration (μM)	Incubation time (min)	Fold Accumulation
Ledipasvir	1	2	1.26
		20	1.25
	5	2	1.33
		20	1.24

Conclusion

Ledipasvir was not transported by or an inhibitor of the OCT1 uptake transporter in transfected CHO cells using the probe substrate TEA.

AD-337-2001: Effect of sofosbuvir and ledipasvir on the bidirectional permeability of tenofovir disoproxil fumarate through Caco-2 monolayers

Introduction

In this study, the effect of ledipasvir (LDV) and sofosbuvir (SOF) on the bidirectional permeability of tenofovir disoproxil fumarate (TDF) was determined using human colon carcinoma (Caco-2) cells and the P-gp and BCRP inhibitor ritonavir (RTV).

Materials and Methods

Caco-2 cells were grown to confluence for at least 21 days on 12-well Transwell plates (Corning). Forward (apical to basolateral, A to B) and reverse (basolateral to apical, B to A) permeability of TDF (50 μM) was determined over 120 minutes. The efflux ratio was calculated as the average reverse apparent permeability (P_{app}) divided by the average forward P_{app} ($P_{\text{app,B:A}}/P_{\text{app,A:B}}$). Digoxin served as the transporter positive control. Experimental conditions were conducted in duplicate and samples were analyzed by LC-MS/MS.

Results

At a concentration of 1 μM , TDF had medium forward permeability with an efflux ratio of 18, indicating significant efflux transport (Table 1). The positive control inhibitor RTV reduced the efflux ratio to 1.3, suggesting involvement of P-gp and/or BCRP in TDF transport. At concentrations of 1, 5, and 25 μM , LDV also reduced the efflux ratio (range: 1.5-2.5) and at a concentration of 1000 μM , SOF reduced the efflux ratio to 2.1.

The increase in forward TDF recovery (from 54% to 80%) in the presence of SOF suggests that SOF may inhibit metabolism of TDF, possibly via shared routes of metabolism as both are nucleotide-based drugs.

Table 1: Effect of LDV, SOF, or RTV on the bidirectional permeability of TDF across Caco-2 monolayers (source: Study Report Table 1)

Assay #	Inhibitor	Direction	Initial Conc. (μM)	Recovery (%)	P_{app} (10^{-6} cm/s)			Efflux Ratio
					R1	R2	Avg	
13GILEP4	--	Cell-Free	35.2	79	18.6	---	18.6	18
		Forward	34.4	54	0.56	0.71	0.63	
		Reverse	38.7	77	11.5	11.0	11.3	
	LDV (1 μM)	Forward	37.3	56	2.11	2.33	2.22	2.5
		Reverse	37.9	68	5.24	5.92	5.58	
	LDV (5 μM)	Forward	37.9	54	3.20	2.12	2.66	1.6
		Reverse	41.1	66	3.69	4.73	4.21	
	LDV (25 μM)	Forward	35.6	53	2.59	2.67	2.63	1.5
		Reverse	38.8	69	3.82	3.91	3.87	
	SOF (1000 μM)	Forward	42.9	80	5.40	5.23	5.31	2.1
		Reverse	40.5	83	10.7	11.9	11.3	
	RTV (20 μM)	Forward	47.7	58	1.53	1.83	1.68	1.3
		Reverse	54.4	85	2.15	2.38	2.26	

Conclusion

Ledipasvir (concentration range: 1-25 uM) inhibited P-gp- and/or BCRP-mediated transcellular transport of TDF in Caco-2 cells, reducing the efflux ratio from 18 without ledipasvir to 1.5-2.5 with ledipasvir. In addition, 1000 uM sofosbuvir inhibited P-gp- and/or BCRP-mediated transcellular transport of TDF, resulting in an efflux ratio from 2.1. Recovery of TDF in the forward direction was increased in the presence of sofosbuvir, suggesting that TDF metabolism may be inhibited by sofosbuvir; this is not surprising, as both are nucleotide drugs and may compete for metabolism and activation. The results of this study indicate that both ledipasvir and sofosbuvir have the potential to increase intestinal absorption of TDF or other P-gp and BCRP substrates drugs that are coadministered with ledipasvir and/or sofosbuvir.

4.2.6 Pharmacometric Review (Jeff)

Application Number	NDA 205834
Submission Number (Date)	February 10, 2014
Drug Name	Ledipasvir/Sofosbuvir
Proposed Indication	Treatment of genotype 1 chronic hepatitis C (CHC) in adults (b) (4)
Clinical Division	DAVP
Primary CP Reviewer	Jenny Zheng, Ph.D.
Primary PM Reviewer	Jeffrey Florian, Ph.D.
Secondary CP Reviewer	Shirley Seo, Ph.D.
Secondary PM Reviewer	Yaning Wang, Ph.D.
Sponsor	Gilead Sciences

Results of Sponsor's Analysis

Introduction

Population PK analyses of SOF, GS-331007, and LDV were performed using intensive and sparse PK samples collected in Phase 1, 2, and 3 studies in healthy and HCV-infected subjects. Intensive sampling entailed serial blood sampling at defined time points, and sparse sampling (single sample) entailed blood collection at all study visits. In the population PK model-building process, various structural, statistical, and error models were tested to determine the base model.

The population PK model development dataset for SOF included measureable PK observations from a total of 1455 subjects (209 healthy subjects and 1246 subjects with HCV infection) across 10 clinical studies. The population PK model development dataset for GS-331007 included measureable PK observations from a total of 1966 subjects (207 healthy subjects and 1759 subjects with HCV infection) across 10 clinical studies. The population PK model development dataset for LDV included measureable PK observations from a total of 2150 subjects (391 healthy subjects and 1759 subjects with HCV infection) across 14 clinical studies. A summary of the data used in these analyses are provided below in **Table 1**.

Table 1: Studies used in the population pharmacokinetic analysis of SOF, GS-331007, and LDV

Study	Population PK Models in Which Data Were Used			Population	Phase	Sampling (Intensive/Sparse)
	SOF	GS-331007	LDV			
GS-US-334-0101	√	√	√	Healthy	1	Intensive
GS-US-334-0111	√	√	√	Healthy	1	Intensive
GS-US-334-0146			√	Healthy	1	Intensive
GS-US-344-0101			√	Healthy	1	Intensive
GS-US-344-0102	√	√	√	Healthy	1	Intensive
GS-US-344-0108			√	Healthy	1	Intensive
GS-US-344-0109			√	Healthy	1	Intensive
GS-US-337-0101	√	√	√	Healthy	1	Intensive
GS-US-337-0127	√	√	√	Healthy	1	Intensive
GS-US-337-0118	√	√	√	HCV-Infected	2	Intensive + Sparse
P7977-0523	√	√	√	HCV-Infected	2a	Intensive + Sparse
GS-US-337-0102	√	√	√	HCV-Infected	3	Intensive substudy + Sparse
GS-US-337-0108	√	√	√	HCV-Infected	3	Sparse
GS-US-337-0109 ^a	√	√	√	HCV-Infected	3	Intensive substudy + Sparse

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N ^a	Number of Subjects with Evaluable Population PK Exposure Parameters		
	SOF	GS-331007	LDV
Healthy Subjects	209	209	391
HCV-Infected Subjects	1576	2147	2147
Total	1785	2356	2538

Sponsors Summary of Clinical Pharmacology, pg 172

SOF Population PK Summary

The final population PK model for SOF best described the plasma concentration data with a 1-compartment PK model with first-order absorption, first-order elimination from the central compartment, and an absorption lag time, with interindividual variability terms on oral clearance (CL/F), first-order absorption rate constant (K_a), and apparent central volume (V_c/F). Covariate analysis indicated statistically significant effects of HCV infection status (i.e., healthy subjects versus HCV-infected subjects) and CL_{cr} on CL/F, and the effect of meal status (fed versus fasted) on K_a.

Values of SOF CL/F, V_c/F, and K_a for the typical HCV-infected subject with CL_{cr} of 106 mL/min under fasted conditions were estimated to be 305 L/h, 299 L, and 3.49 1/h respectively (Table 2).

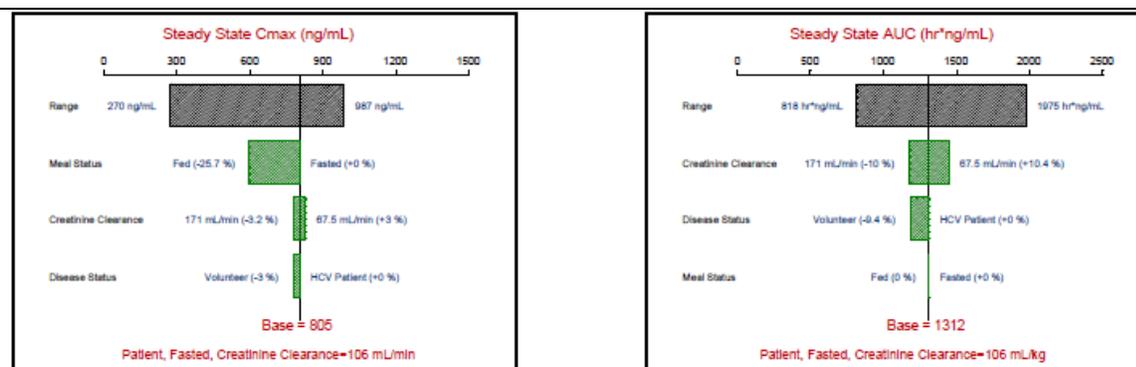
Table 2: Key SOF Population PK Parameter Values and Covariate Effects for Representative Subjects Administered

PK Parameters and Baseline Covariates		Baseline Covariate Value	Estimate	Change from Typical (%)	Inter-individual Variability (%)
Typical CL/F (CLCR=106 mL/min, HCV-infected, L/hr)			304.9	—	40.2
CLCR (mL/min)	5 th Percentile	67.5	292.1	-4.2	—
	95 th Percentile	175	319.8	4.9	—
Healthy volunteer			336.5	10.4	—
Typical V _c /F (L)			298.9	—	33.3
Typical k _a (hr ⁻¹)			3.49	—	115.8
Food	Fasted		3.49	—	—
	Fed		1.55	—	—
Typical lag time (hr)			0.188	—	—
Residual variability as coefficient of variation (%)			95.3	—	—

Sponsors SOF Population PK Report, pg 13

Sensitivity analyses suggested that the magnitude of HCV infection status, CL_{cr}, and meal status on the steady-state AUC_{tau} and C_{max} of SOF was mild (C_{max}: < 26%; AUC_{tau}: < 10.5%) for HCV-infected subjects with extreme covariate values (5th and 95th percentiles) relative to the typical HCV-infected subject. As such, these covariates are not considered to have a clinically meaningful impact on SOF exposure (Figure 1).

Figure 2: Sensitivity plot comparing the effect of covariates on SOF steady state exposure (AUC, C_{min} and C_{max})



Sponsors SOF Population PK Report, pg 14

GS-331007 Population PK Summary

The final population PK model for GS-331007 best described the plasma concentration data with a 2-compartment PK model with first-order absorption, first-order elimination from the central

compartment, and an absorption lag time, with interindividual variability terms on CL/F, K_a , V_c/F , apparent peripheral volume (V_p/F), and apparent intercompartmental clearance (Q/F). Covariate analysis indicated statistically significant effects of CL_{cr} , sex, RBV usage, and race on CL/F, effects of CL_{cr} , RBV usage, and HCV infection status on V_c/F , effects of HCV infection status and meal status (fed versus fasted) on K_a , and effects of meal status on relative bioavailability (F1).

Values of GS-331007 CL/F, V_c/F , V_p/F , Q/F, and K_a for the typical white male HCV-infected subject with CL_{cr} of 106 mL/min under fasted conditions without RBV usage were estimated to be 31.8 L/h, 534 L, 788 L, 51.9 L/h, and 0.32 1/h, respectively (Table 3).

Table 3: Key GS-331007 Population PK Parameter Values and Covariate Effects for Representative Subjects Administered

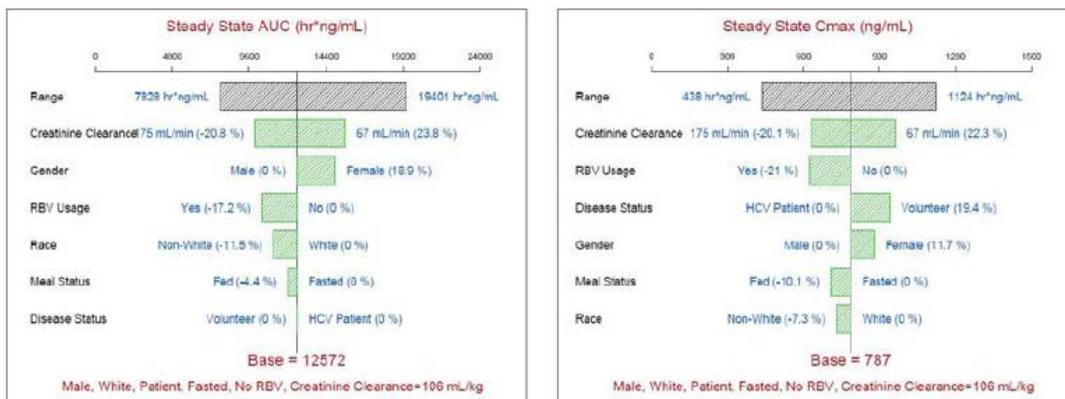
PK Parameters and Baseline Covariates		Baseline Covariate Value	Estimate	Change from Typical (%)	Inter-individual Variability (%)
Typical CL/F ($CL_{CR}=106$ mL/min, white, male, without RBV, L/hr)			31.8	--	22.9
CLCR (mL/min)	5 th Percentile	67	29.0	-8.8	--
	95 th Percentile	171	35.1	10.2	--
Female			26.8	-15.7	
With RBV			38.4	20.8	
Non-White			35.9	12.9	
Typical V_c/F ($CL_{CR}=106$ mL/min, Healthy subject, without RBV, L)			192	--	53.1
HCV-Infected subject			534	178	--
With RBV			269	40.1	
CLCR (mL/min)	5 th Percentile	67	177	-7.8	--
	95 th Percentile	171	211	9.9	--
Typical Q/F (L/hr)			51.9	--	47.5
Typical V_p/F (L)			788	--	32.9
Typical K_a (HV, fasted, hr ⁻¹)			0.320	--	28.7
Patient			0.608	90.0	
Fed			0.225	29.7	
Typical lag time (hr)			0.273	--	--
Relative Bioavailability, Fed (Fasted = 1)			0.956	4.40	--
Residual variability as coefficient of variation (%) – HCV Patient			23.3	--	--
Residual variability as coefficient of variation (%) – Healthy volunteer			18.0	--	--

Sponsors GS-331007 Population PK Report, pg 13

Sensitivity analyses suggested that the effects of covariates other than CrCL are relatively small and do not affect GS-331007 exposure in a clinically meaningful manner when compared to the range of exposures observed in the 5th to 95th percentiles of the population. As such, these covariates are not considered to have a clinically meaningful impact on GS-331007 exposure. CrCL was inversely correlated with GS-331007 AUC_{tau} and C_{max} . GS-331007 AUC_{tau} and C_{max} exhibited a mean difference of 31% and 28%, respectively, between the midpoints of the lowest and highest quartiles of CrCL. Exposure-response analyses did not identify any relationships between GS-331007 exposures in the Phase III trials and efficacy or safety measures. As such,

the impact of CrCL on GS-331007 exposure in subjects with mild or normal renal impairment is not considered clinically relevant nor does it necessitate any dose adjustments (Figure 2).

Figure 3: Sensitivity plot comparing the effect of covariates on GS-331007 steady state exposure (AUC, Cmin and Cmax)



Sponsors GS-331007 Population PK Report, pg 14

LDV Population PK Summary

The final population PK model for LDV best described the plasma concentration data with a 2-compartment PK model with first-order absorption, first-order elimination from the central compartment, and an absorption lag time, with interindividual variability terms on CL/F, K_a , V_c/F , and V_p/F . Covariate analysis indicated statistically significant effects of sex, body weight, RBV usage, and HCV infection status on CL/F; effects of body weight on V_c/F ; and effects of HCV infection status (healthy versus treatment-naïve versus treatment-experienced) on relative bioavailability (F1).

Values of LDV CL/F, V_c/F , V_p/F , Q/F, and K_a for the typical male treatment-naïve HCV-infected subject weighing 80 kg without coadministration of RBV were estimated to be 13.1 L/h, 399 L, 620 L, 28.5 L/h, and 0.326 1/h, respectively (Table 4).

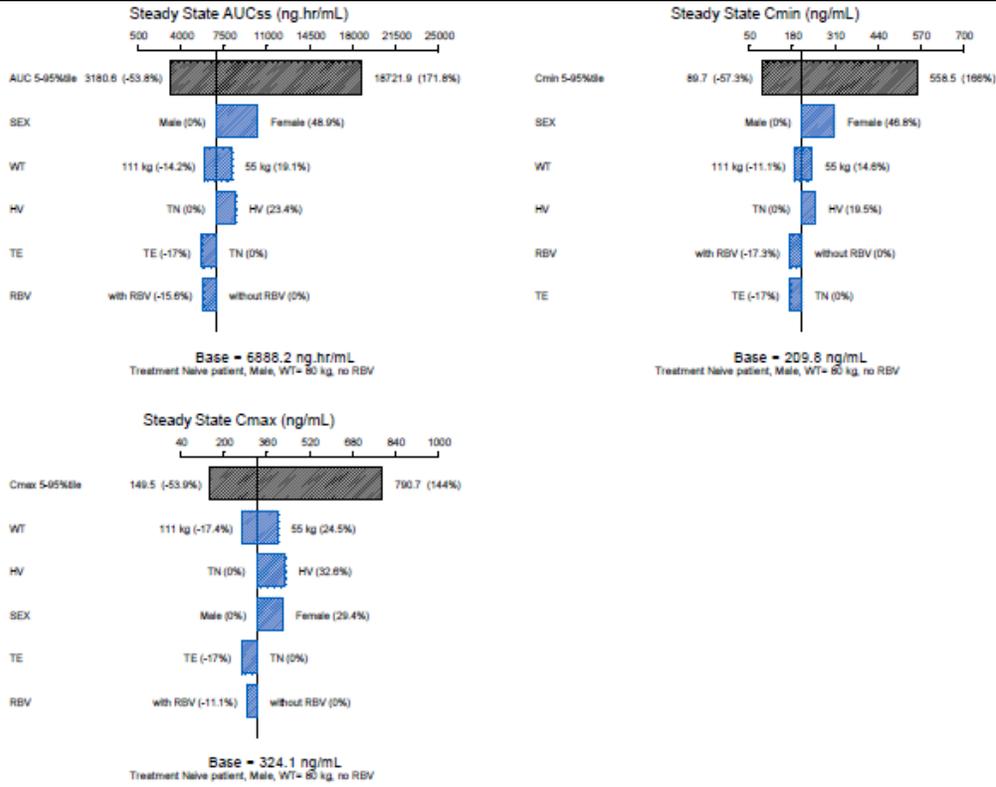
Table 4: Key LDV Population PK Parameter Values and Covariate Effects for Representative Subjects

PK Parameters and Baseline Covariates		Baseline Covariate Value	Estimate	Change from Typical (%)	Inter-Individual Variability
Typical CL (L/hr, Male, WT 80 kg, TN and no RBV)			13.1	—	47.6%
<i>Female</i>			8.78	-33	
<i>WT (kg)</i>	5 th Percentile	55	11	-16	
	95 th Percentile	111	15.2	16	
<i>With RBV</i>			15.5	18.3	
<i>Healthy Volunteer</i>			16.6	26.7	
Typical Vc (L, WT 80 kg)			399	—	56%
<i>WT (kg)</i>	5 th Percentile	55	257	-35.6	
	95 th Percentile	111	588	47.4	
Typical Q (L/hr)			28.5	-	
Typical Vp (L)			620	-	78%
Typical ka (1/hr)			0.326	-	45.9%
Typical lag time (hr)			0.442	-	
Relative Bioavailability, F1 (TN)			1.00	-	
<i>Disease status</i>	<i>Healthy volunteer</i>		1.57	57	
	<i>Treatment experienced HCV-Infected subject</i>		0.83	-17	
Residual variability as coefficient of variation (%)			6.2	-	

Sponsors LDV Population PK Report, pg 11

Typical values of F1 were determined to be 1.00, 0.83, and 1.57 for treatment-naive, treatment-experienced, and healthy subjects respectively. Sensitivity analyses suggested that the magnitude of effects of sex, body weight, disease status, and RBV usage on LDV steady-state AUC, C_{max}, and C_{min} was AUC: < 48.9%; C_{max}: < 32.6%; C_{min}: < 46.8% for subjects with extreme covariate values (5th and 95th percentiles) relative to the typical subject. Ledipasvir AUC_{tau}, C_{max}, and C_{tau} were approximately 77%, 58%, and 75% higher in female subjects compared with male subjects. Ledipasvir AUC_{tau}, C_{max}, and C_{tau} exhibited mean differences of approximately 29%, 31%, and 25%, respectively, between the midpoints of the lower and highest quartiles of BMI. The geometric mean AUC_{tau}, C_{max}, and C_{tau} for LDV were approximately 20%, 18%, and 20% lower, respectively, in treatment-experienced subjects relative to treatment-naive subjects (Figure 3).

Figure 4: Sensitivity plot comparing the effect of covariates on LDV steady state exposure (AUC, Cmin and Cmax)



Sponsors LDV Population PK Report, pg 12

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/s/

HUIMIN ZHENG
07/10/2014

LESLIE W CHINN
07/10/2014

JEFFRY FLORIAN
07/10/2014

YANING WANG
07/10/2014

SHIRLEY K SEO
07/10/2014

BIOPHARMACEUTICS REVIEW			
Office of New Drug Quality Assessment			
Application No.:	NDA 205-834 (000)	Reviewer: Sandra Suarez Sharp, Ph.D.	
Division:	DAP		
Applicant:	Gilead Sciences, Inc.	Team Leader: Angelica Dorantes, Ph.D.	
Trade Name:	-----	Acting Biopharmaceutics Supervisor: Richard Lostritto, Ph.D.	
Generic Name:	Sofosbuvir/Ledipasvir Fixed-Dose Combination Immediate Release Tablets	Date Assigned:	Feb 10, 2014
Indication:	Treatment of chronic hepatitis C (CHC) genotype 1 infection in adults.	Date of Review:	July 08, 2014
Formulation/strength	IR Tablets 400 mg/90 mg tablets (SOF/LDV FDC).		
Route of Administration	Oral		
SUBMISSIONS REVIEWED IN THIS DOCUMENT			
Submission Dates		Date of informal/Formal Consult	Primary Review Due in DARRTS
01/29/14 04/25/14 06/30/14		Feb 10, 2014	July 10, 2014
Type of Submission:	Original NDA (Priority Review)		
Key review points	<ol style="list-style-type: none"> 1. Dissolution method and acceptance criteria 2. Dissolution testing as a tool to monitor for solid state 3. Bridging Across Phases of Drug Development 4. Role of dissolution on supporting several drug product specifications 		

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D.1 BIOWAIVERS

16. Is there a waiver request of in vivo BE data (Biowaiver)? If yes, what is/are the purpose/s of the biowaiver request/s? What data support the biowaiver request/s?
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18. Is there any in vitro alcohol dose-dumping information submitted? What data are provided to support the Applicant's claim (e.g. lack of dose-dumping in the presence of alcohol)?

D.2 SURROGATES IN LIEU OF DISSOLUTION

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19. Are there any manufacturing parameters (e.g. disintegration, drug substance particle size, etc.) being proposed as surrogates in lieu of dissolution testing? What data are available to support the approval of the proposed surrogate test?

D.3 DISSOLUTION AND QBD

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20. Does the application contain QbD elements? If yes, is dissolution identified as a CQA for defining design space?
21. Was dissolution included in the DoE? What raw materials and process variables are identified as having an impact on dissolution? What is the risk assessment been performed to evaluate the criticality of dissolution?
22. What biopharmaceutics information is available to support the clinical relevance of the proposed design space?
23. Is there any dissolution model information submitted as part of QbD implementation? What is the regulatory application of the dissolution model in the submission? What data are provided to support the acceptability of the dissolution model?

BIOPHARMACEUTICS ASSESSMENT

I) SUMMARY OF BIOPHARMACEUTICS FINDINGS

Gilead is seeking approval of ledipasvir (LDV, GS-5885) and sofosbuvir (SOF, GS-7977) as an oral fixed-dose combination (FDC) tablet (SOF/LDV 90 mg/400 mg film-coated tablets) for the treatment of chronic genotype 1 hepatitis C virus (HCV) infection. LDV/SOF was granted Breakthrough Therapy Designation.

Ledipasvir is

(b) (4)

According to the Applicant, data collected on both the LDV (b) (4) and SOF/LDV tablets during clinical development demonstrate (b) (4)

The Applicant believes that dissolution test is

(b) (4)

The SOF/LDV tablet formulation demonstrated similar PK performance to the 2 coadministered single-agent tablets, LDV (b) (4) 90-mg strength and SOF tablet 400-mg strength in Study GS-US-337-0101. The SOF/LDV (b) (4) tablet formulation was subsequently used in all pivotal Phase 3 clinical studies and is the (b) (4) formulation used in all primary and registration stability batches and is the formulation proposed for the commercial drug product.

This review focuses on the evaluation of:

- 1) The acceptability of the dissolution method and acceptance criterion;
- 2) The role of dissolution as a tool to monitor the solid state at release and stability;
- 3) The data supporting appropriate bridging across the phases of drug development; and
- 4) The use of dissolution to support the drug product specification ranges.

1) Dissolution Method and Acceptance Criterion:

The following dissolution method and dissolution acceptance criterion have been found acceptable for both components of the proposed drug product (refer to submission dated June 30, 2014).

USP Apparatus	Spindle Rotation	Medium Volume	Temperature	Medium	Recommended Acceptance Criterion
II	75 rpm	900 mL	37°C	10 mM potassium phosphate, pH 6.0 with 1.5% polysorbate 80 and 0.0075 mg/mL BHT	Q= (b) (4) % in 20 min

The Applicant submitted adequate/sufficient information to support the discriminating ability of the dissolution method. The setting of the dissolution acceptance criterion was based on the mean dissolution profiles of pivotal clinical (BE batches) and stability batches.

2) The Role of Dissolution as a Tool to Monitor for LDV (b) (4)

The Applicant originally proposed the use of dissolution testing as a tool (b) (4)

The data submitted in the original NDA submission and during the review cycle demonstrated the capability of the method to detect for (b) (4) content as low as (b) (4); however, the recommended acceptance criterion for dissolution of Q= (b) (4) % would allow up to about (b) (4) LDV in some tablets when testing is conducted at (b) (4). Therefore, the following recommendations were conveyed to the Applicant during the review cycle:

- Provide information on the effect of (b) (4) content on the systemic exposure of LDV in humans, if available.
- Alternatively, implement as the trigger limit for in lab testing (b) (4) or other analytical methodology, the following additional dissolution acceptance criterion as the quality control limit for LDV (b) (4) content:

Mean dissolution of < (b) (4) % in 20 minutes; Stage (b) (4)

In response to this request, on June 30, 2014, the Applicant stated (b) (4)

The Applicant proposed the following alternative to a dissolution-based trigger in the drug product specification:

- 1) to add a test for (b) (4) LDV using an (b) (4) method to the stability protocol for the first three commercial batches to further demonstrate that LDV

(b) (4)
The testing for (b) (4) LDV will be performed at the initial time point, at 6 months under accelerated storage, and annually upon long-term storage.

According to the Applicant, the control strategies of (b) (4) testing at LDV (b) (4) and dissolution testing for SOF/LDV tablets provide assurance (b) (4)

However, because of the high risk (antiviral product for which BA is highly impacted (b) (4)); the following comments were conveyed to the Applicant on Jul 8, 2014.

1. Given that the dissolution specifications (method and acceptance criterion) would allow up to about (b) (4) ledipasvir in some tablets when testing is conducted at (b) (4) (i.e., at Q-(b) (4) %), we recommend that additional monitoring be added to the control strategy for (b) (4) content. We recommend adding one of these three approaches:
 - a) Test all tablet batches at release, and annually on stability, using the (b) (4) method until the end-to-end studies have reached the maximum expiry periods that are desired for the ledipasvir (b) (4) and the tablets.
 - b) Add (b) (4) testing of tablets when dissolution testing at (b) (4) is needed at release or on stability.
 - c) Add (b) (4) testing of tablets when the mean percent dissolved is less than (b) (4) % at Stage (b) (4) at release or on stability.

Since by the current GRMP date, the above issue has not yet been resolved with the Applicant. After their response is received, evaluated, and agreed on, an Addendum to this original review will be written with a recommendation on the measures that need to be implemented for the monitoring of (b) (4) ledipasvir.

2) Appropriate Bridging Across Phases of Drug Development

There were some major process and formulation changes implemented to the Phase 1 and Phase 2 clinical trial formulations. These changes are supported by the result of several BA studies linking the early formulations to the to-be-marketed formulation as described in formulation development section. These studies have been reviewed by OCP. The SOF/LDV tablet was developed prior to the initiation of Phase 3 clinical studies using SOF (b) (4) drug substance and LDV (b) (4) to support Phase 3 clinical studies. The tablet contains 90 mg of LDV and 400 mg of crystalline SOF (b) (4). According to the Applicant, the SOF/LDV tablet formulation demonstrated similar PK performance to the 2 coadministered single-agent tablets; LDV (b) (4) 90-mg strength and SOF tablet 400-mg strength in Study GS-US-337-0101 (refer to OCP review by Dr. Zheng).

There are two manufacturing sites being proposed, (b) (4) and Gilead in Ireland. Dissolution profile comparisons were submitted in

response to the FDA's request to bridge between the two manufacturing site used. The f_2 similarity values were above 50 indicating that the batches manufactured at either site have similar in vitro and in vivo performance.

3) The Role of Dissolution in Supporting Several Drug Product Specifications

The quality attributes of sofosbuvir drug substance, ledipasvir drug substance, (b) (4) were assessed for potential impact on the SOF/LDV tablet dissolution CQA. In addition, the process parameters for unit operations assessed as high risk of impact to the SOF/LDV tablet dissolution CQA were studied during development to establish PARs. Material attributes and process parameters were not formally identified as critical; however, development studies showed that (b) (4) have significant impacts on SOF/LDV tablet dissolution. Also, SOF particle size affects dissolution of the SOF component in the final product.

Comment to the CMC Review Team

The proposed specifications for film coating weight (b) (4) content (b) (4) and the ranges tested in bulk density ((b) (4) g/mL) are supported by the dissolution data; however, this Reviewer has the following recommendation:

1. *The recommended dissolution acceptance criterion of $Q = \frac{(b)(4)}{(4)}\%$ in 20 min will not be able to reject for batches (b) (4). Specifically, the proposed SOF particle size specification of NMI (b) (4) microns will not be able to reject batch DK1206B, which failed similarity testing. Therefore, it is recommended (b) (4) and a specification for drug substance particle size be implemented to ensure consistent product quality.*

II) RECOMMENDATION

At this time of the review process (GRMP date), ONDQA-Biopharmaceutics cannot provide a recommendation because of the pending resolution on the Applicant's agreement to monitor for the (b) (4) content of the LDV component. After this issue is resolved, an Addendum to this original review will be written including the Biopharmaceutics recommendation for approval of NDA 205-834 for Sofosbuvir/Ledipasvir Fixed-Dose Combination Tablets, 400 mg/90 mg.

Sandra Suarez Sharp, Ph. D.
Biopharmaceutics Reviewer
Office of New Drug Quality Assessment

Angelica Dorantes, Ph.D.
Biopharmaceutics Team Leader
Office of New Drug Quality Assessment

III) QUESTION BASED REVIEW APPROACH

A) GENERAL ATTRIBUTES

1. *What are the highlights of the chemistry and physico-chemical properties of the drug substance (e.g. solubility) and formulation of the drug product?*

Drug Substance

Ledipasvir

The aqueous solubility of ledipasvir (LDV) is pH-dependent. (b) (4)



LDV (b) (4) is produced (b) (4)



(b) (4) As such, the properties of LDV (b) (4) are important to the development of the drug product.



Sofosbuvir

(b) (4) crystalline sofosbuvir (b) (4) is the commercial sofosbuvir drug substance. Sofosbuvir drug substance may contain (b) (4)





Drug Product

Sofosbuvir (SOF)/ledipasvir (LDV) fixed-dose combination tablets (SOF/LDV tablets) are an immediate-release tablet dosage form containing 400 mg of sofosbuvir and 90 mg of ledipasvir. SOF/LDV tablets are orange, diamond-shaped, ^{(b) (4)} film-coated tablets with “GSI” debossed on one side and “7985” on the other side. The quantitative composition of SOF/LDV tablets is listed in Table 2. SOF/LDV tablets contain 40% (400 mg/tablet) of sofosbuvir and 9% (90 mg/tablet) of ledipasvir. The tablet formulation utilizes LDV ^{(b) (4)}



Table 2. Quantitative Composition of Sofosbuvir/Ledipasvir Tablets

Component	Composition (% w/w)	Unit Formula (mg/tablet)	Reference to 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)	(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.	
Colloidal Silicon Dioxide			NF, Ph. Eur.	
Magnesium Stearate			NF, Ph. Eur.	
(b) (4)			NF, Ph. Eur.	
(b) (4)			NF, Ph. Eur.	
(b) (4)			NF, Ph. Eur.	
(b) (4)	--			
(b) (4)	In-house			
(b) (4)	USP, Ph. Eur.			



2. Is there any information on BCS classification? What claim did the applicant make based on BCS classification? What data are available to support this claim?

(b) (4) ledipasvir free base is moderately hygroscopic, with approximately (b) (4) weight gain from 0 to 90% RH at room temperature. Both sofosbuvir and ledipasvir

exhibits low solubility (dose solubility volume of greater than 250 mL) and high apparent permeability. They are thus considered to be a BCS Class 2 compounds.

B) DISSOLUTION INFORMATION

B1) Dissolution Method

3. What is the proposed dissolution method?

The dissolution method proposed as a quality control tool for SOF/LDV IR Tablets is summarized below:

USP Apparatus	Spindle Rotation	Medium Volume	Temperature	Medium
II	75 rpm	900 mL	37°C	10 mM potassium phosphate, pH 6.0 with 1.5% polysorbate 80 and 0.0075 mg/mL BHT

4. What data are provided to support the adequacy of the proposed dissolution method (e.g medium, apparatus selection, etc.)?

Dissolution Method Development

The dissolution method was evaluated to determine the effect varying dissolution parameters would have on the *in vitro* drug release (for more detail refer to pdm-1586 at <\\cdsesub1\evsprod\NDA205834\0000\m3\32-body-data\32p-drug-prod\ldv-sof-tablet\32p5-contr-drug-prod\32p52-analyt-proc>). The following method parameters were evaluated.

- **Dissolution media pH and composition.** The table below summarizes the media tested:

pH (Media)	Solubility (mg/mL)
(b) (4)	

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C) DRUG PRODUCT FORMULATION DEVELOPMENT AND BRIDGING ACROSS PHASES

13. What are the highlights of the drug product formulation development?

Figure 11 gives a Schematic Overview on the LDV/SOF Formulation Development and the data provided to bridge across stages. All the BE studies listed in the diagram are being reviewed by OCP as agreed upon with the OCP reviewer via email communication on March 7, 2014. Note that BE studies 0102 and 0110 are considered not pivotal for the approval of the drug since there used to bridge formulation tested in earlier phases of development and there is PK data for the phase 3/TBM formulation.

SOF single-agent formulation development included several formulations of drug product: (b) (4)

LDV single-agent formulation development included several formulations of drug product (b) (4)

The SOF/LDV tablet was developed prior to the initiation of Phase 3 clinical studies using SOF (b) (4) drug substance and LDV (b) (4) to support Phase 3 clinical studies. The

tablet contains 90 mg of LDV and 400 mg of crystalline SOF (b)(4). According to the Applicant, the SOF/LDV tablet formulation demonstrated similar PK performance to the 2 coadministered single-agent tablets, LDV (b)(4) 90-mg strength and SOF tablet 400-mg strength in Study GS-US-337-0101.

The SOF/LDV (b)(4) tablet formulation was subsequently used in all pivotal Phase 3 clinical studies (GS-US-337-0102 [ION-1], GS-US-337-0109 [ION-2], and GS-US-337-0108 [ION-3]). This is the (b)(4) formulation used in all primary and registration stability batches and is the formulation proposed for the commercial drug product



Figure 11. Schematic Overview on the SOF/LDV Formulation Development

14. Are there any manufacturing changes implemented (e.g. formulation changes, process changes, site change, etc.) to the clinical trial formulation? What information is available to support these changes?

There are two manufacturing sites being proposed, (b)(4) and Gilead in Ireland (Table 6).

Table 6. Manufacturing History of Sofosbuvir/Ledipasvir Tablets

Lot Number	Batch Size (kg)	Date of Manufacture ^a	Manufacturer	Use
DK1201B	(b)(4)	May 2012	(b)(4)	Clinical, BA Study (GS-US-337-0101)
DK1202B	(b)(4)	May 2012	(b)(4)	Clinical, Stability
DK1204B	(b)(4)	May 2012	(b)(4)	Clinical, Stability
DK1205B	(b)(4)	Jun 2012	(b)(4)	Clinical, Stability

DK1206B	(b) (4)	June 2012	(b) (4)	Clinical, Stability
DK1208B		June 2012		Clinical, Stability
DK1209B		June 2012		Clinical, Stability
13SFC001R		December 2012		Clinical, Stability
13SFC002R		December 2012		Clinical, Stability
13SFC003R		December 2012		Clinical, Stability
13SFC004R		April 2013		Clinical, Stability
13SFC005R		June 2013		Clinical, Stability
13SFC006R		August 2013		Scale-Up
DK1309B		August 2013		Scale-Up

Source: Table 1, [\\cdsesub1\evsprod\NDA205834\0000\m3\32-body-data\32p-drug-prod\ldv-sof-tablet\32p5-contr-drug-prod\32p54-batch-analys](#) ;

Since no data were submitted on the original NDA submission, the following IR was sent to the Applicant as part of the 74-day letter:

To support the approval of the alternate manufacturing site provide:

- ***Dissolution profiles comparisons in three different media for the batches (at least 3) manufactured at (b) (4) vs. those manufactured at Gilead in Ireland.***

On a submission dated April 25, 2014 the Applicant submitted dissolution profiles comparisons for which statistical testing is summarized in Tables 7 and 8.

Table 7. f₂ Comparison for Ledipasvir Dissolution Profiles at pH 4.5

Lot No.	DK1204B	DK1205B	DK1208B
13SFC001R	(b) (4)		
13SFC004R	(b) (4)		
13SFC005R	(b) (4)		

Table 8. f₂ Comparison for Ledipasvir Dissolution Profiles at pH 6.0

Lot No.	DK1204B	DK1205B	DK1208B
13SFC001R	(b) (4)		
13SFC004R	(b) (4)		
13SFC005R	(b) (4)		

Reviewer's Comments

Among the three medium tested, f_2 values failed at pH 4.5 and 6. According to the Applicant, the difference in the dissolution profiles between the two manufacturing sites was attributed to slight differences in manufacturing process parameters used at the two sites during clinical development (e.g., Gilead GSL used (b) (4) during clinical manufacturing). The Applicant claims that the two manufacturing sites have now adopted the same manufacturing process parameters. However, it was not clear which manufacturing parameters were revised. Therefore, the following comment was conveyed to the Applicant on an IR letter dated May 26, 2014:

On a submission dated April 30, 2014, it is suggested that the failing of similarity in dissolution between the batches manufactured at (b) (4) vs. those manufactured at Gilead Ireland is due to variations on the process parameters and that this variation will be resolved by harmonizing the manufacturing process parameters. Provide a list of the manufacturing parameters that will be harmonized and the specification ranges.

On a submission dated June 30, 2014 the Applicant provided table comparing the equipment and process parameters that were harmonized. In a phone conversation with the CMC review team, Drs. Lunn and Miller agreed that the updated process parameters are appropriate. In addition, the Applicant provided additional profile comparisons between batches manufactured in the two sites showing that similar in vitro performance (e.g. $f_2 > 50$).

Therefore, the alternate manufacturing site is acceptable from biopharmaceutics perspective.

15. Are all the strengths evaluated in the pivotal clinical trials? What data are available to support the approval of lower strengths?

There is only one strength being proposed which was tested in phase 3 trials.

D) DISSOLUTION APPLICATIONS

D.1 BIOWAIVERS

16. Is there a request for waiver of in vivo BE data (Biowaiver)? What is/are the purpose/s of the biowaiver request/s? What data support the biowaiver request/s?

There were no biowaivers being request in this submission.

17. Is there any IVIVC information submitted? What is the regulatory application of the IVIVC in the submission? What data is provided to support the acceptability of the IVIVC?

There were no IVIVC models included.

18. Is there any in vitro alcohol dose-dumping information submitted? What data are provided to support the Applicant's claim (e.g. lack of dose-dumping in the presence of alcohol)?

Not applicable.

D.2 SURROGATES IN LIEU OF DISSOLUTION

19. Are there any manufacturing parameters (e.g. disintegration, drug substance particle size, etc.) being proposed as surrogates in lieu of dissolution testing? What data is available to support this claim?

No. In laboratory dissolution testing is being implemented.

D.3 DISSOLUTION AND QBD

20. If the application contains QbD elements, is dissolution identified as a CQA for defining design space?

This NDA does not claim the presence of a QbD approach; however, some elements of Quality by Design and Quality Risk Management were applied to the development of the drug product. According to the Applicant, quality risk assessments and design of experiments (DOE) were performed to increase understanding of the robustness of the proposed commercial formulation and the parameters affecting the core and coating manufacturing steps of the proposed commercial drug product process. Dissolution was identified as CQA.

19. Was dissolution included in the DoE? What raw materials and process variables are identified as having an impact on dissolution? What is the risk assessment been performed to evaluate the criticality of dissolution?

The quality attributes of sofosbuvir drug substance, ledipasvir drug substance (b) (4) were assessed for potential impact on the SOF/LDV tablet dissolution CQA. In addition, the process parameters for unit operations assessed as high risk of impact to the SOF/LDV tablet dissolution CQA were studied during development to establish PARs. Material attributes and process parameters were not formally identified as critical; however, development studies showed that LDV (b) (4) have significant impacts on SOF/LDV tablet dissolution. Also, as mentioned before SOF particle size affects dissolution of the SOF component in the final product.

a. Drug Substance Particle size

Figure 12 (reproduction of Figure 5 above) shows the effect of particle size on dissolution. According to the Applicant, (b) (4)



Figure 12. Dissolution Profile of Sofosbuvir and Ledipasvir from Sofosbuvir/Ledipasvir Tablets.

Reviewer's Comments

The recommended dissolution acceptance criterion of $Q = \frac{(b)}{(4)}\%$ in 20 min will not be able to reject a batch $\frac{(b)}{(4)}$ like batch DK1206B, which does not meet similarity testing. Therefore, it is recommended $\frac{(b)}{(4)}$ and a specification for drug substance particle size be implemented to ensure consistent product quality.

b. $\frac{(b)}{(4)}$ Bulk Density

The dissolution profiles for both sofosbuvir and ledipasvir from SOF/LDV $\frac{(b)}{(4)}$ with bulk densities of $\frac{(b)}{(4)}$ g/mL were generated. The dissolution method discriminates for batches with density values above $\frac{(b)}{(4)}$ g/mL. Therefore, the ranges of bulk density implemented are appropriate from biopharmaceutics perspective.

c. $\frac{(b)}{(4)}$ Content

No significant differences in the dissolution profiles were observed for batches manufactured with $\frac{(b)}{(4)}$ content in the range of $\frac{(b)}{(4)}$ to $\frac{(b)}{(4)}$. For details refer to the following link. <\\cdsesub1\evsprod\NDA205834\0010\m1\us\111-info-amendment>.

d. Tablet Harness

Dissolution data demonstrate that the tablet hardness range of $\frac{(b)}{(4)}$ to $\frac{(b)}{(4)}$ kp does not affect the dissolution profile of sofosbuvir and ledipasvir for SOF/LDV tablet.

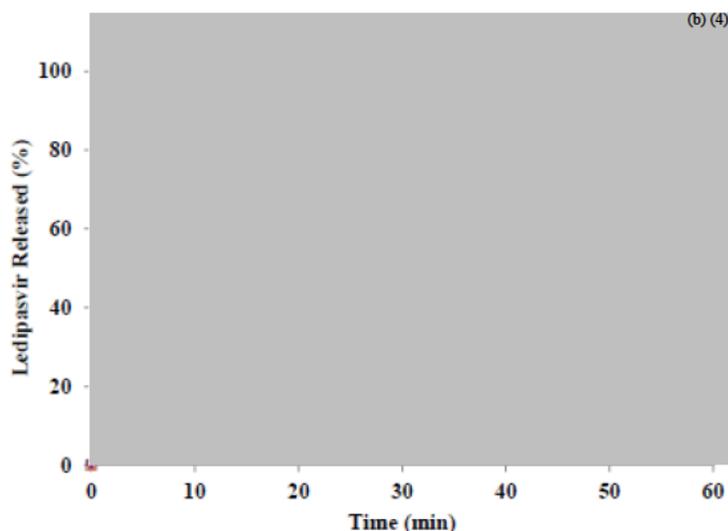


Figure 13. Dissolution Profiles of LDV from Tablets Used in Hardness Evaluation Study.

e. Film Coating Weight

Dissolution profiles of SOF and LDV were not affected for batches manufactured with coating weight values in the range of (b) (4) %.

Reviewer’s Comments

The proposed specifications for film coating weight (b) (4) content (b) (4) and the ranges tested in bulk density ((b) (4) g/mL) are supported by the dissolution data; however this Reviewer has the following recommendations:

- *The recommended dissolution acceptance criterion of $Q = \frac{(b)}{(4)}\%$ in 20 min will not be able to reject a batch (b) (4) like batch DK1206B, which does not meet similarity testing. Therefore, it is recommended (b) (4) and a specification for drug substance particle size be implemented to ensure consistent product quality.*

20. What biopharmaceutics information is available to support the clinical relevance of the proposed design space?

There is no design space being proposed.

21. Is there any dissolution model information submitted as part of QbD implementation? What is the regulatory application of the dissolution model in the submission? What data are provided to support the acceptability of the dissolution model?

No dissolution models were proposed.

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

/s/

SANDRA SUAREZ
07/10/2014

ANGELICA DORANTES
07/10/2014

**CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS
FILING FORM/CHECKLIST FOR NDA/BLA or Supplement**

Office of Clinical Pharmacology

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA/BLA Number	205834	Brand Name	Not available
OCP Division (I, II, III, IV, V)	IV	Generic Name	Ledipasvir/Sofosbuvir
Medical Division	DAVP	Drug Class	HCV NS5A/NS5B inhibitor
OCP Reviewer	Jenny H Zheng	Indication(s)	Treatment of chronic hepatitis C genotype 1 in adults
OCP Team Leader	Shirley Seo	Dosage Form	90 mg ledipasvir and 400 mg sofosbuvir fixed-Dose combination tablets
Pharmacometrics Reviewer	Jeff Florian	Dosing Regimen	One tablet once daily with or without food (b) (4)
Pharmacogenomics Reviewer	Sarah Dorff		
Date of Submission	2/10/2014	Route of Administration	Oral
Estimated Due Date of OCP Review	7/10/2014	Sponsor	Gilead
Medical Division Due Date	10/10/2014	Priority Classification	Priority
PDUFA Due Date	10/10/2014		

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	Number of studies submitted	Number of studies being reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	x			
Tabular Listing of All Human Studies	x			
HPK Summary	x			
Labeling	x			
Reference Bioanalytical and Analytical Methods	x			
I. Clinical Pharmacology				
Mass balance:	x	2	1	GS-US-256-0108
Isozyme characterization:	x	2	2	
Transporter characterization:	x	1	1	
Blood/plasma ratio:	x	1	1	
Plasma protein binding:	x	2	1	
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	x	4	3	GS-US-256-0101, GS-US-334-0111, GS-US-169-0105
multiple dose:				
Patients-				
single dose:				
multiple dose:	x	2	1	GS-US-256-0102
Dose proportionality -				
fasting / non-fasting single dose:	x			
fasting / non-fasting multiple dose:				

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CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

Drug-drug interaction studies -		18	7	GS-US-256-0129, GS-US-248-0104, GS-US-334-0101, GS-US-334-0146, GS-US-344-0102, GS-US-337-0128, GS-US-337-0127
In-vivo effects on primary drug:	x			
In-vivo effects of primary drug:	x			
In-vitro:	x	7	7	
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				
geriatrics:				
renal impairment:	x	2	1	GS-US-344-0108
hepatic impairment:	x	3	1	GS-US-344-0101
PD -				
Phase 2:				
Phase 3:				
PK/PD -				
Phase 1 and/or 2, proof of concept:	x	9	4	P7977-0724, GS-US-344-0109, P7977-0523, GS-US-337-0118
Phase 3 clinical trial:	x	3	3	GS-US-337-0102, GS-US-337-0109, GS-US-337-0108
Population Analyses -	x			
Data rich:	x	3	3	SOF, GS-331007, and LDV PopPK reports from the FDC regiments
Data sparse:				
II. Biopharmaceutics				
Absolute bioavailability				
Relative bioavailability -	x	5	2	GS-US-256-0110, GS-US-337-0101
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:				
replicate design; single / multi dose:				
Food-drug interaction studies				
Bio-waiver request based on BCS				
BCS class				
Dissolution study to evaluate alcohol induced dose-dumping				
III. Other CPB Studies				
Genotype/phenotype studies	x			
Chronopharmacokinetics				
Pediatric development plan	x			
Literature References				
Total Number of Studies		64	38	

On **initial** review of the NDA/BLA application for filing:

	Content Parameter	Yes	No	N/A	Comment
Criteria for Refusal to File (RTF)					
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?			x	
2	Has the applicant provided metabolism and drug-drug interaction information?	x			
3	Has the sponsor submitted bioavailability data satisfying the CFR requirements?	x			
4	Did the sponsor submit data to allow the evaluation of the validity of	x			

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CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

	the analytical assay?				
5	Has a rationale for dose selection been submitted?	x			
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive review to begin?	x			
7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can begin?	x			
8	Is the electronic submission searchable, does it have appropriate hyperlinks and do the hyperlinks work?	x			
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					
Data					
9	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?	x			
10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?	x			
Studies and Analyses					
11	Is the appropriate pharmacokinetic information submitted?	x			
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?	x			
13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?	x			
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?	x			
15	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?			x	
16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			x	
17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?	x			
General					
18	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	x			
19	Was the translation (of study reports or other study information) from another language needed and provided in this submission?			x	

IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE?

Yes

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

File name: 5_Clinical Pharmacology and Biopharmaceutics Filing Form/Checklist for NDA_BLA or Supplement 090808

**CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS
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Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None.

Reviewing Clinical Pharmacologist

Date

Team Leader/Supervisor

Date

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

/s/

HUIMIN ZHENG
03/11/2014

JEFFRY FLORIAN
03/11/2014

SARAH E DORFF
03/11/2014

SHIRLEY K SEO
03/11/2014