CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208341Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

CLINICAL PHARMACOLOGY REVIEW MEMO

NDA: 208341	Submission Date(s): February 10, 2014
Brand Name	EPCLUSA™
Generic Name	Sofosbuvir/Velpatasvir
Clinical Pharmacology Reviewers	Jenny H. Zheng, Ph.D.
Secondary Reviewer	Shirley K. Seo, Ph.D.
OCP Division	Division 4
OND division	DAVP
Applicant	Gilead Sciences
Formulation; Strength(s)	Fixed dose combination tablets; 400 mg/100 mg
Indication	Treatment of chronic hepatitis C (CHC) (b) (4) infection in adults

The Clinical Pharmacology Review for NDA208341 was completed at March 25, 2016. When the review was completed, a Clinical Pharmacology issue was pending:

- Can proton-pump inhibitors (PPIs) be coadministered with EPCLUSA™?

Based on the rationales provided by the applicant and consultation with the gastroenterology products team leader in the Office of Clinical Pharmacology (OCP), the Agency ultimately revised the proposed labeling recommendation as follows (final, agreed-upon wording):

"Coadministration of omeprazole or other proton pump inhibitors is not recommended. If it is considered medically necessary to coadminister, EPCLUSA should be administered with food and taken 4 hours before omeprazole 20 mg. Use with other proton pump inhibitors has not been studied."

As stated in the original NDA review, the applicant's original labeling proposal was (b) (4) The Agency did not agree with the applicant's proposal because of the concern that coadministration of Sofosbuvir/Velpatasvir (SOF/VEL) with proton-pump inhibitors under the proposed conditions decreases the concentration of velpatasvir (VEL), which may lead to reduced therapeutic effect of SOF/VEL; therefore, coadministration is not recommended.

Because SOF/VEL+ribavirin (RBV) is a highly effective treatment for HCV infected patients with decompensated cirrhosis, and PPIs are commonly prescribed in patients with decompensated cirrhosis for prophylaxis of variceal hemorrhage, the applicant insisted (b) (4)

(b) (4)

In the absence of a large clinical dataset for the use of SOF/VEL with PPI, a more cautious approach may be warranted. The Agency and the applicant have reached the following agreements:

- Allow coadministration of omeprazole with SOF/VEL only when it is medically necessary.
    - In ASTRAL 3, VEL AUC exposures were 30% lower in relapsers compared to non-relapsers. Thus, even this degree of decrease in VEL AUC may be problematic.
    - Since SOF/VEL is administered under fed conditions and omeprazole should be administered under fasted conditions (based on recommendations in the omeprazole label), there is concern over the practicality of dosing in the real world when a patient needs to consider the dose of omeprazole to take (20 mg), time of day to take two different drugs (SOF/VEL 4 hours before omeprazole), and prandial condition of each drug administration. In considering the worst case scenario, there were significant decreases in exposure for both SOF and VEL that could impact efficacy for patients with any HCV genotype.
- SOF/VEL should not be used with other PPIs, because the use of SOF/VEL with other proton pump inhibitors has not been studied.
- SOF/VEL should be administered with food and taken 4 hours before omeprazole 20 mg, because the impact of omeprazole on VEL is relatively less as compared to when omeprazole is administered 2 hours ahead of SOF/VEL as shown in the following table. There is 33% and 28% reduction on VEL Cmax and AUC, respectively, as compared to that when SOF/VEL is administered under fasted conditions without omeprazole. Because administration of SOF/VEL with a high-fat/high-calorie or a moderate-fat/moderate-calorie meal resulted in a 21% and 34% increase in VEL AUC, and SOF/VEL can be given with or without food, for patients who take SOF/VEL under fed conditions, the true effect of omeprazole on VEL exposures could actually be more.

Therefore, use of omeprazole or other PPIs with SOF/VEL is not recommended unless medically necessary.

Drug Interactions: Changes in Pharmacokinetic Parameters for Velpatasvir in the Presence of Omeprazole

Dose of Omeprazole (mg)	SOF Dose (mg)[a]	VEL Dose (mg)[a]	N	Mean Ratio (90% CI) of Velpatasvir PK With/Without Coadministered Drug No Effect=1.00	
				C_{max}	AUC
20 once daily 2 hours prior to SOF/VEL	400 single dose fed	100 single dose fed	40	0.52 (0.43, 0.64)	0.62 (0.51, 0.75)
20 once daily 4 hours after SOF/VEL	400 single dose fed	100 single dose fed	38	0.67 (0.58, 0.78)	0.74 (0.63, 0.86)

[a] SOF/VEL was administered under fasted conditions in the reference arms.

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/s/

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06/17/2016

SHIRLEY K SEO
06/17/2016

OFFICE OF CLINICAL PHARMACOLOGY REVIEW

NDA: 208341	Submission Date(s): October 28, 2015
Brand Name	To be Determined
Generic Name	Sofosbuvir/Velpatasvir (GS-7977/GS-5816)
Primary Clinical Pharmacology Reviewers	Jenny H. Zheng, Ph.D. Abhay Joshi, Ph.D.
Secondary Reviewer	Shirley K. Seo, Ph.D.
PM Reviewer	Fang Li, Ph.D.
Secondary PM Reviewer	Jeffry Florian, Ph.D.
OCP Division	Division 4
OND division	DAVP
Applicant	Gilead Sciences
Relevant IND(s) and NDA(s)	INDs 118605, 106739, 115670 and NDAs 204671, 205834
Submission Type	Priority
Formulation; Strength(s)	Fixed dose combination tablets; 400 mg/100 mg
Indication	Treatment of chronic hepatitis C (CHC) infection in adults

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1. EXECUTIVE SUMMARY

Gilead Sciences is seeking approval of sofosbuvir (SOF, GS-7977) and velpatasvir (VEL, GS-5816) together as an oral fixed-dose combination (FDC) tablet (SOF/VEL 400 mg/100 mg) for the treatment of chronic hepatitis C virus (HCV) infection. SOF/VEL was granted Breakthrough Therapy Designation on May 18, 2015.

VEL is a novel HCV NS5A inhibitor that has demonstrated potent anti-HCV activity against genotypes 1 through 6 HCV infection. SOF is a nucleotide NS5B polymerase inhibitor that inhibits HCV RNA replication in vitro, and has been approved for use in combination with other agents for the treatment of chronic HCV infection in adults (Sovaldi®, NDA 204671 and Harvoni® [ledipasvir (LDV)/SOF], NDA 205834).

The proposed SOF/VEL dosage regimen is one 400 mg/100 mg tablet, taken orally, once daily with or without food. The following treatment regimens are proposed by the applicant, regardless of HCV genotype:

Patient Population	Proposed Treatment Regimen
Patients without cirrhosis and patients with compensated cirrhosis	SOF/VEL for 12 weeks
Patients with decompensated cirrhosis	SOF/VEL + ribavirin for 12 weeks

The consideration for approval of this NDA is based on efficacy data from three Phase 3 trials (ASTRAL-1, ASTRAL-2, and ASTRAL-3) in subjects with genotypes 1 through 6 HCV infection with or without compensated cirrhosis and one Phase 3 trial (ASTRAL-4) in subjects with genotypes 1 through 6 HCV infection with decompensated cirrhosis.

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 SOF/VEL.

1.2 Phase IV Commitments

Post Marketing Requirement (PMR): The sponsor will be asked to conduct a drug interaction study to evaluate the interaction between sofosbuvir/velpatasvir and atorvastatin.

Rationale: The results from the rosuvastatin study indicate that velpatasvir can significantly increase the concentration of substrates of organic anion transporting polypeptides (OATP) and breast cancer resistance protein (BCRP), such as atorvastatin. Although the results from the rosuvastatin drug interaction study cannot be directly extrapolated to atorvastatin, there is a mechanistic basis for a potentially clinically significant interaction with this commonly used statin. Furthermore, a serious safety event (rhabdomyolysis) has been identified in postmarketing reports with use of ledipasvir/sofosbuvir and atorvastatin. Based on the evidence from *in vitro* studies, ledipasvir and velpatasvir may have similar drug interaction potential for inhibition of OATP1B and BCRP transport, so the potential exists for sofosbuvir/velpatasvir to increase atorvastatin exposures, leading to serious adverse events. Thus, a PMR is needed to study the interaction between sofosbuvir/velpatasvir and atorvastatin in order to derive appropriate dosing recommendations for concomitant use.

1.3 Summary of Important Clinical Pharmacology Findings

A comprehensive program of Phase 1 and Phase 2 clinical studies and in vitro studies characterized the PK of SOF, its predominant inactive metabolite GS-331007, and VEL when administered either as single agents or as the FDC. Additionally, both intensive and sparse plasma concentration data from 331 healthy subjects and 1694 HCV-infected subjects who received SOF/VEL FDC, SOF and VEL administered together as single agents, or VEL as a single agent from 11 clinical studies (four Phase 1, three Phase 2, and four Phase 3 studies) were used for population PK evaluation of SOF, its predominant circulating metabolite GS-331007, and VEL.

Mechanism of Action (MOA), General Pharmacokinetics (PK) and Pharmacodynamics (PD)

MOA: SOF/VEL is a fixed-dose combination of SOF (an inhibitor of the HCV NS5B RNA-dependent RNA polymerase) and VEL (an inhibitor of the HCV NS5A protein) which are direct-acting antiviral agents against the hepatitis C virus.

Pharmacokinetics: The following table characterizes the absorption, distribution, metabolism, and excretion (ADME) of the components of SOF/VEL

	Sofosbuvir	**Velpatasvir**
Absorption		
Tmax (h)	0.5-1	3
Effect of moderate meal (relative to fasting)[a]	↑ 60%	↑ 34%
Effect of high fat meal (relative to fasting)[a]	↑ 78%	↑ 21%
Distribution		
% Bound to human plasma proteins	61-65%	>99.5%
Blood-to-plasma ratio	0.7	0.52-0.67
Metabolism		
Metabolism	Cathepsin A CES1 HINT1	CYP2B6 (minor) CYP2C8 (minor) CYP3A4 (minor)
Elimination		
Major route of elimination	SOF: metabolism GS-331007[b]: glomerular filtration and active tubular secretion	Biliary excretion as parent (77%)
t1/2 (h)[c]	SOF: 0.5 GS-331007[b]: 25	15
% Of dose excreted in urine[d]	80%[e]	0.4%
% Of dose excreted in feces[d]	14%	94%

CES1 = carboxylesterase 1; HINT1 = histidine triad nucleotide-binding protein 1
[a] Values refer to mean systemic exposure. Moderate meal = ~600 kcal, 30% fat; high fat meal = ~800 kcal, 50% fat. SOF/VEL can be taken with or without food.
[b] GS-331007 is the primary circulating nucleoside metabolite of SOF.
[c] $t_{1/2}$ values refer to median terminal plasma half-life.
[d] Single dose administration of [^{14}C] SOF or [^{14}C] VEL in mass balance studies.
[e] Predominantly as GS-331007.

Pharmacodynamics: At a dose three times the maximum recommended dose for SOF and a dose five times the maximum recommended dose for VEL, neither SOF nor VEL prolong QTc to a clinically relevant extent. Please refer to the review conducted by Interdisciplinary Review Team for QT Studies Consultation (IND 115670, 4/15/2015) for details.

Exposure-Response: No exposure-response relationships for safety or efficacy were identified for either of the components of SOF/VEL at the recommended dosage. No dose-response was identified between SOF 400 mg + VEL 25 mg and SOF 400 mg + VEL100 mg for treatment-naïve, non-cirrhotic subjects. However, higher efficacy was observed with SOF 400 mg + VEL 100 mg (SVR12 (sustained virologic response at 12 weeks following completion of all

treatment): 94%) compared to SOF 400 mg + VEL 25 mg (SVR12: 71%) in Genotype 3, treatment-experienced subjects (Phase 2 study GS-US-342-0109).

Drug Interaction Potential

- VEL solubility decreases as pH increases. Drugs that increase gastric pH are expected to decrease systemic concentrations of VEL.
- SOF and VEL are substrates of drug transporters P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) while GS-331007 is not. Drugs that are inducers of P-gp may decrease plasma concentrations of SOF and VEL leading to reduced therapeutic effect of SOF/VEL.
- In vitro, slow metabolic turnover of VEL by CYP2B6, CYP2C8, and CYP3A4 was observed. Drugs that are moderate to potent inducers of CYP2B6, CYP2C8, or CYP3A4 may decrease plasma concentrations of VEL leading to reduced therapeutic effect of SOF/VEL.
- VEL is an inhibitor of drug transporters P-gp, breast cancer resistance protein (BCRP), OATP1B1 and OATP1B3. Coadministration of SOF/VEL with drugs that are substrates of these transporters may increase the exposure of such drugs.
- Serious symptomatic bradycardia has been observed when amiodarone is coadministered with a SOF-containing regimen (with another HCV direct acting antiviral). Therefore, coadministration of amiodarone with SOF/VEL may also result in serious symptomatic bradycardia. The mechanism of this effect is unknown.

The following table provides a listing of reviewer-proposed changes (in blue) to established or potentially clinically significant drug interactions. The table is not all inclusive.

Concomitant Drug Class: Drug Name	**Effect on Concentration[a]**	**Clinical Comment**
Acid Reducing Agents:	↓ VEL	VEL solubility decreases as pH increases. Drugs that increase gastric pH are expected to decrease concentration of VEL.
Antacids (e.g., aluminum and magnesium hydroxide)		Separate antacid and SOF/VEL administration by 4 hours.
H_2-receptor antagonists[b] (e.g., famotidine)		H_2-receptor antagonists may be administered simultaneously with or 12 hours apart from SOF/VEL at a dose that does not exceed doses comparable to famotidine 40 mg twice daily.
Proton-pump inhibitors[b] (e.g., omeprazole)		Coadministration of SOF/VEL with proton-pump inhibitors decrease the concentration of velpatasvir, which may lead to reduced therapeutic effect of SOF/VEL. Coadministration is not recommended.
Antiarrhythmics: amiodarone	Effect on amiodarone, SOF, and VEL concentrations unknown	Coadministration of amiodarone with SOF/VEL may result in serious symptomatic bradycardia. The mechanism of this effect is unknown. Coadministration of amiodarone with SOF/VEL is not recommended; if coadministration is required, cardiac monitoring is recommended.
digoxin[b]	↑ digoxin	Measure serum digoxin concentrations before initiating SOF/VEL. Reduce digoxin concentrations by decreasing the dose by approximately 15-30% or by modifying the dosing frequency and continue monitoring.

Anticancers: topotecan	↑ topotecan	Coadministration is not recommended.
Anticonvulsants: carbamazepine phenytoin phenobarbital oxcarbazepine	↓ SOF ↓ VEL	Coadministration is not recommended.
Antimycobacterials: rifabutin rifampin[b] rifapentine	↓ SOF ↓ VEL	Coadministration is not recommended.
HIV Antiretrovirals:		
efavirenz[b]	↓ VEL	Coadministration of SOF/VEL with efavirenz-containing regimens is not recommended.
Regimens containing tenofovir DF (b) (4)	↑ tenofovir	Monitor for tenofovir-associated adverse reactions in patients receiving SOF/VEL concomitantly with a regimen containing tenofovir DF. Refer to (b) (4) prescribing information for recommendations on renal monitoring.
(b) (4)		
tipranavir/ritonavir	↓ SOF ↓ VEL	Coadministration is not recommended.
Herbal Supplements: St. John's wort *(Hypericum perforatum)*	↓ SOF ↓ VEL	Coadministration is not recommended.
HMG-CoA Reductase Inhibitors: rosuvastatin[b]	↑ rosuvastatin	Coadministration of SOF/VEL with rosuvastatin may significantly increase the concentration of rosuvastatin which is associated with increased risk of myopathy, including rhabdomyolysis. Rosuvastatin may be administered with SOF/VEL at a dose that does not exceed 10 mg.
atorvastatin	↑ atorvastatin	Coadministration of SOF/VEL with atorvastatin is expected to increase the concentrations of atorvastatin, which is associated with increased risk of myopathy, including rhabdomyolysis. Monitor closely for HMG-CoA reductase inhibitor-associated adverse events, such as myopathy.

a. ↓ = decrease, ↑ = increase
b. These interactions have been studied in healthy adults.

Although the drug interaction potential was not studied between SOF/VEL and atorvastatin, atorvastatin is a sensitive substrate of OATP1B1, and has been reported as a substrate of P-gp

and BCRP. There have been postmarketing reports of rhabdomyolysis cases associated with the coadministration of Harvoni® (LDV/SOF) and atorvastatin. VEL and LDV have similar drug interaction potential profiles. Therefore, there is a potential for a clinically relevant drug interaction (DDI) between SOF/VEL and atorvastatin. Because it is difficult to extrapolate the results from one statin to another, a postmarketing requirement (PMR) is necessary to study the interaction between SOF/VEL and atorvastatin.

Use in Specific Populations

- No dosage adjustment is recommended based on gender, race, or age (≥18 to ≤82 years).
- *Renal Impairment:* No dosage adjustment of SOF/VEL is required for patients with mild or moderate renal impairment. The safety and efficacy of SOF/VEL have not been established in patients with severe renal impairment (eGFR less than 30 mL/min/1.73m^2) or ESRD requiring hemodialysis. No dosage recommendation can be given for patients with severe renal impairment or ESRD.
- *Hepatic Impairment:* No dosage adjustment of SOF/VEL is required for patients with mild, moderate, or severe hepatic impairment (Child-Pugh Class A, B, or C). Safety and efficacy of SOF/VEL have been established in patients with decompensated cirrhosis.

Safety and Efficacy

The SOF/VEL pivotal studies consisted of three randomized Phase 3 trials (ASTRAL-1, ASTRAL-2, and ASTRAL-3) in subjects with genotypes 1 through 6 HCV infection with or without compensated cirrhosis and one Phase 3 trial (ASTRAL-4) in subjects with genotypes 1 through 6 HCV infection with decompensated cirrhosis. Overall, 1035 subjects were treated with SOF/VEL for 12 weeks in Studies GS-US-342-1138, GS-US-342-1139, and GS-US-342-1140 including 328, 238, 277, 116, 35, and 41 subjects with genotype 1, 2, 3, 4, 5, or 6 HCV infection, respectively. In Study GS-US-342-1137, 267 subjects with genotype 1, 2, 3, 4, or 6 HCV infection with decompensated cirrhosis were treated with SOF/VEL±RBV for 12 weeks or SOF/VEL for 24 weeks.

For all subjects, regardless of cirrhosis status, SVR12 rates > 95% (range: 95.3% to 100.0% across genotypes) were achieved across all HCV genotypes. Among subjects with cirrhosis, the SVR12 rates were > 91% (range: 91.3% to 100.0% across HCV genotypes) and among subjects with prior treatment experience, the SVR12 rates also were > 90% (range: 90.1% to 100.0% across HCV genotypes). For subjects with decompensated cirrhosis, numerically higher SVR 12 rates were achieved following treatment with SOF/VEL+RBV for 12 weeks (SVR12: 94.3) as compared to treatment with SOF/VEL for 12 weeks (SVR 83.3%) or 24 weeks (SVR12: 85.6%). The most common adverse reaction (incidence at least 5%) were headache, fatigue, and nausea in subjects treated with 12 weeks of SOF/VEL. The most common adverse reactions (all grades with frequency of at least10% in subjects with decompensated cirrhosis who received SOF/VEL plus RBV for 12 weeks were fatigue (32%), anemia (26%), nausea (15%), headache (11%), insomnia (11%), and diarrhea (10%).

2. QUESTION BASED REVIEW

2.1 General Attributes

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

Sofosbuvir/velpatasvir (SOF/VEL) fixed-dose combination tablets contain 400 mg of sofosbuvir (SOF) and 100 mg of velpatasvir (VEL).

Please refer to the Clinical Pharmacology Review for NDA 204671 for additional details on the physico-chemical properties of SOF. The following shows physico-chemical properties of VEL.

Empirical Formula: C49H54N8O8
Molecular Weight: 883.0g/mol
pKa: pKa1 = 3.2; pKa2 = 4.6

Solubility of VEL in Aqueous Media at 25 °C

Aqueous Media	Solubility (mg/mL)	USP/Ph. Eur. Solubility Description
Water, pH 1.2[a]	> 36	Soluble
Water, pH 2.0[a]	3.6	Slightly soluble
Sodium acetate buffer, pH 5.0	< 0.1	Practically insoluble
Phosphate buffer, pH 6.8	< 0.1	Practically insoluble
FeSSIF, pH 5.0[b]	0.1	Very slightly soluble
FaSSIF, pH 6.5[c]	< 0.1	Practically insoluble

[a] pH established using HCl
[b] FeSSIF, Fed-state simulated small intestine fluid contains 15 mM bile salts (1:1 aurocholate: glycocholate, 3.75 mM lecithin)
[c] FaSSIF, Fasted-state simulated small intestine fluid contains 5 mM bile salts (1:1 taurocholate: glycocholate, 0.75 mM lecithin)

The quantitative composition of SOF/VEL tablets is shown in the following table. SOF/VEL tablets contain (b) (4) (400 mg) SOF and (b) (4) (100 mg) VEL. The tablet formulation utilizes (b) (4).

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

a (b) (4)

b

c

d

e

f

g

h

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

SOF is a HCV NS5B polymerase nucleotide inhibitor that displays potent inhibition of HCV RNA replication in vitro. SOF is a nucleotide prodrug that undergoes intracellular metabolism to form the pharmacologically active uridine analog triphosphate (GS-461203). VEL is a novel HCV NS5A inhibitor that has displayed potent in vitro inhibition of HCV RNA replication. VEL exhibits broad genotypic (genotypes 1-6) potency and selectivity in multiple cell-based replicon assays and specificity for HCV. The proposed indication for SOF/VEL FDC tablet is the treatment of chronic HCV infection in adults.

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

The proposed dose of SOF/VEL is 400 mg/100 mg, taken orally, once daily with or without food. The proposed treatment regimen is based on patient population:

- For patients without cirrhosis and patients with compensated cirrhosis the recommended treatment regimen is SOF/VEL for 12 weeks.
- For patients with decompensated cirrhosis the recommended treatment regimen is SOF/VEL +ribavirin for 12 weeks.

When administered with SOF/VEL the recommended dose of ribavirin is based on weight: 1000 mg per day for patients less than 75 kg and 1200 mg for those weighing at least 75 kg, divided and administered twice daily with food.

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 program of Phase 1 and Phase 2 studies characterized the PK of SOF/VEL, VEL, and SOF. Additionally, intensive and/or sparse plasma concentration data from 331 healthy subjects and 1694 HCV-infected subjects who received SOF/VEL, VEL, or SOF from 11 clinical studies (4 Phase 1, 3 Phase 2, and 4 Phase 3 studies) were used for population PK evaluations of VEL and SOF and its predominant circulating metabolite GS-331007, as appropriate.

Dose Selection: SOF 400 mg is the approved marketed dose of SOF (Sovaldi®) for the treatment of HCV infection and, as such, was selected for coformulation with VEL in an FDC tablet for evaluation in the SOF/VEL development program.

VEL:
The Phase 1b Study GS-US-281-0102 evaluated the antiviral and safety activity of administration of VEL for 3 days at doses ranging from 5 to 150 mg in subjects with genotype 1, 2, 3, or 4 HCV infection. The median maximal decline in HCV RNA across all HCV genotypes at all VEL doses evaluated was > 3 log10 IU/mL. The Phase 2 studies (Studies GS-US-342-0102, GS-US-342-0109, and GS-US-337-0122 [ELECTRON-2, Cohort 4]) evaluated the efficacy and safety of coadministration of SOF and VEL in subjects with genotype 1 to 6 HCV infection; 2 treatment durations (8 and 12 weeks), 2 VEL doses (25 and 100 mg), and the contribution of RBV to efficacy and safety were assessed. Results of the Phase 2 studies indicated that a regimen of SOF 400 mg with VEL 100 mg for 12 weeks was well tolerated and resulted in the highest SVR12 rates in subjects with genotype 1 to 6 HCV infection including subjects with prior treatment failure and compensated cirrhosis, while a regimen of SOF 400 mg with VEL 25 mg for 12 weeks resulted in a lower SVR12 rate for treatment-experienced subjects with genotype 3 (see Section 2.2.4.1 for detailed discussion).

Phase 3 Clinical Efficacy and Safety Evaluation:

The SOF/VEL Phase 3 clinical development program included four Phase 3 studies: GS-US-342-1138 (ASTRAL-1), GS-US-342-1139 (ASTRAL-2), GS-US-342-1140 (ASTRAL-3), and GS-US-342-1137 (ASTRAL-4).

Study GS-US-342-1138 evaluated 12 weeks of SOF/VEL in subjects with chronic genotype 1, 2, 4, 5, or 6 HCV infection; Study GS-US-342-1139 compared SOF/VEL with SOF+RBV for 12 weeks in subjects with chronic genotype 2 HCV infection; Study GS-US-342-1140 compared SOF/VEL for 12 weeks with SOF+RBV for 24 weeks in subjects with chronic genotype 3 HCV infection; and Study GS-US-342-1137 evaluated SOF/VEL for 12 weeks, SOF/VEL+RBV for 12 weeks, and SOF/VEL for 24 weeks in subjects with chronic HCV infection and Child-Pugh-Turcotte (CPT) B cirrhosis.

The following table summarizes the overall virologic outcomes across the Phase 3 studies.

Study	GS-US-342-1138 (ASTRAL-1)		GS-US-342-1139 (ASTRAL-2)		GS-US-342-1140 (ASTRAL-3)		GS-US-342-1137 (ASTRAL-4)		
Genotype(s)	1, 2, 4, 5, or 6		2		3		1, 2, 3, 4, or 6		
Duration	12 Weeks		12 Weeks		12 Weeks	24 Weeks	12 Weeks	12 Weeks	24 Weeks
Regimen	SOF/VEL (N = 624)	Placebo (N = 116)	SOF/VEL (N = 134)	SOF+RBV (N = 132)	SOF/VEL (N = 277)	SOF+RBV (N = 275)	SOF/VEL (N = 90)	SOF/VEL+RBV (N = 87)	SOF/VEL (N = 90)
SVR12	99.0%	—	99.3%	93.9%	95.3%	80.4%	83.3%	94.3%	85.6%
95% CI	97.9% to 99.6%	—	95.9% to 100.0%	88.4% to 97.3%	92.1% to 97.5%	75.2% to 84.9%	74.0% to 90.4%	87.1% to 98.1%	76.6% to 92.1%
n/N	618/624	—	133/134	124/132	264/277	221/275	75/90	82/87	77/90
Virologic Failure	0.3%	—	0	4.5%	4.0%	14.2%	12.2%	3.4%	8.9%
n/N	2/624	—	—	6/132	11/277	39/275	11/90	3/87	8/90
Other	0.6%	—	0.7%	1.5%	0.7%	5.5%	4.4%	2.3%	5.6%
n/N	4/624	—	1/134	2/132	2/227	15/275	4/90	2/87	5/90

LLOQ = lower limit of quantitation
HCV RNA was analyzed using COBAS® AmpliPrep/COBAS® TaqMan HCV Quantitative Test v2.0 with limit of quantitation of 15 IU/mL.
Relapse = confirmed HCV RNA ≥ LLOQ during the posttreatment period having achieved HCV RNA < LLOQ at last on-treatment visit.
On-treatment virologic failure = breakthrough (confirmed HCV RNA ≥ LLOQ after having previously had HCV RNA < LLOQ while on treatment), rebound (confirmed > 1 $\log_{10}$ IU/mL increase in HCV RNA from nadir while on treatment), or nonresponse (HCV RNA persistently ≥ LLOQ through 8 weeks of treatment). Other = subject who did not achieve SVR12 and did not meet virologic failure criteria.

For all subjects, regardless of cirrhosis status, SVR12 rates > 95% (range: 95.3% to 100.0% across genotypes) were achieved across all HCV genotypes. Among subjects with cirrhosis, the SVR12 rates were > 91% (range: 91.3% to 100.0% across HCV genotypes) and among subjects with prior treatment experience, the SVR12 rates also were > 90% (range: 90.1% to 100.0% across HCV genotypes). For subjects with decompensated cirrhosis, numerically higher SVR 12 rate were achieved following treatment with SOF/VEL+RBV for 12 weeks (SVR12: 94.3) as compared to treatment with SOF/VEL for 12 weeks (SVR 83.3%) or 24 weeks (SVR12: 85.6%).

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 sustained virologic response or SVR (i.e., undetectable virus [LLOQ or limit of detection for assay] 12 [SVR12] or 24 [SVR24] weeks after the completion of therapy). SVR12 was selected as the primary endpoint for Phase 2/3 clinical trials for SOF/VEL 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 VEL, SOF, and GS-331007 were used to assess PK parameters and exposure-response relationships.

VEL is an HCV inhibitor targeting the HCV NS5A protein, and thus is an active moiety. Following single-dose oral administration of [^{14}C]VEL to healthy human males, unchanged VEL contributed the major portion (approximately 99%) of circulating radioactivity in plasma. Therefore, VEL plasma concentrations are appropriate to evaluate VEL 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 showing 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 measureable 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, although it is not in the metabolic pathway to form the active triphosphate moiety (see Section 2.2.5.6). GS-566500 PK profiles are similar to SOF and thus PK for GS-566500 was not analyzed in all 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?

There was no VEL dose-response relationship for efficacy in treatment-naïve subjects. As shown in the following table, in Phase 2 study GS-US-342-0102, the SVR12 rates after 12-week treatment with SOF 400 mg + VEL 100 mg (N =77) and SOF 400 mg + VEL 25 mg (N=76) were almost identical. Both treatment arms showed a highly effective SVR12 of 96%. Despite a 5-fold difference in exposure (AUC) between 100 mg and 25 mg dose of VEL, the 100 mg dose did not demonstrate greater efficacy than the 25 mg dose in the combination treatment in treatment naïve patients.

SVR12 Rate in Treatment-Naïve Subject after 12-Week Combinational Treatment with SOF 400 mg + VEL 100 or 25 mg (Phase 2 Study: GS-US-342-0102)

ARM*	Duration	Total	SVR12-Yes	SVR12-No	VEL AUCτ (h.ng/mL)	VEL Cmax (ng/mL)
SOF 400 mg + VEL 100 mg	12 weeks	77	74 (96.1%)	3(3.90%)	2833.6 (N=9)	353.4 (N=9)
SOF 400 mg + VEL 25 mg	12 weeks	76	73 (96.05%)	3 (3.95%)	487.1 (N=15)	78.9 (N=15)

*Genotype 1 2 3 4 5 6 combined

However, a dose-response relationship for efficacy was evident in treatment-experienced subjects with HCV genotype 3 infection. As shown in the following tables, in study GS-US-342-0109, the SVR12 rate after 12-week treatment with SOF 400 mg + VEL 100 mg was 96.3% (77/80) while the SVR12 rate was only 81% (64/79) after treatment with SOF 400 mg + VEL 25 mg. All subjects who failed had HCV genotype 3. The SVR12 rate in subjects with genotype 3 who received SOF + VEL 25 mg was 71% and it was 94% in subjects who received SOF + VEL 100 mg. The data suggest that the higher dose (100 mg) is needed to treat genotype 3 subjects with previous treatment failure.

SVR12 Rate in Treatment-Experienced Subject after 12-Week Combinational Treatment with SOF 400 mg + VEL 100 or 25 mg (Phase 2 Study: GS-US-342-0109)

ARM*	Duration	Total	SVR12-Yes	SVR12-No	VEL AUCτ (h.ng/mL)	VEL Cmax (ng/mL)
SOF 400 mg + VEL 100 mg	12 weeks	80	77 (96.3%)	3(3.7%)	2053.4 (N=11)	285.0 (N=11)
SOF 400 mg + VEL 25 mg	12 weeks	79	64 (81.0%)	15 (19.0%)	478.8 (N=5)	71.6 (N=5)

*Genotype 1 and 3 combined.

SVR12 Rate in Treatment-Experienced Subject after 12-Week Combinational Treatment with SOF 400 mg + VEL 100 or 25 mg Stratified by Genotype (Phase 2 Study: GS-US-342-0109)

ARM*	Genotype	Total	SVR12-Yes	SVR12-No	VEL AUCτ (h.ng/mL)	VEL Cmax (ng/mL)
SOF 400 mg + VEL 100 mg 12 weeks	genotype 1	27	27(100%)	0(0%)	2053.4 (N=11)	285.0 (N=11)
	genotype 3	53	50(94.3%)	3(5.7%)		
SOF 400 mg + VEL 25 mg 12 weeks	genotype 1	27	27(100%)	0(0%)	478.8 (N=5)	71.6 (N=5)
	genotype 3	52	37(71.1%)	15(28.9%)		

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

No dose- or exposure-response relationship for safety was explored for VEL and SOF. The phase 3 studies only tested one single dose of SOF/VEL (400 mg/100 mg), and the OCP team did not identify any prominent adverse events or adverse events of special interest for further investigation.

Thorough QT studies have been conducted for SOF and VEL.

The effect of SOF 400 mg (maximum recommended dosage) and 1200 mg (three times the maximum recommended dosage) on QTc interval was evaluated in a randomized, single-dose, placebo-, and active-controlled (moxifloxacin 400 mg) four period crossover thorough QT trial in 59 healthy subjects. At a dose three times the maximum recommended dose, SOF does not prolong QTc to any clinically relevant extent.

The effect of VEL 500 mg was evaluated in a randomized, single-dose, placebo-, and active-controlled (moxifloxacin 400 mg) four period crossover thorough QT trial in 48 healthy subjects. At a dose five times the maximum recommended dose, and the AUC 3.7-fold of the observed value for VEL 100 mg, VEL does not prolong QTc interval to any clinically relevant extent.

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 SOF as a single agent or as a component of SOF/VEL, the majority of SOF-related drug exposure is attributable to GS-331007. For SOF/VEL, the median time at which maximum SOF plasma concentrations were achieved (Tmax) occurred at 0.5 hours. Consistent with the short half-life (median, 0.5 hours), SOF was not detectable at 24 hours postdose. GS-331007 median Tmax occurred later (3 hours) relative to that of SOF and GS-331007 exhibited a half-life of approximately 25 hours.

Accumulation of SOF following once-daily dosing of SOF/VEL is not expected based on the short half-life relative to the once-daily dosing schedule. Evaluation of accumulation ratios using cross-study comparisons from these studies shows modest accumulation for GS-331007 of approximately 32% and 40% for Cmax and AUC, respectively.

Following administration of a single dose of SOF/VEL, the median Tmax for VEL was approximately 3 hours. The median half-life was estimated to be approximately 15 hours, supporting once-daily dosing. Consistent with a moderate half-life relative to a once-daily dosing schedule, VEL accumulation of < 2-fold was observed following multiple-dose administration of VEL or SOF/VEL.

Population PK exposures for SOF, GS-331007 and VEL following once-daily administration of VEL and SOF in HCV-infected subjects are shown in the next section (Section 2.2.5.2)

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

Based on population PK modeling, mean VEL exposure observed in HCV-infected subjects was lower (AUCtau: 37%↓, Cmax: 41%↓, and Ctau: 38%↓) than observed in healthy subjects. These observations are likely due to lower relative bioavailability and higher CL and Vc (which results in the net effect of shorter t1/2) for HCV-infected subjects. Mean SOF and GS-331007 exposures (AUCtau and Cmax) observed in HCV-infected subjects and healthy subjects were similar. This table includes subjects who were coadministered SOF and VEL with and without RBV. RBV use has no effect on SOF or VEL exposure, but decreases GS-331007 AUC by 20%. Therefore GS-331007 results were reanalyzed with or without RBV by the review team. The relative ratio of the GS-331007 exposures for HCV-infected subjects vs. healthy subjects is similar following coadministration of SOF and VEL with or without RBV.

SOF, GS-331007 and VEL PK Parameters after Once-Daily Administration of VEL and SOF in HCV-Infected Subjects (Population PK Analysis from Phase 2 and 3 Studies) or Healthy Subjects (Population PK Analysis from Phase 1 Studies) (Source: Applicant's Summary of Clinical Pharmacology)

PK Parameter	Mean (%CV)		%GMR (90% CI)
	HCV-Infected Subjects (Population PK)	Healthy Subjects (Population PK)	
SOF	**N = 982**	**N = 331**	
AUCtau (h*ng/mL)	1262 (37.2)	1272 (24.3)	96.0 (92.7, 99.3)
Cmax (ng/mL)	566 (31.4)	550 (33.3)	103.9 (100.4, 107.5)
GS-331007	**N = 1428**	**N = 331**	
AUCtau (h*ng/mL)	13967 (28.0)	12040 (19.5)	113.8 (110.9, 116.8)
Cmax (ng/mL)	868 (27.6)	817 (21.1)	104.6 (101.9, 107.4)
VEL	**N = 1425**	**N = 331**	
AUCtau (h*ng/mL)	2967 (50.2)	4556 (38.7)	62.8 (59.9, 65.9)
Cmax (ng/mL)	259 (53.9)	421 (39.0)	58.5 (55.4, 61.7)
Ctau (ng/mL)	41 (65.0)	65 (54.2)	62.1 (58.7, 65.8)

Analyses included all HCV-infected subjects who received SOF 400 mg and VEL 100 mg as either single agents or a FDC in Study GS-US-337-0122, GS-US-342-0102, GS-US-342-0109, GS-US-342-1138, GS-US-342-1139, or GS-US-342-1140 and healthy subjects in Study GS-US-342-0104, GS-US-342-1167, GS-US-342-1326, or GS-US-342-1346.

2.2.5.3 What are the characteristics of drug absorption?

The pharmacokinetic properties of SOF, GS-331007 and VEL have been evaluated in healthy adult subjects and in subjects with chronic hepatitis C. Following oral administration of SOF/VEL, SOF was absorbed with a peak median plasma concentration 0.5–1.0 hour post-dose. Median peak plasma concentration of GS-331007 was observed 3.0 hours post-dose. VEL median peak concentration was observed 3.0 hours post-dose.

Food increases the exposure of both SOF and VEL. The effect of food on the absorption of SOF and VEL is discussed in See Section 2.5.3.

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

SOF and VEL are substrates of P-gp and BCRP. Therefore, drugs that are inducers or inhibitors of P-gp or BCRP may affect the absorption of SOF and VEL. See Section 2.4.2 (Drug-Drug Interaction) for detailed discussion.

2.2.5.4 What are the characteristics of drug distribution?

SOF is approximately 61–65% bound to human plasma proteins and the binding is independent of drug concentration over the range of 1 microgram/mL to 20 microgram/mL. Protein binding of

GS-331007 was minimal in human plasma. After a single 400 mg dose of [^{14}C]-SOF in healthy subjects, the blood to plasma ratio of ^{14}C-radioactivity was approximately 0.7.

VEL is greater than 99.5% bound to human plasma proteins and binding is independent of drug concentration over the range of 0.09 microgram/mL to 1.8 microgram/mL. After a single 100 mg dose of [^{14}C]-VEL in healthy subjects, the blood to plasma ratio of ^{14}C-radioactivity ranged between 0.52 and 0.67.

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. 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.

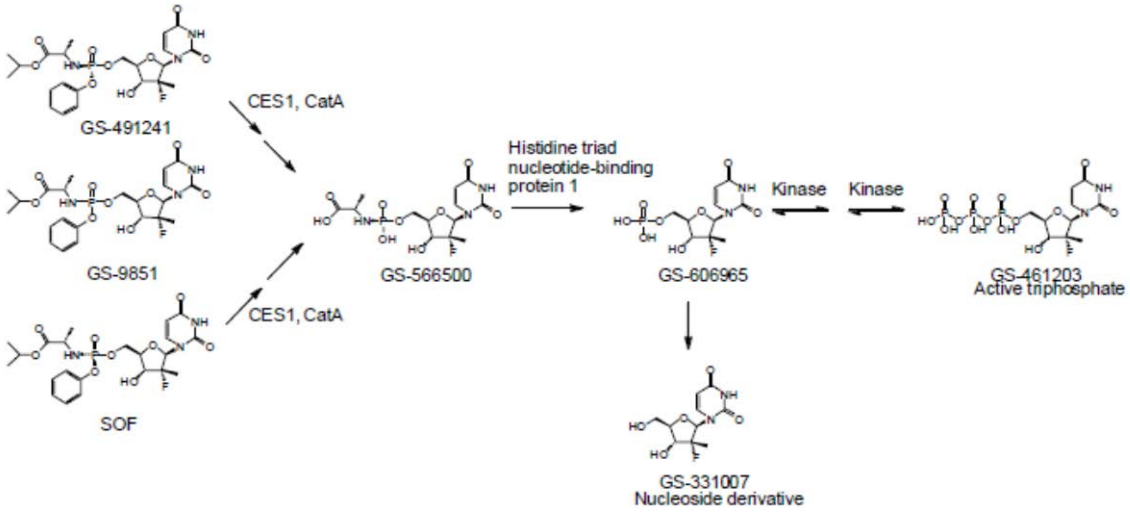
Biliary excretion of parent drug is a major route of elimination for VEL. Following single-dose oral administration of [^{14}C]VEL to healthy human males, unchanged VEL contributed the major portion (approximately 99%) of circulating radioactivity in plasma, with 0.4% and 0.7% of the total radioactivity attributed to metabolites [^{14}C]M18 and [^{14}C]M19, respectively. The major route of excretion of radioactivity was via feces, with approximately 94% of the administered radioactivity recovered in feces. Unchanged VEL was the major component excreted in feces and accounted for a mean of 77% of the administered radioactivity. Approximately 0.4% of the dose was recovered in urine through 24 hours postdose as unchanged VEL and metabolites. The results from bile duct-cannulated rats and dogs showed unchanged parent drug accounted for 11.7% and 55.5% of the radioactivity was recovered in bile, respectively, which supports the hypothesis that biliary excretion of parent drug is a major route of elimination for VEL in human. In agreement with the mass-balance study results, VEL AUC and Cmax were only 50% and 11% higher, respectively, in subjects with severe renal impairment compared with control subjects with normal renal function. Exploratory analyses showed no statistically significant correlation between eGFR and primary VEL PK parameters (AUC or Cmax).

2.2.5.6 What are the characteristics of drug metabolism?

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 or inducers 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.

Intracellular Metabolic Pathway of Sofosbuvir



VEL is a substrate of CYP2B6, CYP2C8, and CYP3A4 with slow turnover. Following a single dose of 100 mg [^{14}C]-VEL, the majority (greater than 98%) of radioactivity in plasma was parent drug. The monohydroxylated and desmethylated VEL were the metabolites identified in human plasma. Unchanged VEL is the major species present in feces.

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 AUCinf and Cmax 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 supratherapeutic (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 AUCinf and Cmax, and GS-331007 AUCinf, with GS-331007 Cmax 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.

Results of Phase 1 studies evaluating VEL plasma PK parameters following fasted single- and multiple-dose administration of VEL or SOF/VEL in healthy subjects indicate that VEL exposure is not dose proportional over a large dose range. VEL exposure increases in a greater than proportional manner from 5 to 50 mg and in a less than proportional manner from 50 to 450 mg, suggesting that VEL absorption is solubility or permeability limited. VEL PK was similar following administration of VEL single-agent tablet or SOF/VEL. However, the results from Phase 1b and Phase 2 studies in the patient population showed that VEL exhibited more than or near dose-proportional increases in exposure from 25 mg to 150 mg when coadministered with SOF. The mechanism behind the difference in the PK linearity of VEL between healthy volunteers and HCV-infected patients is unclear.

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

The PK of single- and multiple-ascending doses of SOF (100, 200, and 400 mg for 28 days; 100-mg tablet formulation) were evaluated in treatment-naive subjects with genotype 1 HCV infection. SOF and its metabolites exhibited time-independent PK with minimal accumulation (accumulation ratio is near 1).

The PK of single- and multiple-ascending doses of VEL (5, 25, 50, 100, and 150 mg) was evaluated in subjects with genotype 1a, 1b, 2, 3, or 4 HCV infection. VEL exhibited a time-independent PK profile across the evaluated dose range. Across all doses, accumulation was limited (≤ 44% for any exposure parameter), a finding consistent with the half-life of VEL (15 hours).

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, inter-subject variability expressed as percent coefficient of variation (%CV) was moderate for SOF oral clearance (CL/F) but high for apparent central volume of distribution (Vc/F).

Based on the population pharmacokinetic analysis, the inter-individual variability for the apparent oral clearance (CL/F) and apparent volume of distribution (Vc/F) was 48% and 95%, respectively. Hepatic impairment, sex, and food were identified as covariates that contributed significantly to the variability of CL/F.

Female subjects had a 14% lower CL/F than male subjects. Food was predicted to decrease CL/F by 30%. No additional causes of SOF variability were identified as significant from the population pharmacokinetic analysis. However, these sources of variability in PK parameters were not found to impact exposures to a clinically relevant extent in clinical efficacy trials. 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 (24%) for apparent oral clearance (CL/F) and moderate (44%) for apparent volume of distribution based on a population pharmacokinetic analysis.

This analysis identified a statistically significant impact of baseline creatinine clearance (4% drop in GS-331007 clearance for a 10% decrease in creatinine clearance), gender (16% lower CL/F in women), race (8% higher clearance in Hispanic or Latino patients), RBV use (23% increase in clearance with RBV use), and statin use (11% lower clearance) on the apparent clearance (CL/F).

Similarly, the analysis identified a significant impact of body weight, RBV use (46% increase in Vc/F), and gender (18% decrease in Vc/F in women) on Vc/F. However, these sources of variability in PK parameters were not found to impact exposures to a clinically relevant extent in clinical efficacy trials. No additional significant 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.

VEL
The inter-individual variability for VEL was 51% for apparent oral clearance (CL/F), 69% for apparent central volume of distribution (Vc/F), 51% for apparent peripheral volume of distribution (Vp/F), and 54% for Ka.

Sex, disease status (healthy volunteer versus HCV-infected), and hepatic impairment, were identified as significant covariates on CL/F. Sex, disease status, and hepatic impairment were identified as significant covariates on Vc/F.

Female gender was associated with a 30% lower CL/F and a 26% lower Vc/F compared to males. Healthy volunteers had a 38% lower CL/F compared to HCV-infected subjects. Moderate and severe hepatic impairment (CTP-B/C) was associated with 35% higher CL/F relative to normal hepatic function or mild hepatic impairment (CPT-A). Food decreases drug absorption rate (k_a) by about 35%. There was insufficient data available to characterize the intra-subject variability of VEL from the available pharmacokinetic data.

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?

Renal Impairment:

No dosage adjustment of SOF/VEL is required for patients with mild or moderate renal impairment. The safety and efficacy of SOF/VEL have not been established in patients with severe renal impairment (eGFR less than 30 mL/min/1.73m^2) or ESRD requiring hemodialysis. No dosage recommendation can be given for patients with severe renal impairment or ESRD. The following summarizes the rationale for the Agency's recommendation.

SOF:
The PK of SOF were studied in HCV-negative subjects with mild (eGFR ≥ 50 and < 80 mL/min/1.73 m2), moderate (eGFR ≥ 30 and < 50 mL/min/1.73 m^2), and severe renal impairment (eGFR < 30 mL/min/1.73 m^2) following a single dose of SOF 400 mg and subjects with ESRD requiring hemodialysis following a single dose of SOF 400 mg prior to dialysis and following a single dose of SOF 400 mg after dialysis (Study P7977-0915). Compared with subjects with normal renal function (eGFR > 80 mL/min/1.73 m2), the SOF AUCinf was approximately 61%, 107%, and 171% higher and the GS-331007 AUCinf was approximately 55%, 88% and 451% higher in subjects with mild, moderate, and severe renal impairment, respectively (Study P7977-0915). In subjects with ESRD, compared with subjects with normal renal function, SOF and GS-331007 AUCinf was approximately 28% and 1283% higher when SOF was dosed 1 hour before hemodialysis compared with approximately 60% and 2072% higher when SOF was dosed 1 hour after hemodialysis.

VEL:
No dose adjustment is warranted for VEL in subjects with mild, moderate, or severe renal impairment. However, because SOF is not recommended for patients with severe renal impairment or ESRD, SOF/VEL FDC should not be used in patients with severe renal impairment or ESRD.

The effect of severe renal impairment (eGFR: < 30 mL/min) on the PK of VEL 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) (Study GS-US-281-1056). The following table presents VEL plasma PK parameters following the single-dose administration of VEL 100 mg in subjects with severe renal impairment compared with subjects with normal renal function.

GS-US-281-1056: Effect of Severe Renal Impairment on the PK of VEL (Source: Study Report for GS-US-281-1056)

VEL PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI)[a]
	Severe Renal Impairment (N = 10)	Normal Renal Function (N = 9)	
AUC_{inf} (h*ng/mL)	8108.3 (32.4)	5651.6 (31.2)	149.9 (117.0, 192.1)
AUC_{last} (h*ng/mL)	7971.7 (31.8)	5597.8 (31.2)	149.1 (116.6, 190.5)
C_{max} (ng/mL)	732.4 (24.1)	702.7 (28.1)	110.9 (90.8, 135.4)

a Matched pairs only (N = 9/9) were used in the comparison analysis.
%GLSM ratios (90% CI) are expressed as impaired/normal, with an increase in 90% CI for PK parameters of ≥100% considered significant.

Although VEL is eliminated primarily in the feces, results of the renal impairment study were consistent with the current knowledge that renal impairment can alter the PK of compounds by changes in intestinal and hepatic metabolism and transport. Exploratory analyses showed no statistically significant correlation between eGFR and primary VEL PK parameters (AUC or Cmax). Since the exposure of VEL was not significantly impacted in the setting of severe renal impairment, evaluation of VEL PK in subjects with mild or moderate renal impairment was not specifically conducted.

In HCV-infected subjects in the population PK analyses, the median (first quartile [Q1], third quartile [Q3]) CLcr was 105.4 (87.2, 125.6) mL/min, with a range of 38.7 to 253.2 mL/min. The following table presents VEL PK parameters by CLcr (calculated by the Cockcroft-Gault equation) following administration of VEL and SOF in HCV-infected subjects.

VEL PK Parameters across Quartiles of Creatinine Clearance Following Administration of VEL and SOF in HCV-Infected Subjects (Source: Ad Hoc Table 7556.3)

VEL PK Parameter[a]	Mean (%CV)			
	Creatinine Clearance (mL/min) [min, median, max]			
	Q1 (N = 359)	Q2 (N = 358)	Q3 (N = 359)	Q4 (N = 358)
	[38.7, 77.1, 87.2]	[87.2, 96.8, 105.4]	[105.4, 114.9, 125.6]	[125.6, 143.6, 253.2]
Sex, N (%)	Female: 224 (62.4) Male: 135 (37.6)	Female: 134 (37.4) Male: 224 (62.6)	Female: 106 (29.5) Male: 253 (70.5)	Female: 103 (28.8) Male: 255 (71.2)
AUC_{tau} (h*ng/mL)	3362 (47.6)[a]	2901 (48.3)[b]	2789 (50.5)[c]	2819 (52.3)
C_{max} (ng/mL)	295 (51.3)[a]	253 (52.0)[b]	245 (55.1)[c]	243 (54.9)
C_{tau} (ng/mL)	48 (61.1)[a]	40 (62.5)[b]	38 (65.8)[c]	39 (68.2)

Analyses included all HCV-infected subjects who received SOF 400 mg and VEL 100 mg as either single agents or a FDC in Study GS-US-337-0122, GS-US-342-0102, GS-US-342-0109, GS-US-342-1138, GS-US-342-1139, or GS-US-342-1140.
a N = 356
b N = 354
c N = 357

Population PK modeling did not identify CLcr as a clinically relevant covariate for VEL. VEL exposure parameters (AUCtau, Cmax, and Ctau) were slightly higher in Q1. This is reasonable because subjects in Q2 to Q4 are mainly subjects with normal renal function, while Q1 represents subjects with mild and moderate renal impairment. This finding is consistent with the results from the renal impairment study. It needs to be noted that there was a higher proportion of females within Q1 relative to the other quartiles, which drove the higher VEL exposures in Q1 (see following Demographic Factors section for the effect of gender on the PK of VEL) .

SOF/VEL: Although a dedicated renal impairment study was not conducted for the combination of SOF and VEL, the effect of renal impairment on the PK of SOF and VEL when administered as individual agents or as SOF/VEL FDC tablet is expected to be similar. Based on the following considerations, no dose adjustment is required for SOF/VEL for patients with mild or moderate renal impairment while no dose recommendations can be made for patients with a worse degree of renal impairment.

- The safety and efficacy of SOF/VEL have not been established in subjects with severe renal impairment (eGFR < 30 mL/min) or ESRD requiring hemodialysis.

- Only limited data are available from subjects with moderate renal impairment in Phase 2/3 trials. Subjects with an $eGFR_{CG}$ of < 50 mL/min (Study GS-US-342-1137) or < 60 mL/min and (Studies GS-US-342-1138, GS-US-342-1139, and GS-US-342-1140) at screening were excluded from SOF/VEL Phase 3 studies. In 3 Phase 2 trials, Subjects with an $eGFR_{CG}$ of < 60 mL/min at screening were also excluded. Therefore, most of the patients in Phase 2 and 3 had normal renal function or mild renal impairment; Across all Phase 2 and 3 trials, only 53 out of 1434 subjects (3.7%) had an $eGFR_{CG}$ of < 60 mL/min, with the majority of these subjects (42) having an $eGFR_{CG}$ between 50 to 60 mL/min. Only 1 subject had an $eGFR_{CG}$ of < 30 mL/min. Supporting data are available from Harvoni® (LDV/SOF) program. For SOF/VEL FDC, VEL increases SOF AUC by approximately 2.4-fold, due to the inhibition of P-gp and BCRP, which is similar to the effect of LDV on SOF AUC. LDV/SOF has been approved for use in HCV patients with mild or moderate renal impairment. A Phase 2 study (GS-US-334-0154) evaluating the safety, efficacy, and PK of SOF and ledipasvir (LDV/SOF in subjects with severe renal impairment or ESRD) is ongoing.

Hepatic Impairment

SOF/VEL FDC can be administered in patients with mild, moderate, or severe hepatic impairment. The following summarizes the rationale for the Agency's recommendation.

SOF:
The PK of SOF and GS-331007 were evaluated following the multiple-dose administration of SOF for 7 days of SOF in HCV-infected subjects with moderate (CPT B) and severe (CPT C) hepatic impairment.

Compared with subjects with normal hepatic function, the SOF AUCtau was 126% and 143% higher in moderate and severe hepatic impairment and the GS-331007 AUCtau was 18% and 9% higher, respectively (Study P2938-0515).

VEL:
The effects of moderate (CPT B) and severe (CPT C) hepatic impairment on the PK of VEL were evaluated in non-HCV-infected subjects and a matching cohort of subjects with normal

hepatic function (matched for age, sex, and BMI) (Study GS-US-281-0112). Subjects received a single dose of VEL 100 mg.

The following table shows the effect of hepatic impairment on the PK of VEL in subjects with moderate and severe hepatic impairment compared with subjects with normal hepatic function.

GS-US-281-0112: Effect of Moderate and Severe Hepatic Impairment on the PK of VEL
(Source: Sponsor's Study Report)

VEL PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI)
	Hepatic Impairment	Normal Hepatic Function	
Moderate Hepatic Impairment, N	10	10	
AUC_{inf} (h*ng/mL)	4104.6 (37.9)	5199.0 (42.5)	83.03 (57.53, 119.83)
C_{max} (ng/mL)	343.8 (49.0)	582.9 (36.5)	59.41 (39.78, 88.71)
Severe Hepatic Impairment, N	10	10	
AUC_{inf} (h*ng/mL)	5403.7 (50.3)	4619.4 (41.0)	113.64 (74.72, 172.82)
C_{max} (ng/mL)	268.4 (54.5)	523.5 (35.1)	47.19 (29.32, 75.96)

%GLSM ratios (90% CI) are expressed as impaired/normal, with an increase in 90% CI for PK parameters of ≥ 100% considered significant.

Hepatic impairment had no clinically relevant impact on VEL PK. Similar AUCinf and a modestly lower Cmax (approximately 41% to 53%) were observed in subjects with hepatic impairment compared with subjects with normal hepatic function. A reduction in Cmax in the absence of a change in AUC was not deemed clinically important. The mean percent free fraction (unbound concentrations) for VEL increased with severity of hepatic impairment; it was approximately 0.5% in subjects with normal hepatic function and 0.7% and 1.2% in subjects with moderate and severe hepatic impairment, respectively. Exploratory analyses indicated no clinically significant correlations between various measures of hepatic function at baseline (CPT scores, serum albumin, total bilirubin, prothrombin time, and international normalized ratio) and overall VEL exposure (AUC).

SOF/VEL:
The effect of hepatic impairment on the PK of SOF and VEL when administered as SOF/VEL FDC tablet was evaluated based on Population PK Analyses from Phase 2 and 3 Studies. The following tables present SOF, GS-331007 and VEL PK parameters following once-daily administration of VEL and SOF in HCV-infected subjects with and without cirrhosis.

SOF and GS-331007 PK Parameters by Cirrhosis Status Following Once-Daily Administration of VEL and SOF in HCV-Infected Subjects (Population PK Analysis from Phase 2 and 3 Studies, Source: Sponsor's Summary of Clinical Pharmacology)

PK Parameter	Mean (%CV)		%GMR (90% CI)
	Cirrhotic	Noncirrhotic	
Compensated Cirrhosis (CPT A)			
SOF	**N = 209**	**N = 769**	
AUC_{tau} (h*ng/mL)	1298 (37.7)	1252 (37.0)	102.9 (98.3, 107.6)
C_{max} (ng/mL)	574 (32.5)	565 (31.2)	101.2 (97.1, 105.4)
GS-331007	**N = 287**	**N = 1135**	
AUC_{tau} (h*ng/mL)	13,116 (27.1)	14,189 (28.0)	92.5 (89.9, 95.3)
C_{max} (ng/mL)	830 (28.8)	878 (27.3)	94.2 (91.4, 97.0)
Decompensated Cirrhosis (CPT B)			
SOF	**N = 206**	**N = 769**	
AUC_{tau} (h*ng/mL)	2219 (35.9)	1252 (37.0)	177.3 (169.5, 185.4)
C_{max} (ng/mL)	755 (34.5)	565 (31.2)	131.4 (125.9, 137.2)
GS-331007	**N = 266**	**N = 1135**	
AUC_{tau} (h*ng/mL)	13,856 (38.1)	14,189 (28.0)	94.7 (91.7, 97.8)
C_{max} (ng/mL)	819 (36.3)	878 (27.3)	90.9 (88.0, 93.9)

Analyses included all HCV-infected subjects who received SOF 400 mg and VEL 100 mg as either single agents or a FDC in Study GS-US-337-0122, GS-US-342-0102, GS-US-342-0109, GS-US-342-1137, GS-US-342-1138, GS-US-342-1139, or GS-US-342-1140.
Subjects with missing cirrhotic status were excluded from this analysis.

VEL PK Parameters by Cirrhosis Status Following Once-Daily Administration of VEL and SOF in HCV-Infected Subjects (Population PK Analysis from Phase 2 and 3 Studies, Source: Applicant's Summary of Clinical Pharmacology)

VEL PK Parameter	Mean (%CV)		%GMR (90% CI)
	Cirrhotic	Noncirrhotic	
Compensated Cirrhosis (CPT A)	**N = 285**	**N = 1134**	
AUC_{tau} (h*ng/mL)	2748 (52.2)	3027 (49.6)	90.8 (86.1, 95.8)
C_{max} (ng/mL)	235 (53.3)	265 (53.7)	89.0 (83.9, 94.5)
C_{tau} (ng/mL)	38 (69.8)	42 (63.8)	91.2 (85.7, 97.1)
Decompensated Cirrhosis (CPT B)	**N = 266**	**N = 1134**	
AUC_{tau} (h*ng/mL)	2183 (55.8)	3027 (49.6)	70.1 (66.3, 74.2)
C_{max} (ng/mL)	145 (59.4)	265 (53.7)	53.4 (50.2, 56.9)
C_{tau} (ng/mL)	44 (57.0)	42 (63.8)	105.8 (99.3, 112.8)

Analyses included all HCV-infected subjects who received SOF 400 mg and VEL 100 mg as either single agents or a FDC in Study GS-US-337-0122, GS-US-342-0102, GS-US-342-0109, GS-US-342-1137, GS-US-342-1138, GS-US-342-1139, or GS-US-342-1140.

Compensated cirrhosis did not have an effect on SOF or GS-331007 exposures in HCV-infected subjects and was not a clinically relevant covariate based on population PK analyses. Study GS-US-342-1137 evaluated subjects who had decompensated (CPT B) cirrhosis only. In the Phase 2 and 3 study population used for population PK analyses, decompensated cirrhosis was

identified as a statistically significant covariate for SOF, but not GS-331007. Compared with subjects without cirrhosis, subjects with decompensated cirrhosis had higher SOF exposure (AUCtau: 77.3%↑, Cmax: 31.4%↑) and similar GS-331007 exposures. These results are consistent with the findings from the dedicated hepatic impairment study for SOF, which demonstrated modestly higher SOF exposure in subjects with moderate and severe hepatic impairment and no significant change in the PK of GS-331007 (Study P2938-0515). As these increased exposures were not clinically relevant, no dose adjustment of SOF is recommended for patients with mild, moderate, or severe hepatic impairment.

Compensated cirrhosis did not have a significant effect on VEL exposures in HCV-infected subjects and was not a clinically relevant covariate based on population PK analyses. VEL exposure parameters were approximately 10% lower in subjects with compensated cirrhosis compared with subjects without cirrhosis. Compared with subjects without cirrhosis in the Phase 2 and 3 study population, subjects with decompensated cirrhosis (Study GS-US-342-1137) had lower VEL Cmax (47%↓) and modestly lower VEL AUCtau (30%↓), with no significant change in Ctau (%GMR: 105.8%). These results are consistent with the findings from the dedicated hepatic impairment study for VEL, which demonstrated a decrease in VEL Cmax and a prolonged t1/2 in subjects with moderate (CPT B) or severe (CPT C) hepatic impairment (Study GS-US-281-0112). Although 30% reduction on VEL AUCtau was observed in subjects with decompensated cirrhosis, high response rates observed in this population and lack of any definitive PK/PD relationship (AUCtau vs SVR12) in Study GS-US-342-1137 as well across the overall population in the Phase 2 or 3 studies suggest that the decrease in VEL AUC and Cmax is not clinically meaningful. Accordingly, no dose adjustment for VEL is recommended for patients with mild, moderate, or severe hepatic impairment.

Demographic Factors

Several demographic factors, such as age, gender, race, and body mass index (BMI), have been evaluated to determine if these factors have effect on the PK of SOF, GS-331007 and VEL. No effect has been found for age, race or BMI. Gender was identified as a statistically significant covariate for SOF, GS-331007, and VEL PK, based on the population PK analyses. SOF AUCtau and Cmax in female subjects are approximately 19% and 18% higher, respectively, compared with male subjects. Female subjects had approximately 27% to 28% higher AUCtau and Cmax for GS-331007, respectively, compared with male subjects. VEL AUCtau, Cmax, and Ctau were approximately 47%, 43%, and 69% higher in female subjects compared with male subjects. Considering the favorable safety profile of SOF/VEL and high response rates in male and female subjects (SVR12 rates of 97.3% and 99.3%, respectively), the relationships between sex and the exposures of SOF, GS-331007 or VEL were not considered clinically relevant.

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 VEL are substrates for P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) while GS-331007 is not. In addition, VEL is a substrate for CYP2B6, CYP2C8 and CYP3A with slow turnover. Drugs that are inducers of P-gp may decrease plasma concentrations of SOF and VEL, while drugs that are inducers of either P-gp or CYP2B6, CYP2C8, or CYP3A4 may decrease plasma concentrations of VEL. In addition, in vitro studies showed that VEL is not a substrate for OATP1B1 or OATP1B3. However, a clinical study with a single dose of the perpetrator drug rifampin indicates that VEL can be modestly affected by OATP1B1 and/or OATP1B3 inhibition. There are no known BCRP or OATP1B inducers at present. Drugs that inhibit CYP2B6, CYP2C8, or CYP3A4 may increase the plasma concentrations of velpatasvir.

VEL is an inhibitor of drug transporter P-gp, BCRP, and OATP2B1 and may increase intestinal absorption of coadministered substrates for these transporters. The absorption potential of SOF and VEL in the context of SOF/VEL has been studied in vitro by assessing the effect of VEL 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 VEL. Results suggest that SOF intestinal absorption may be increased in the context of the SOF/VEL FDC tablet due to inhibition of intestinal transporters by VEL, which was observed *in vivo*. In addition, VEL is an inhibitor of OATP1B1 and OATP1B3. Coadministration of SOF/VEL with drugs that are substrates of these transporters may increase the exposure of such drugs.

The in vivo drug interactions are discussed in sections 2.4.2.4 and 2.4.2.8.

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. See the Clinical Pharmacology review for NDA 204671 for additional details.

The in vitro metabolic stability of VEL is consistent with the slow rates of hepatic biotransformation observed during PK studies. In vitro, slow metabolic turnover of VEL by CYP2B6, CYP2C8, and CYP3A4 was observed. Poor metabolizers of these enzymes may have higher VEL concentrations. However, because of the slow metabolic turnover of VEL by these enzymes and the great safety margin for SOF/VEL, we do not expect a significant effect of the polymorphism of these enzymes.

2.4.2.3 Is the drug an inhibitor and/or inducer of CYP enzymes or UGT1A1?

SOF and GS-331007 are not inhibitors or inducers of CYP or UGT1A1 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).

VEL does not inhibit the activities of any of the tested human enzymes, including CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4 and UGT1A1. Small increases in CYP2B6 and 3A4 activity and mRNA levels was observed for VEL in vitro at the highest concentration tested of 10 µM were

less than 20% of those caused by the positive controls. No evidence for CYP-based induction has been observed for VEL during clinical drug-drug interaction studies.

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.

Sofosbuvir and GS-331007 are not inhibitors of drug transporters P-gp, BCRP, OATP1B1, OATP1B3 and OCT1 and GS-331007 is not an inhibitor of OAT1, OAT3, OCT2, and MATE1.

VEL is a substrate of drug transporters P-gp and BCRP. An *in vitro* study showed that VEL is not a substrate of OATP1B1 or OATP1B3. However, a clinical study with a single dose of the perpetrator drug rifampin indicates that VEL is weakly affected by OATP1B1 and/or OATP1B3.

VEL is an inhibitor of drug transporter P-gp, BCRP, OATP2B1, OATP1B1 and OATP1B3. At clinically relevant concentrations, VEL is not an inhibitor of hepatic transporters OATP1A2 or OCT1, renal transporters OCT2, OAT1, OAT3 or MATE1.

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.

CES1 based drug interaction potential was also evaluated in vitro between SOF and irinotecan. Irinotecan is hydrolyzed by CES1 to a putatively active metabolite, SN-38. The results show no clinically relevant CES1 based drug-drug interactions are expected between SOF and irinotecan.

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?

Yes. SOF/VEL as a FDC will be coadministered with ribavirin when used in HCV-infected patients with decompensated cirrhosis. Population PK analyses indicated that ribavirin use had no effects on the exposures of SOF or VEL, but has an effect on GS-331007 exposures. Ribavirin use decreased GS-331007 AUC, Cmax and Cmin by 20%, 25% and 18%, respectively. Because coadministration of SOF/VEL with ribavirin has been studied in Phase 2/3 trials, the magnitudes of reduction of GS-331007 exposures are not considered clinically significant.

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

Medications that are likely to be co-administered in HCV-infected patients include antiretroviral agents for the treatment of HIV, analeptics, antiarrythmics, anticonvulsants, antimycobacterials, HMG-CoA reductase inhibitors, methadone, immunosuppressants, antidepressants and other mood-stability medications, combined oral contraceptives, acid-reducing agents, and some

herbal supplements. DDI studies have been conducted with SOF, VEL, or SOF/VEL in combination with these drugs, which are discussed in detail in 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, VEL, or SOF/VEL in combination with representative antiretrovirals, and other drugs as victims or perpetrators. The tables shown in this section show DDIs that are statistically significant (90% confidence interval is outside of 80%-125%). A list of reviewer-proposed established or potentially clinically significant drug interactions is shown in the Summary section.

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

The following table shows statistically significant changes in PK parameters for SOF, GS-331007, and VEL in the presence of the coadministered drug(s)

Drug Interactions: Changes in Pharmacokinetic Parameters for Sofosbuvir, GS-331007, and Velpatasvir in the Presence of the Coadministered Drug[a]

Coadministered Drug	Dose of Coadministered Drug (mg)	VEL Dose (mg)	SOF Dose (mg)	N	Mean Ratio (90% CI) of SOF, GS-331007 and VEL PK With/Without Coadministered Drug No Effect=1.00			
						C_{max}	AUC	C_{min}
Atazanavir/ ritonavir + emtricitabine/ tenofovir DF	300/100 + 200/300 once daily	100 once daily	400 once daily	24	SOF	1.12 (0.97,1.29)	1.22 (1.12, 1.33)	NA
					GS-331007	1.21 (1.12, 1.29)	1.32 (1.27, 1.36)	1.42 (1.37, 1.49)
					VEL	1.55 (1.41, 1.71)	2.42 (2.23, 2.64)	4.01 (3.57, 4.50)
Cyclosporine	600 single dose	ND	400 single dose	19	SOF	2.54 (1.87, 3.45)	4.53 (3.26, 6.30)	NA
					GS-331007	0.60 (0.53, 0.69)	1.04 (0.90, 1.20)	NA
		100 single dose	ND	12	VEL	1.56 (1.22, 2.01)	2.03 (1.51, 2.71)	NA
Darunavir/ ritonavir + emtricitabine/ tenofovir DF	800/100 + 200/300 once daily	100 once daily	400 once daily	29	SOF	0.62 (0.54, 0.71)	0.72 (0.66, 0.80)	NA
					GS-331007	1.04 (0.99,1.08)	1.13 (1.08, 1.18)	1.13 (1.06, 1.19)
					VEL	0.76 (0.65, 0.89)	0.84 (0.72, 0.98)	1.01 (0.87, 1.18)
Efavirenz/ emtricitabine/ tenofovir DF[b]	600/200/300 once daily	100 once daily	400 once daily	14	SOF	1.38 (1.14, 1.67)	0.97 (0.83, 1.14)	NA
					GS-331007	0.86 (0.80, 0.93)	0.90 (0.85, 0.96)	1.01 (0.95, 1.07)
					VEL	0.53 (0.43, 0.64)	0.47 (0.39, 0.57)	0.43 (0.36, 0.52)
Elvitegravir/ cobicistat/ emtricitabine/ tenofovir	150/150/200/10 once daily	100 once daily	400 once daily	23	SOF	1.23 (1.07, 1.42)	1.37 (1.24, 1.52)	NA
					GS-331007	1.29 (1.25, 1.33)	1.48 (1.43, 1.53)	1.58 (1.52, 1.65)

alafenamide[c]					VEL	1.30 (1.17, 1.45)	1.50 (1.35, 1.66)	1.60 (1.44, 1.78)
Elvitegravir/ cobicistat/ emtricitabine/ tenofovir DF[d]	150/150/200/300 once daily	100 once daily	400 once daily	24	SOF	1.01 (0.85, 1.19)	1.24 (1.13, 1.37)	NA
					GS-331007	1.13 (1.07, 1.18)	1.35 (1.30, 1.40)	1.45 (1.38, 1.52)
					VEL	1.05 (0.93, 1.19)	1.19 (1.07, 1.34)	1.37 (1.22, 1.54)
Famotidine	40 single dose simultaneously with SOF/VEL	100 single dose	400 single dose	60	SOF	0.92 (0.82, 1.05)	0.82 (0.74, 0.91)	NA
					GS-331007	0.84 (0.78, 0.89)	0.94 (0.91, 0.98)	NA
					VEL	0.80 (0.70, 0.91)	0.81 (0.71, 0.91)	NA
	40 single dose 12 hours prior to SOF/VEL			60	SOF	0.77 (0.68, 0.87)	0.80 (0.73, 0.88)	NA
					GS-331007	1.20 (1.13, 1.28)	1.04 (1.01, 1.08)	NA
					VEL	0.87 (0.76, 1.00)	0.85 (0.74, 0.97)	NA
Ketoconazole	200 twice daily	100 single dose	ND	12	VEL	1.29 (1.02, 1.64)	1.71 (1.35, 2.18)	NA
Lopinavir/ ritonavir + emtricitabine/ tenofovir DF	4x200/50 + 200/300 once daily	100 once daily	400 once daily	24	SOF	0.59 (0.49, 0.71)	0.71 (0.64, 0.78)	NA
					GS-331007	1.01 (0.98, 1.05)	1.15 (1.09, 1.21)	1.15 (1.07, 1.25)
					VEL	0.70 (0.59, 0.83)	1.02 (0.89, 1.17)	1.63 (1.43, 1.85)
Methadone	30 to 130 daily	ND	400 once daily	14	SOF	0.95 (0.68, 1.33)	1.30 (1.00, 1.69)	NA
					GS-331007	0.73 (0.65, 0.83)	1.04 (0.89, 1.22)	NA
Omeprazole	20 once daily simultaneously with SOF/VEL	100 single dose fasted	400 single dose fasted	60	SOF	0.66 (0.55, 0.78)	0.71 (0.60, 0.83)	NA
					GS-331007	1.18 (1.10, 1.26)	1.00 (0.95, 1.05)	NA
					VEL	0.63 (0.50, 0.78)	0.64 (0.52, 0.79)	NA
	20 once daily 12 hours prior to SOF/VEL	100 single dose fasted	400 single dose fasted	60	SOF	0.55 (0.47, 0.64)	0.56 (0.49, 0.65)	NA
					GS-331007	1.26 (1.18, 1.34)	0.97 (0.94, 1.01)	NA
					VEL	0.43 (0.35, 0.54)	0.45 (0.37, 0.55)	NA
	20 once daily 2 hours prior to SOF/VEL	100 single dose fed[e]	400 single dose fed[e]	40	SOF	0.84 (0.68, 1.03)	1.08 (0.94, 1.25)	NA
					GS-331007	0.94 (0.88, 1.02)	0.99 (0.96, 1.03)	NA
					VEL	0.52 (0.43, 0.64)	0.62 (0.51, 0.75)	NA
	20 once daily 4 hours after	100 single	400 single	38	SOF	0.79 (0.68, 0.92)	1.05 (0.94, 1.16)	NA

	SOF/VEL	dose fed[e]	dose fed[e]		GS-331007	0.91 (0.85, 0.98)	0.99 (0.95, 1.02)	NA
					VEL	0.67 (0.58, 0.78)	0.74 (0.63, 0.86)	NA
	40 once daily 4 hours after SOF/VEL	100 single dose fed[e]	400 single dose fed[e]	40	SOF	0.70 (0.57, 0.87)	0.91 (0.76, 1.08)	NA
					GS-331007	1.01 (0.96, 1.07)	0.99 (0.94, 1.03)	NA
					VEL	0.44 (0.34, 0.57)	0.47 (0.37, 0.60)	NA
Rifampin	600 once daily	ND	400 single dose	17	SOF	0.23 (0.19, 0.29)	0.28 (0.24, 0.32)	NA
					GS-331007	1.23 (1.14, 1.34)	0.95 (0.88, 1.03)	NA
		100 single dose	ND	12	VEL	0.29 (0.23, 0.37)	0.18 (0.15, 0.22)	NA
	600 single dose	100 single dose	ND	12	VEL	1.28 (1.05, 1.56)	1.46 (1.17, 1.83)	NA
Tacrolimus	5 single dose	ND	400 single dose	16	SOF	0.97 (0.65, 1.43)	1.13 (0.81, 1.57)	NA
					GS-331007	0.97 (0.83, 1.14)	1.00 (0.87, 1.13)	NA

NA = not available/not applicable, ND = not dosed.

a. All interaction studies conducted in healthy volunteers.
b. Administered as Atripla (efavirenz, emtricitabine and tenofovir DF fixed-dose combination).
c. Administered as Genvoya (elvitegravir, cobicistat, emtricitabine and tenofovir alafenamide fixed-dose combination).
d. Administered as Stribild (elvitegravir, cobicistat, emtricitabine and tenofovir DF fixed-dose combination).
e. SOF/VEL was administered under fasted conditions in the reference arms.

No effect on the pharmacokinetic parameters of SOF, GS-331007 or VEL was observed with dolutegravir and the combination of emtricitabine, rilpivirine, and tenofovir DF; or emtricitabine, raltegravir, and tenofovir DF.

Clinical Implication of the Effect of the Coadministered Drugs on SOF/VEL:

As stated in Section 2.4.2.1, SOF is a substrate of P-gp and BCRP. Therefore, inhibitors of P-gp or BCRP may increase SOF concentrations while inducers of P-gp may decrease SOF concentrations. The effects of coadministered drugs on SOF and GS-331007 when SOF is used as a single agent were reviewed previously for the Sovaldi® NDA. The effects of coadministered P-gp/BCRP inhibitors or P-gp inducers on SOF and GS-331007 when SOF was used with VEL were generally similar to the effects observed when SOF was used with ledipasvir (LDV). Therefore, there were sufficient safety data to support the use of SOF with P-gp/BCRP inhibitors without dose adjustment, and the warning and precaution for not recommending the coadministration of SOF with P-gp inducers should be kept for SOF/VEL FDC. Reduced SOF AUC and Cmax have been observed when SOF/VEL was coadministered with omeprazole, which was not observed when LDV/SOF was coadministered with omeprazole. The mechanism of this difference is not clear.

As stated in Section 2.4.2.1, VEL is a substrate of P-gp and BCRP. In addition, VEL is a substrate of CYP2B6, CYP2C8 and CYP3A with slow turnover. The % metabolism rate of the

positive control was 6.6%, 5.5% and 18% for CYP2B6, CYP2C8 and CYP3A, respectively. Therefore, strong CYP3A and P-gp inhibitors, such as atazanavir/ritonavir and cyclosporine, increased VEL concentration to the highest extent. The agency asked the applicant to provide the long-term safety data for SOF/VEL in humans to support the coadministration of SOF/VEL with strong CYP3A or P-gp inhibitors. The following safety data were provided by the applicant:

- In Phase 3 trials (ASTRAL-1, -2, -3, and -4), the following CYP3A and/or P-gp inhibitors were used as concomitant medications: azithromycin, carvedilol, clarithromycin, erythromycin, felodipine, fluvoxamine, ketoconazole, quercetin, and verapamil.
    - A total of 36 subjects used a CYP3A and/or P-gp inhibitor chronically (>14 days) and 33 subjects reported short term (≤ 14 days) use.
- In ASTRAL-5 (HIV/HCV coinfection), safety was compared based on the preliminary data from 20 subjects receiving ATV/r and 86 not on ATV/r

Based on the preliminary safety assessment by the clinical reviewers, the data provided do not suggest safety concerns with the coadministration of SOF/VEL and ATV/r or other strong CYP3A or P-gp inhibitors. Therefore, no dose adjustment is required when SOF/VEL is coadministered with boosted ATV or other strong CYP3A and/or P-gp inhibitors.

The impact of inducers of P-gp or potent or moderate inducers of CYP2B6, CYP2C8, or CYP3A4 on the PK of SOF/VEL was evaluated with rifampin (a strong/moderate inducer of CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A and P-gp) or efavirenz (a moderate inducer of CYP2B6 and CYP3A). A decrease in VEL AUC by 82% and 53% was observed when VEL was coadministered with rifampin and efavirenz, respectively. Inducers of P-gp or potent or moderate inducers of CYP2B6, CYP2C8, or CYP3A4 were not permitted in Phase 2 or 3 trials. Coadministration of drugs that are inducers of P-gp and/or moderate to potent inducers of CYP2B6, CYP2C8, or CYP3A4 with SOF/VEL is not recommended.

A 46% increase of VEL AUC was observed when VEL was coadministered with OATP1B1/3 inhibitor rifampin (single dose), although the in vitro study indicated that VEL is not a substrate of OATP1B1 or OATP1B3. The magnitude of increase in VEL AUC when coadministered with the OATP1B1/3 probe inhibitor was not considered clinically significant.

Drug-drug interactions were evaluated between omeprazole (OME) and SOF/VEL under the following administration conditions: simultaneous, 12 hours staggered administration, OME administered 2 hours prior to SOF/VEL, and OME administered 4 hours after SOF/VEL, under fasted or fed conditions. Two doses of OME were used: 20 mg or 40 mg. The studies show that greater effects of OME on VEL concentrations were observed when OME was staggered with SOF/VEL or when higher OME doses were used. In addition, when SOF/VEL was administered under fed conditions, increased VEL concentration caused by food reduced the overall effect of OME on VEL. The lowest reduction of VEL was observed when 20 mg OME was used and SOF/VEL was administered under fed conditions. Food also increases SOF concentrations. Under fasted conditions, OME reduced SOF AUC and Cmax by 29%-44%, and 34%-45%, respectively. Under fed conditions, administration of SOF/VEL with food and omeprazole (regardless of timing or dose of omeprazole) resulted in similar overall AUC of SOF, as compared to when SOF/VEL was administered alone under fasted conditions, .The applicant proposes (b) (4) As indicated in Sections 1.3 and 2.2.4.1, although no dose-response relationship was observed for treatment-naïve subjects across all genotypes and treatment-experienced subjects with genotype 1, higher efficacy was

observed in SOF 400 mg + VEL 100 mg (SVR12: 94%) compared to SOF 400 mg + VEL 25 mg (SVR12: 71%) in genotype 3, treatment-experienced subjects (Phase 2 study GS-US-342-0109). Because patients will not be screened for genotype, (b) (4) reduction in VEL concentrations may reduce the efficacy of SOF/VEL. Therefore, coadministration of PPIs with SOF/VEL should not be recommended.

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

The following table shows the statistically significant effects of SOF, VEL or SOF/VEL on coadministered drugs.

Changes in Pharmacokinetic Parameters for Coadministered Drug in the Presence of SOF, VEL, or SOF/VEL[a]

Co-administered Drug	Dose of Co-administered Drug (mg)	VEL dose (mg)	SOF Dose (mg)	N	Mean Ratio (90% CI) of Coadministered drug PK With/Without SOF, VEL or SOF/VEL No Effect=1.00		
					C_{max}	AUC	C_{min}
Atazanavir/ ritonavir + emtricitabine/ tenofovir DF[b]	atazanavir 300 once daily	100 once daily	400 once daily	24	1.09 (1.00, 1.19)	1.20 (1.10, 1.31)	1.39 (1.20, 1.61)
	ritonavir 100 once daily				0.89 (0.82, 0.97)	0.97 (0.89, 1.05)	1.29 (1.15, 1.44)
	emtricitabine 200 once daily				1.01 (0.96, 1.06)	1.02 (0.99, 1.04)	1.06 (1.02, 1.11)
	tenofovir DF 300 once daily				1.55 (1.43, 1.68)	1.30 (1.24, 1.36)	1.39 (1.31, 1.48)
Darunavir/ ritonavir + emtricitabine/ tenofovir DF[c]	darunavir 800 once daily	100 once daily	400 once daily	29	0.90 (0.86, 0.95)	0.92 (0.87, 0.98)	0.87 (0.79, 0.95)
	ritonavir 100 once daily				1.07 (0.97, 1.17)	1.12 (1.05, 1.19)	1.09 (1.02, 1.15)
	emtricitabine 200 once daily				1.05 (1.01, 1.08)	1.05 (1.02, 1.08)	1.04 (0.98, 1.09)
	tenofovir DF 300 once daily				1.55 (1.45, 1.66)	1.39 (1.33, 1.44)	1.52 (1.45, 1.59)
Digoxin	0.25 single dose	100	ND	21	1.88 (1.71, 2.08)	1.34 (1.13, 1.60)	NA
Efavirenz/ emtricitabine/ tenofovir DF[d]	efavirenz 600 once daily	100 once daily	400 once daily	15	0.81 (0.74, 0.89)	0.85 (0.80, 0.91)	0.90 (0.85, 0.95)
	emtricitabine 200 once daily				1.07 (0.98, 1.18)	1.07 (1.00, 1.14)	1.10 (0.97, 1.25)
	tenofovir DF 300 once daily				1.77 (1.53, 2.04)	1.81 (1.68, 1.94)	2.21 (2.00, 2.43)
Elvitegravir/ cobicistat/ emtricitabine/ tenofovir alafenamide[e]	elvitegravir 150 once daily	100 once daily	400 once daily	24	0.87 (0.80, 0.94)	0.94 (0.88, 1.00)	1.08 (0.97, 1.20)
	cobicistat 150 once daily				1.16 (1.09, 1.23)	1.30 (1.23, 1.38)	2.03 (1.67, 2.48)
	emtricitabine 200 once daily				1.02 (0.97, 1.06)	1.01 (0.98, 1.04)	1.02 (0.97, 1.07)
	tenofovir alafenamide 10 once daily				0.80 (0.68, 0.94)	0.87 (0.81, 0.94)	NA
Elvitegravir/ cobicistat/ emtricitabine/ tenofovir DF[f]	elvitegravir 150 once daily	100 once daily	400 once daily	24	0.93 (0.86, 1.00)	0.93 (0.87, 0.99)	0.97 (0.91, 1.04)
	cobicistat 150 once daily				1.11 (1.06, 1.17)	1.23 (1.17, 1.29)	1.71 (1.54, 1.90)
	emtricitabine 200 once daily				1.02 (0.97, 1.08)	1.01 (0.98, 1.04)	1.06 (1.01, 1.11)
	tenofovir DF 300				1.36	1.35	1.45

	once daily				(1.25, 1.47)	(1.29, 1.42)	(1.39, 1.51)
Emtricitabine/ rilpivirine/ tenofovir DF[g]	emtricitabine once 200 daily	100 once daily	400 once daily	24	0.95 (0.90, 1.00)	0.99 (0.97, 1.02)	1.05 (0.99, 1.11)
	rilpivirine 25 once daily				0.93 (0.88, 0.98)	0.95 (0.90, 1.00)	0.96 (0.90, 1.03)
	tenofovir DF 300 once daily				1.44 (1.33, 1.55)	1.40 (1.34, 1.46)	1.84 (1.76, 1.92)
Norelgestromin	norgestimate 0.180/0.215/0.25/ ethinyl estradiol 0.025 once daily	100 once daily	ND	13	0.97 (0.88, 1.07)	0.90 (0.82, 0.98)	0.92 (0.83, 1.03)
		ND	400 once daily	15	1.07 (0.94, 1.22)	1.06 (0.92, 1.21)	1.07 (0.89, 1.28)
Norgestrel		100 once daily	ND	13	0.96 (0.78, 1.19)	0.91 (0.73, 1.15)	0.92 (0.73, 1.18)
		ND	400 once daily	15	1.18 (0.99, 1.41)	1.19 (0.98, 1.45)	1.23 (1.00, 1.51)
Ethinyl estradiol		100 once daily	ND	12	1.39 (1.17, 1.66)	1.04 (0.87, 1.24)	0.83 (0.65, 1.06)
		ND	400 once daily	15	1.15 (0.97, 1.36)	1.09 (0.94, 1.26)	0.99 (0.80, 1.23)
Pravastatin	pravastatin 40 single dose	100 once daily	ND	18	1.28 (1.08, 1.52)	1.35 (1.18, 1.54)	NA
Rosuvastatin	rosuvastatin 10 single dose	100 once daily	ND	18	2.61 (2.32, 2.92)	2.69 (2.46, 2.94)	NA
Raltegravir+ emtricitabine/ tenofovir DF	emtricitabine 200 once daily	100 once daily	400 once daily	30	1.08 (1.04, 1.12)	1.05 (1.03, 1.07)	1.02 (0.97, 1.08)
	tenofovir DF 300 once daily				1.46 (1.39, 1.54)	1.40 (1.34, 1.45)	1.70 (1.61, 1.79)
	raltegravir 400 twice daily				1.03 (0.74, 1.43)	0.97 (0.73, 1.28)	0.79 (0.42, 1.48)
Tacrolimus	5 single dose	ND	400 single dose	16	0.73 (0.59, 0.90)	1.09 (0.84, 1.40)	NA

NA = not available/not applicable, ND = not dosed.

a. All interaction studies conducted in healthy volunteers.
b. Comparison based on exposures when administered as atazanavir/ritonavir + emtricitabine/tenofovir DF.
c. Comparison based on exposures when administered as darunavir/ritonavir + emtricitabine/tenofovir DF.
d. Administered as Atripla (efavirenz, emtricitabine and tenofovir DF fixed-dose combination).
e. Administered as Genvoya (elvitegravir, cobicistat, emtricitabine and tenofovir alafenamide fixed-dose combination). Tenofovir Cmax, AUC and Cmin were increased by 77%, 81%, and 121%, respectively.
f. Administered as Stribild (elvitegravir, cobicistat, emtricitabine and tenofovir DF fixed-dose combination).
g. Administered as Complera (emtricitabine, rilpivirine and tenofovir DF fixed-dose combination).

No effect on the pharmacokinetic parameters of the following coadministered drugs was observed with sofosbuvir or velpatasvir: cyclosporine, dolutegravir, lopinavir/ritonavir (only studied with VEL), or methadone (only studied with SOF).

Clinical Implication of the Effects of SOF/VEL, SOF, or VEL on Coadministered Drugs:

As stated in Section 2.4.2.1, VEL is an inhibitor of drug transporter P-gp, BCRP, and OATP2B1 and may increase intestinal absorption of coadministered substrates for these transporters. In

addition, VEL is an inhibitor of OATP1B1 and OATP1B3. Coadministration of SOF/VEL with drugs that are substrates of these transporters may increase the systemic exposure of such drugs. As shown in the table above, most of the effects of VEL or SOF/VEL on coadministered drugs, except the effects on tenofovir (TFV) (following coadministration of tenofovir disoproxil fumarate (TDF) with SOF/VEL) or rosuvastatin, were minimal and are not considered clinically significant.

The percent of TFV AUC increase is similar when SOF/VEL is coadministered with TDF containing regimens including ritonavir-boosted protease inhibitors (PI), cobicistat (COBI), or without boosted PI/COBI (not including EFV-based regimen). However, because of the effect of boosted PI's or COBI on TFV and additional increased TFV exposures caused by food, the absolute TFV values are highest when SOF/VEL is coadministered with a boosted PI or COBI. The following statement should be included in the US Package Insert: "Monitor for TFV-associated adverse reactions in patients receiving SOF/VEL concomitantly with a regimen containing TDF. Refer to VIREAD or TRUVADA prescribing information for recommendations on renal monitoring." Limited safety data are available from ASTRAL-5 (HIV/HCV coinfection) where SOF/VEL was coadministered with a boosted PI or Stribild® (Elvitegravir/ cobicistat/emtricitabine/tenofovir DF) in the study. Therefore, the statement "(b) (4) is recommended for coadministration of SOF/VEL with regimens containing tenofovir DF and either ritonavir or cobicistat, until additional safety data from ASTRAL-5 become available.

VEL increased rosuvastatin (a substrate of BCRP and OATP1B1) Cmax and AUC by approximately 2.7-fold. SOF/VEL only increased pravastatin (a substrate of OATP1B1) Cmax and AUC by 28% and 35%, respectively, because although pravastatin is a selective substrate for OATP1B1, it is not a sensitive OATP1B1 substrate. A large effect of OATP1B1 inhibition on pravastatin has not been observed. The Agency recommends the applicant to conduct a drug interaction study to evaluate the drug interaction between sofosbuvir/velpatasvir and atorvastatin, because the results from the rosuvastatin study indicated that velpatasvir can significantly increase the concentration of substrates of OATP and BCRP, such as atorvastatin. While the results from rosuvastatin drug interaction results cannot be directly extrapolated to atorvastatin and serious safety events (rhabdomyolysis) have been reported with use of ledipasvir/sofosbuvir and atorvastatin, a PMR is needed to study the interaction between sofosbuvir/velpatasvir and atorvastatin. See Dr. Sang Chang's consult review for details.

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 (PD) drug-drug interactions (DDI) for VEL. Serious symptomatic bradycardia has been observed when SOF and another HCV direct acting antiviral (DAA) are coadministered with amiodarone. Therefore, coadministration of amiodarone with SOF/VEL may also result in serious symptomatic bradycardia and concomitant use is not recommended.

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.

VEL exhibits a pH-dependent solubility profile, and is practically insoluble (less than 0.1 mg/mL) above pH 5, slightly soluble (3.6 mg/mL) at pH 2, and soluble (greater than 36 mg/mL) at pH 1.2. See the Biopharmaceutics Reviewer's review for details. The in vitro permeability studies on VEL were carried out in Caco-2 cell monolayers. However, permeability values of VEL could not be reliably obtained due to low recovery of the compound and poor reproducibility. The report for this in vitro study was not submitted.

VEL increased the forward permeability of SOF by inhibiting its efflux transport across Caco-2 cell monolayers in vitro. These results are consistent with increases in SOF exposures observed clinically in the context of the FDC.

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 or bioanalytical inspection is needed for the relative BA study. The to-be-marketed SOF/VEL FDC tablets were used in the Phase 3 studies. (The formulation composition and manufacturing process for the SOF/VEL tablets used in Phase 3 studies are identical to the proposed commercial product.) Some Phase 1 and Phase 2 studies have utilized SOF and VEL single agents or a combination of these 2 single agents. Relative BA studies have shown that similar plasma exposures of SOF, GS-331007 and VEL were achieved following administration of SOF/VEL FDC and coadministration of SOF+VEL as single agents.

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 VEL and SOF when administered as the SOF/VEL FDC tablet is based on the results of Study GS-US-342-0104. Administration of SOF/VEL with a high-fat/high-calorie or a moderate-fat/moderate-calorie meal resulted in a 21% and 34% increase in VEL AUC, with no change to 31% increases in VEL Cmax. Food slowed the rate of absorption of SOF within SOF/VEL, with only modest alterations in bioavailability, as evidenced by < 2-fold higher mean AUC and no change in mean Cmax. For GS-331007, an approximately 25% to 37% lower Cmax was observed following SOF/VEL administration with food, with no change in AUC (Study GS-US-342-0104). These changes in exposure are not considered clinically significant for any moiety. Accordingly, SOF/VEL has been administered without regard to food in the Phase 2 and Phase 3 trials.

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 and intermediate metabolite GS-566500 in humans, but not GS-461203. 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. 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.

VEL itself is the active moiety. It is the predominant species circulating in plasma (~99%). VEL was characterized in clinical pharmacology studies and used for exposure-response analysis. LC/MS/MS with positive ionization was utilized to determine VEL concentrations in plasma and urine.

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, urine and dialysate assay methods for SOF, GS-566500, GS-331007 and VEL (no dialysate) were precise and accurate.

Two bioanalytical labs have been used for SOF/VEL bioanalytical analyses: (b) (4) . Cross validation of bioanalytical methods between 2 different bioanalytical laboratories was done using spiked quality control (QC) samples and pooled study samples. All of the cross validation met the cross validation precision and accuracy acceptance criteria stated in cross validation standard operating procedures.

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 VEL was measured. This is acceptable because protein binding of VEL 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, VEL, and SOF/VEL 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/1.73m^2) or with end stage renal disease (ESRD) due to higher exposures (up to 20-fold) of the predominant sofosbuvir metabolite.
- No dose adjustment of SOF/VEL is required for patients with mild or moderate renal impairment.
- No dose adjustment of SOF/VEL is required for patients with mild, moderate or severe hepatic impairment (Child-Pugh Class A, B or C).
- Drugs that are inducers of P-gp may decrease plasma concentrations of SOF. Drugs that are inducers of P-gp and/or moderate to potent inducers of CYP2B6, CYP2C8, or CYP3A4 (including efavirenz) may decrease plasma concentrations of VEL, leading to reduced therapeutic effect of SOF/VEL. The use of these agents with SOF/VEL is not recommended.
- VEL solubility decreases as pH increases. Drugs that increase gastric pH are expected to decrease concentration of VEL.
    - Coadministration of SOF/VEL with proton pump inhibitors (PPI) is not recommended.
    - SOF/VEL should be coadministered with H2-receptor antagonist (H2RA) either simultaneously or 12 hours apart.
- VEL increases tenofovir concentration when SOF/VEL is coadministrated with tenofovir disoproxil fumarate (TDF). TFV-associated adverse reactions should be monitored in patients receiving SOF/VEL concomitantly with a regimen containing TDF.
- Coadministration of SOF/VEL with rosuvastatin may significantly increase the concentration of rosuvastatin which is associated with increased risk of myopathy, including rhabdomyolysis. Rosuvastatin may be administered with SOF/VEL at a dose that does not exceed 10 mg.
- Coadministration of SOF/VEL with atorvastatin is expected to increase the concentrations of atorvastatin, which is associated with increased risk of myopathy, including rhabdomyolysis. Monitor closely for HMG-CoA reductase inhibitor-associated adverse events, such as myopathy.

4. APPENDICES

4.1 Individual Study Review

4.1.1 Biopharmaceutics

4.2.1.1 GS-US-342-0104: A Phase 1, Randomized, Open-Label, Single-Dose Study to Evaluate the Relative Bioavailability and Effect of Food on Sofosbuvir/GS-5816 (Velpatasvir, VEL) Fixed-Dose Combination Tablets in Healthy Volunteers

Objectives:

- To evaluate the relative bioavailability (BA) of sofosbuvir (SOF)/VEL fixed-dose combination (FDC) tablets relative to individual tablet formulations in healthy subjects
- To evaluate the effect of food on the pharmacokinetics (PK) of SOF/VEL FDC tablets in healthy subjects

Study Design: This is a Phase 1, randomized, open-label, single-dose study, proceeded in 2 parts (Parts A and B).

Part A: Relative Bioavailability of SOF/VEL Compared with SOF 400 mg + VEL 25 mg or 100 mg (Cohorts 1 and 2): Part A consisted of 2 randomized cohorts.

Subjects (n=26) in Cohort 1 were randomized to receive single doses of SOF 400 mg +VEL 25 mg coadministered under fasted conditions followed by a 9-day washout period and then a single dose of SOF/VEL (400 mg/25 mg) under fasted conditions (Sequence AB) or to receive a single dose of SOF/VEL (400 mg/25 mg) under fasted conditions followed by a 9-day washout period and then single doses of SOF 400 mg +VEL 25 mg coadministered under fasted conditions (Sequence BA).

Subjects (n=26) in Cohort 2 were randomized to receive single doses of SOF 400 mg + VEL 100 mg coadministered under fasted conditions followed by a 9-day washout period and then a single dose of SOF/VEL (400 mg/100 mg) under fasted conditions (Sequence CD) or to receive a single dose of SOF/VEL (400 mg/100 mg) under fasted conditions followed by a 9-day washout period and then coadministration of SOF 400 mg + VEL 100 mg under fasted conditions (Sequence DC).

Part B: Food Effect (Cohorts 3 and 4): Part B of the study consisted of 2 adaptive, randomized cohorts, Cohorts 3 and 4.

Cohort 3 was not evaluated. Subjects in Cohort 4 were randomized to 1 of 6 treatment sequences (Sequences HIJ, IJH, JHI, JIH, HJI, or IHJ) consisting of the following 3 treatments each separated by a 9-day washout period:

- Single dose of SOF/VEL (400 mg/100 mg) administered under fasted conditions (Treatment H)
- Single dose of SOF/VEL (400 mg/100 mg) administered with a moderate-fat meal (approximately 600 kcal, approximately 30% fat) (Treatment I)
- Single dose of SOF/VEL (400 mg/100 mg) administered with a high-calorie, high-fat meal (approximately 800 kcal, approximately 50% fat) (Treatment J)

Formulation: SOF 400-mg tablets (400 mg), Lot # DC1205B1; VEL 25-mg tablet, Lot #DL1301C1; VEL 100-mg tablet, Lot#DL1301D1; SOF/VEL [400 mg/25 mg], Lot # DU1302B1; SOF/VEL [400 mg/100 mg], Lot #DU1301B1

PK Sampling: Intensive PK sampling was performed on Days 1 and 11 predose (≤ 5 min) and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 18, 24, 48, 72, and 96 hours postdose.

Analytical Methods: Concentrations of SOF, GS-566500 and GS-331007, and VEL 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, and VEL were all performed and validated by (b) (4).

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

Pharmacokinetic Results:

Cohort 1: The statistical comparisons of PK parameters following the administration of SOF 400 mg + VEL 25 mg and SOF/VEL (400/25 mg) in Cohort 1 are presented in the table below.

	GLSM		%GLSM Ratio (90% CI)
PK Parameter	SOF 400 mg + VEL 25 mg (Reference) (N = 26)	SOF/VEL (400/25 mg) (Test) (N = 26)	SOF/VEL (400/25 mg) vs SOF 400 mg + VEL 25 mg
SOF			
AUC_{last} (h•ng/mL)	1227.48	1394.75	113.63 (97.98, 131.78)
AUC_{inf} (h•ng/mL)	1235.14	1402.76	113.57 (98.01, 131.60)
C_{max} (ng/mL)	1074.81	1142.98	106.34 (87.32, 129.51)
GS-566500			
AUC_{last} (h•ng/mL)	1391.54	1491.26	107.17 (95.05, 120.82)
AUC_{inf} (h•ng/mL)	1452.63	1548.13	106.57 (95.43, 119.02)
C_{max} (ng/mL)	369.47	387.14	104.78 (95.14, 115.40)
GS-331007			
AUC_{last} (h•ng/mL)	10779.90	11073.50	102.72 (96.85, 108.95)
AUC_{inf} (h•ng/mL)	11524.36	11797.25	102.37 (96.48, 108.62)
C_{max} (ng/mL)	1012.29	977.14	96.53 (90.10, 103.42)
VEL			
AUC_{last} (h•ng/mL)	793.24	1112.98	140.31 (115.04, 171.13)
AUC_{inf} (h•ng/mL)	840.92	1165.54	138.60 (114.90, 167.19)
C_{max} (ng/mL)	110.78	153.09	138.19 (112.90, 169.14)

Similar plasma exposures of SOF and its metabolites GS-566500 and GS-331007 (predominant circulating nucleoside metabolite) were achieved upon administration of SOF/VEL (400/25 mg) and SOF 400 mg + VEL 25 mg. Velpatasvir plasma exposures were approximately 40% higher following administration of SOF/VEL (400/25 mg) compared with coadministration of SOF 400 mg + VEL 25 mg.

Cohort 2: The statistical comparisons of PK parameters following the administration of SOF/VEL (400/100 mg) and SOF 400 mg + VEL 100 mg in Cohort 2 are presented in the table below.

	GLSM		%GLSM Ratio (90% CI)
PK Parameter	SOF 400 mg + VEL 100 mg (Reference) (N = 26)[a]	SOF/VEL (400/100 mg) (Test) (N = 26)	SOF/VEL (400/100 mg) vs SOF 400 mg + VEL 100 mg
SOF			
AUC_{last} (h•ng/mL)	1585.90	1408.21	88.80 (78.09, 100.96)
AUC_{inf} (h•ng/mL)	1592.19	1425.58	89.54 (78.78, 101.76)
C_{max} (ng/mL)	1454.61	1309.21	90.00 (74.72, 108.42)
GS-566500			
AUC_{last} (h•ng/mL)	1717.90	1536.72	89.45 (79.36, 100.83)
AUC_{inf} (h•ng/mL)	1781.36	1603.08	89.99 (80.40, 100.73)
C_{max} (ng/mL)	446.61	412.05	92.26 (82.98, 102.58)
GS-331007			
AUC_{last} (h•ng/mL)	11138.53	11169.92	100.28 (95.79, 104.98)
AUC_{inf} (h•ng/mL)	11949.15	11936.70	99.90 (95.42, 104.58)
C_{max} (ng/mL)	869.41	933.71	107.40 (101.12, 114.06)
VEL			
AUC_{last} (h•ng/mL)	3137.58	3263.13	104.00 (75.02, 144.18)
AUC_{inf} (h•ng/mL)	3213.10	3326.48	103.53 (75.67, 141.65)
C_{max} (ng/mL)	404.57	416.57	102.97 (74.47, 142.37)

Similar plasma exposures of SOF, its metabolites GS-566500 and GS-331007, and VEL were achieved upon administration of SOF/VEL (400/100 mg) compared with coadministration of SOF 400 mg + VEL 100 mg.

Cohort 4: The statistical comparisons of PK parameters following the administration of SOF/VEL (400/100 mg) under fasting conditions, with a moderate-fat meal and with a high-calorie, high-fat meal, are presented in the table below.

PK Parameter	GLSM			%GLSM Ratio (90% CI)	
	High-Calorie, High-Fat Meal (N = 30)	Moderate-Fat Meal (N = 30)	Fasted (N = 30)	High-Calorie, High-Fat Meal/Fasted	Moderate-Fat Meal/Fasted
SOF					
AUC_{last} (h•ng/mL)	2566.66	2312.04	1442.22	177.97 (157.32, 201.32)	160.31 (141.72, 181.35)
AUC_{inf} (h•ng/mL)	2589.02	2324.73	1452.76	178.21 (157.87, 201.18)	160.02 (141.75, 180.65)
C_{max} (ng/mL)	1275.69	1367.09	1434.41	88.94 (75.26, 105.10)	95.31 (80.65, 112.63)
GS-566500					
AUC_{last} (h•ng/mL)	2713.48	2297.60	1492.73	181.78 (162.65, 203.16)	153.92 (137.72, 172.02)
AUC_{inf} (h•ng/mL)	2786.88	2375.16	1568.54	177.67 (160.43, 196.78)	151.43 (136.73, 167.70)
C_{max} (ng/mL)	532.84	472.88	389.03	136.97 (123.85, 151.47)	121.55 (109.91, 134.42)
GS-331007					
AUC_{last} (h•ng/mL)	11846.01	11672.17	11993.94	98.77 (94.43, 103.30)	97.32 (93.05, 101.78)
AUC_{inf} (h•ng/mL)	13155.93	12860.93	12875.58	102.18 (97.75, 106.80)	99.89 (95.56, 104.41)
C_{max} (ng/mL)	605.38	722.65	963.39	62.84 (58.35, 67.68)	75.01 (69.65, 80.79)
VEL					
AUC_{last} (h•ng/mL)	4574.49	5054.54	3758.60	121.71 (99.09, 149.49)	134.48 (109.49, 165.17)
AUC_{inf} (h•ng/mL)	4637.86	5130.92	3827.71	121.17 (99.01, 148.28)	134.05 (109.53, 164.05)
C_{max} (ng/mL)	510.48	638.74	487.44	104.73 (86.61, 126.63)	131.04 (108.38, 158.44)

In Cohort 4, food slowed the rate of absorption of SOF (Tmax 0.5 hours under fasted conditions vs 2 hours under fed conditions) and GS-566500 (Tmax of 1.5 hours under fasted conditions vs 3 hours under fed conditions) with modest alteration in the bioavailability. The effects are not considered clinically significant. For GS-331007, an approximately 25% (following a moderate-fat meal) to 37% (following a high-calorie, high-fat meal) lower Cmax was observed upon SOF/VEL (400/100 mg) administration with food with no change in AUC. The 90% CIs of the GLSM ratios for AUC of GS-331007 remained within the bounds of 80% to 125%. Since the decrease in GS-331007 Cmax was modest and the AUC parameters met PK equivalence criteria, the effect of food on GS-331007 PK was not considered clinically significant.

These results were consistent with the data from previous Phase 1 studies (Studies P7977-1318, P7977-0111, and GS-US-337-0101), which demonstrated that SOF as a single agent or as part of ledipasvir (LDV)/SOF FDC (90/400 mg) could be administered without regard to food.

Compared with fasted conditions, a modest increase in VEL plasma exposure was achieved upon administration of SOF/VEL (400/100 mg) with a high-calorie, high-fat meal (AUC increased by 21% and Cmax increased by 5%) or with a moderate-fat meal (AUC increased by 34% and Cmax increased by 31%).

Conclusion:

- Similar plasma exposures of SOF and its metabolites GS-566500 and GS-331007, and higher exposure of VEL, were achieved following administration of SOF/VEL (400 mg/25 mg) compared with coadministration of SOF 400 mg + VEL 25 mg.
- Similar plasma exposures of SOF, its metabolites GS-566500 and GS-331007, and VEL were achieved following administration of SOF/VEL (400 mg/100 mg) compared with coadministration of SOF 400 mg + VEL 100 mg.
- Administration of SOF/VEL (400 mg/100 mg) with a moderate-fat meal or with a high-calorie, high-fat meal does not substantially alter the plasma exposure of SOF, its metabolites GS-566500 and GS-331007, or VEL.
- SOF/VEL can be used without regard to food.

4.1.2 General Pharmacokinetics/Pharmacodynamics

4.1.2.1 GS-US-281-0101: A Phase 1 Study in Healthy Volunteers to Evaluate the Safety, Tolerability, and Pharmacokinetics of Velpatasvir, the Effect of Food on Velpatasvir Pharmacokinetics, and the Pharmacokinetic Interactions between Velpatasvir and Sofosbuvir (GS-7977) and its Metabolites

Objectives:

- To characterize the single- and multiple-dose pharmacokinetics (PK) of velpatasvir (VEL)
- To perform a preliminary evaluation of the effect of concomitant food intake on VEL PK
- To evaluate the effect of VEL on the PK of sofosbuvir (SOF) and metabolites, and the effect of SOF on the PK of VEL

Study Design: The study was to proceed in 4 parts (Parts A, B, C, and D).

Part A: Single- and Multiple-Ascending Doses (Cohorts 1 to 4): Part A consisted of 4 randomized, double-blind cohorts of single- and multiple-ascending doses of VEL. Within each cohort, unique subjects were randomized to receive either VEL (N = 12) or matching placebo (N = 3). Subjects received a single dose of VEL or placebo in the fasted state on Day 1, followed by a 6-day washout, and then subjects received once-daily doses of VEL or placebo in the fasted state on Days 8 to 14. The doses of VEL evaluated 50 mg (Cohort 1), 150 mg (Cohort 2), 5 mg (Cohort 3) and 450 mg (Cohort 4).

Part B: Food Effect (Cohorts 5 and 6): Part B consisted of 2 open-label, parallel cohorts, including a fasted/light breakfast cohort (Cohort 5) and a fasted/high-fat breakfast cohort (Cohort 6). The 100-mg dose of VEL was selected for evaluation in Part B. Within each cohort, 12 subjects received a single dose of VEL in the fasted state on Day 1, followed by a 6-day washout, and then subjects received a second single dose of VEL in the fed state (light breakfast or high-fat breakfast for Cohorts 5 and 6, respectively) on Day 8.

Part C: Drug-Drug Interaction (Cohorts 7: VEL 150 mg was administered under fed conditions (light breakfast). Eighteen subjects received a single dose of SOF 400 mg on Day 1, followed by a 3-day washout; then subjects received once-daily doses of VEL 150 mg on Days 5 to 13; and then subjects were coadministered single doses of SOF 400 mg + VEL 150 mg on Day 14.

Part D (Cohort 9) was not initiated.

Formulation:
VEL 5-mg tablets (Lot#: DL1202B1),
VEL 50-mg (Lot# DL1203B1),
SOF 400-mg tablets (Lot# DC1202B2),
VEL matching placebo tablets (Lot#DL1201B1)

PK Sampling:

Part A: Intensive PK sampling occurred relative to VEL dosing on Day 1 and Day 14 at the following time points: 0 (predose), 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 16, 20, 24, 48 (only Day 1), 72 (only Day 1), and 96 (only Day 1) hours postdose.

Part B: Intensive PK sampling occurred relative to VEL dosing on Days 1 and 8 at the following time points: 0 (predose), 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 16, 20, 24, 48, 72, and 96 hours postdose.

Part C: Intensive PK sampling occurred relative to the morning dose as follows: Day 1 (SOF): 0 (predose), 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 16, 20, 24, 48, 72, and 96 hours postdose. Day 13 (VEL): 0 (predose), 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 16, 20, and 24 hours postdose. Day 14 (SOF+VEL): 0 (predose), 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 16, 20, 24, 48, 72, and 96 hours postdose.

Analytical Methods: Concentrations of SOF, GS-566500 and GS-331007, and VEL 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, and VEL were all performed and validated by (b) (4)

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

Pharmacokinetic Results:

VEL PK parameters after administration of single and multiple doses of VEL under fasted conditions are presented in Table 1.

Table 1: Velpatasvir Pharmacokinetics after Administration of Single and Multiple Doses of VEL under Fasted Conditions

VEL PK Parameter	Mean (%CV)				
	VEL 5 mg (N = 12)	VEL 50 mg (N = 12)	VEL 100 mg (N = 24)	VEL 150 mg (N = 12)	VEL 450 mg (N = 12)
Single Dose (Cohorts 1-6[b])					
AUClast (h*ng/mL)	134.2 (69.6)	2970.7 (40.1)	4985.6 (44.8)	4925.9 (48.0)	9503.8 (34.5)
AUCinf (h*ng/mL)	158.9 (64.0)	3017.2 (40.1)	5055.0 (45.3)	4978.3 (47.8)	9578.1 (34.3)
Cmax (ng/mL)	22.4 (55.4)	371.3 (32.7)	574.9 (37.2)	608.4 (46.7)	1121.6 (31.7)
Clast (ng/mL)	1.40 (26.9)	2.34 (61.4)	2.85 (80.3)	2.23 (40.1)	3.28 (50.5)
Tmax (h)[a]	1.50 (1.50, 2.00)	2.50 (2.00 3.00)	2.50 (2.50, 3.00)	2.75 (2.50, 3.50)	3.25 (2.50, 3.75)
Tlast (h) [a]	24.00 (14.00, 36.00)	72.00 (48.00, 96.00)	95.00 (71.50, 96.00)	96.00 (84.02, 96.00)	96.00 (96.00, 96.00)
t1/2 (h) [a]	11.20 (5.40, 16.89)	13.62 (10.62, 16.47)	15.73 (12.63, 17.11)	16.16 (14.55, 17.55)	14.97 (12.91, 16.73)
CL/F (mL/h)	58,398.0 (124.4)	19,188.4 (39.2)	24,617.9 (50.8)	72,185.5 (196.4)[c]	53,676.4 (42.5)
Multiple Dose (Cohorts 1-4b)					
AUCtau (h*ng/mL)	172.3 (51.7)	3032.6 (40.4)	—	4890.8 (45.4)	9511.2 (40.9)
Cmax (ng/mL)	28.3 (49.3)	411.4 (40.7)	—	669.4 (48.1)	1195.7 (38.0)
Ctau (ng/mL)	2.2 (76.0)	37.9 (59.5)	—	63.4 (42.8)	127.7 (44.3)
Tmax (h) a	2.00 (1.25, 2.50)	2.50 (2.25, 3.00)	—	2.50 (2.50, 3.50)	3.00 (2.50, 4.25)
Tlast (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)
t1/2 (h) a	13.73 (13.19, 15.88)	13.02 (11.43, 16.23)	—	15.15 (12.03, 15.63)	11.74 (10.64, 13.12)
CLss/F (mL/h)	36,095.7 (46.4)	19,593.0 (50.5)	—	45,082.3 (88.3)	58,804.6 (57.3)

a Tmax, Tlast, and t1/2, were reported as median (Q1, Q3).
b VEL dosing by cohort: Cohort 1 = 50 mg, Cohort 2 = 150 mg, Cohort 3 = 5 mg, Cohort 4 = 450 mg, Cohorts 5 and 6 (pooled in the fasted state) = 100 mg.
c Mean (%CV) CL/F, AUCinf, and Cmax for the VEL 150-mg group excluding Subject 2687-2004 was 31,403.8 (40.5) mL/h, 5404 (36.2) h*ng/mL, and 660.5 (34.8) ng/mL, respectively.

Following single and multiple oral doses of VEL, maximum plasma concentrations occurred between 1.50 and 3.25 hours (median Tmax). VEL exhibited nonlinear PK across the dose range of 5 to 450 mg. Increases in exposure, as assessed by AUC and Cmax, were greater than dose-proportional from 5 to 50 mg. Similar mean plasma exposures were observed upon administration of GS-5816 100 and 150 mg and were driven by unexpectedly low exposures (more than 10-fold lower than the values observed for other subjects) in a single subject (Subject 2687-2004) in the 150-mg dose group. Mean (%CV) AUCinf and Cmax for the VEL 150-mg group excluding Subject 2687-2004 was 5404 (36.2) h*ng/mL and 660.5 (34.8) ng/mL, respectively. Exclusion of this subject from PK analyses resulted in a less than dose-

proportional increase in exposure from 50 to 450 mg. Consistent with the half-life of VEL, modest accumulation was observed with time. After multiple once-daily doses of VEL ≥ 5 mg, the mean plasma concentrations of VEL at 24 hours postdose were above the protein-adjusted concentration of a compound inhibiting virus replication by 50% [EC50: 0.027(0.024 ng/mL) to 1.7 nM (1.5ng/mL)] for genotype 1 to 6 HCV replicons.

The VEL PK parameters after administration of a single dose of VEL 100 mg under fasted and fed conditions are presented in Table 2.

Table 2: Food Effects on Velpatasvir

VEL PK Parameter	GLSM		% GLSM Ratio (90% CI)
	VEL 100 mg Fed (N = 12)	VEL 100 mg Fasted (N = 12)	Fed/Fasted
Light Breakfast (Cohort 5)			
AUC_{last} (ng*h/mL)	6728.66	5389.63	124.84 (110.02, 141.67)
AUC_{inf} (h*ng/mL)	6820.80	5469.11	124.72 (109.94, 141.48)
C_{max} (ng/mL)	784.7	581.72	134.89 (116.84, 155.74)
High-Fat Breakfast (Cohort 6)			
AUC_{last} (h*ng/mL)	3222.57	3746.30	86.02 (73.17, 101.12)
AUC_{inf} (h*ng/mL)	3267.75	3786.61	86.30 (73.43, 101.42)
C_{max} (ng/mL)	364.3	485.72	75.02 (62.56, 89.97)

Food slowed the rate of absorption of VEL without significantly impacting bioavailability. Administration of VEL with a light breakfast resulted in 25% and 35% increases in AUC and Cmax, respectively. Administration of VEL with a high-fat/high-calorie breakfast resulted in 14% and 25% decreases in AUC and Cmax, respectively. These changes were not expected to have a clinical consequence. It is noted that the exposures under fasted conditions were 20%-46% higher in Cohort 5 compared to Cohort 6. It is not clear what factors contributed to the large inter-cohort variability.

The statistical comparisons of the primary PK parameters for VEL, after administration of VEL alone and in combination with a single dose of SOF and the primary PK parameters for SOF, GS-566500, and GS-331007 after administration of SOF alone and in combination with VEL are presented in the table below.

Table 3: Drug-Drug Interaction between Sofosbuvir and Velpatasvir

VEL PK Parameter	GLSM		%GLSM Ratio (90% CI)
	VEL 150 mg (N = 18)	SOF 400 mg + VEL 150 mg (N = 18)	SOF+VEL/VEL
AUCtau(h*ng/mL)	7284.95	8138.22	111.71 (107.54, 116.04)
Cmax (ng/mL)	932.27	987.69	105.94 (101.86, 110.20)
Ctau (ng/mL)	101.09	118.90	117.61 (111.94, 123.57)
SOF PK Parameter	**SOF 400 mg (N = 18)**	**SOF 400 mg + VEL 150 mg (N = 18)**	**SOF+SOF/VEL**
AUClast(h*ng/mL)	1154.59	2749.10	238.10 (214.62, 264.16)
AUCinf (h*ng/mL)	1159.50	2756.96	237.77 (214.27, 263.85)
Cmax (ng/mL)	880.28	1593.80	181.06 (149.43, 219.38)
GS-566500 PK Parameter	**SOF 400 mg (N = 18)**	**SOF 400 mg + VEL 150 mg (N = 18)**	**SOF+SOF/VEL**
AUClast(h*ng/mL)	1615.7	2946.3	182.35 (167.52, 198.50)
AUCinf (h*ng/mL)	1671.8	2999.5	179.42 (165.03, 195.06)
Cmax (ng/mL)	395.6	639.6	161.68 (145.27, 179.94)
GS-331007 PK Parameter	**SOF 400 mg (N = 18)**	**SOF 400 mg + VEL 150 mg (N = 18)**	**SOF+SOF/VEL**
AUClast(h*ng/mL)	11,173.87	12,610.86	112.86 (107.90, 118.05)
AUCinf (h*ng/mL)	11,842.52	13,774.96	116.32 (110.99, 121.90)
Cmax (ng/mL)	1080.97	693.62	64.17 (58.45, 70.44)

VEL plasma exposures (AUCtau, Cmax, and Ctau) were not affected by the coadministration of SOF, and thus no dose adjustment is required for VEL. SOF plasma exposures increased approximately 1.8- (Cmax) and 2.4-fold (AUC) when coadministered with VEL. GS-566500 Cmax and AUC increased approximately 1.6- and 1.8-fold, respectively, when SOF was coadministered with VEL. GS-331007 Cmax decreased approximately 36%, but AUC was unaffected by coadministration of SOF+VEL.

The effect of VEL on SOF (and GS-566500) exposure is likely due to VEL inhibition of the intestinal efflux drug transporters P-glycoprotein (P-gp) and possibly breast cancer resistance protein (BCRP), as SOF is known to be a substrate of these transporters. The increase in the systemic exposure of SOF (and GS-566500) by VEL was similar to that seen previously with P-gp and/or BCRP inhibitors and does not warrant any SOF dose modification.

Conclusion:

- VEL exhibited nonlinear PK across the evaluated single- or multiple-dose range. Increases in exposure were more than dose-proportional up to 50 mg, and less than dose-proportional above 50 mg; modest accumulation was seen with time, consistent with half-life.
- Food did not substantially alter the exposure of VEL; the clinical relevance of any changes in PK were assessed within the clinical development program for VEL.
- SOF plasma exposures increased approximately 1.8- (Cmax) and 2.4-fold (AUC) when coadministered with VEL. GS-566500 Cmax and AUC increased approximately 1.6- and 1.8-fold, respectively, when SOF was coadministered with VEL. GS-331007 Cmax decreased approximately 36%, but AUC was unaffected by coadministration of SOF+VEL. VEL pharmacokinetics is not affected by coadministration with SOF. VEL and SOF may be coadministered, with no dose adjustment required for either agent.

4.1.2.2 GS-US-281-1055: A Phase 1 Study to Evaluate the Pharmacokinetics, Metabolism, and Excretion of GS-5816

Objectives:

- To determine the mass balance of GS-5816 using a single dose of radiolabeled [^{14}C]GS-5816
- To evaluate the pharmacokinetics (PK) of GS-5816 and, where possible, its metabolites, using a dose of radiolabeled [^{14}C]GS-5816
- To determine the metabolite profile of GS-5816 in humans using a dose of radiolabeled [^{14}C]GS-5816

Study Design: This was a Phase 1, open-label, mass balance study designed to evaluate the PK, metabolism, and excretion of GS-5816 in a total of 8 healthy male subjects. After an overnight fast and light breakfast, each subject received a single, 100-mg dose of GS-5816 administered orally as 1 capsule containing [^{14}C] GS-5816 100 µCi on Day 1.

Formulation:
Lot number for GS-5816 100-mg capsule (containing [^{14}C] GS-5816 100 µCi (equivalent to 1.45 mg of GS-5816) and 98.55 mg of nonradiolabeled GS-5816): DL1401A

PK Sampling:
Whole blood and plasma samples were collected for radioactivity analysis at each of the following time points: 0 (predose, ≤ 5 minutes prior to dosing), 0.5, 1, 2, 2.5, 3, 3.5, 4, 6, 8, 12, 18, 24, 48, 72, 96, and 120 hours postdose. After the 120-hour postdose time point, additional blood samples for whole blood and/or plasma were collected at 24-hour intervals up to Day 22 (504 hours) or until 1 of the following 2 conditions was met: (1) liquid scintillation counting (LSC) indicated that the radioactivity levels in 2 consecutive samples had decreased to less than or equal to twice the level of background radioactivity, or (2) both the urine and fecal collections were discontinued.

Plasma samples were collected for metabolite identification/profiling at each of the following time points: 0.5, 1, 2.5, 3, 3.5, and 96 hours postdose. After the 96-hour postdose time point, up to 3 additional plasma samples may have been collected for metabolite profiling/identification at 24-hour intervals up to the morning of Day 8 (168 hours postdose) or until 1 of the following 2 conditions were met: (1) LSC indicated that the radioactivity levels in 2 consecutive samples had decreased to less than or equal to twice the level of background radioactivity, or (2) both the urine and fecal collections were discontinued.

Plasma samples were collected for PK analysis at each of the following time points: 0 (predose, ≤ 5 minutes prior to dosing), 0.5, 1, 2, 2.5, 3, 3.5, 4, 6, 8, 12, 18, 24, 48, 72, and 96 hours postdose.

All urine voided was collected and pooled for radioactivity and metabolite profiling/identification analysis, starting 12 hours predose and continuing over the following collection intervals: 0-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-72, 72-96, and 96-120 hours postdose. All feces were collected for radioactivity and metabolite profiling/identification analysis, starting predose (within a 24-hour period prior to Day-1 dose) and continuing over the following collection intervals: 0-24, 24-48, 48-72, 72-96, and 96-120 hours postdose and at 24-hour intervals until subjects met the discharge criteria. After the 120-hour postdose time point (morning of Day 6), all urine voided

and all feces were continued to be collected for radioactivity and metabolite profiling/identification analysis over 24-hour collection intervals up to Day 22 (504 hours) or until LSC indicated that the radioactivity levels in samples from 2 consecutive 24-hour collection intervals were ≤ 1% of the administered dose and the cumulative ^{14}C radioactivity recovered in urine plus feces was ≥ 90% of the administered dose.

Analytical Methods: Concentrations of GS-5816 in human plasma and 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 timeframe supported by frozen stability storage data. The assays for GS-5816 were all performed and validated by (b) (4).

The standard curve and QC data indicated that the plasma and urine assay methods for GS-5816 was precise and accurate.

The LSC for ^{14}C radioactivity was performed on all whole blood, plasma, urine and fecal samples for real-time monitoring of the recovery of the ^{14}C radioactive dose and determination of when to halt the collection of each sample matrix.

Quantitation of the metabolites present in plasma, urine, and feces was based on high performance liquid chromatography (HPLC) with radiodetection.

Pharmacokinetic Results: One subject did not have detectable radioactivity in whole blood and plasma samples. The investigation conducted by the applicant resulted in no technical or conduct-related findings. The applicant suggested that because the majority of radioactivity (89.6% of the 91.6% total radioactivity recovered for this subject) was collected in the first day of pooled fecal samples (0 to 24 hours), it was possible that hypermotility within the gastrointestinal tract or that the capsule progressed through the upper gastrointestinal tract intact limited availability for absorption. The total recovery of ^{14}C radioactivity in feces and urine following single oral dose administration of [^{14}C] GS-5816 was approximately 95% (n=8), with most of the radioactive dose recovered in the feces (approximately 94%). Consistent with nonclinical species, renal excretion of GS-5816 was a minor pathway for elimination; approximately 0.4% (n= 8) of the administered dose was present in the urine as parent and metabolites.

Quantifiable concentrations of ^{14}C radioactivity were observed for up to 12 hours (except 1 subject) in blood and up to 24 hours in plasma following administration of [^{14}C] GS-5816, after which concentrations remained BLQ. Using LSC, median maximum concentrations of ^{14}C radioactivity were observed 4 hours postdose in both blood and plasma.

Following administration of [^{14}C] GS-5816, systemic exposure was almost exclusively parent drug (approximately 98.9%). The whole blood-to-plasma concentration ratio through 12 hours ranged from 0.517 to 0.670, indicating that total radioactivity was excluded from erythrocytes. Overall, the human data were consistent with the established nonclinical profile of GS-5816.

GS-5816 was the major species identified in feces, accounting for a mean of 76.6% of the administered dose, followed by one known oxidative metabolite M18 (hydroxy-GS-5816-1, 5.9%) and one known dealkylated metabolite M19 (desmethyl-GS-5816, 3.0%). Metabolite profiles of radioactivity in urine consisted of 4 minor unknown components, in addition to

unchanged GS-5816. All radioactive components detected in urine each accounted for less than 0.1% of the dose. Low concentrations of unchanged GS-5816 were detected in urine.

The results indicated that, in humans, metabolism of GS-5816 is minor, as GS-5816 was primarily eliminated as unchanged parent drug via the feces, and unchanged GS-5816 accounted for nearly all exposure in plasma.

Conclusions:

- GS-5816 was primarily eliminated in the feces, with renal excretion as a minor pathway.
- The predominant species circulating in plasma was GS-5816 (~99%).
- GS-5816 was not subject to significant metabolism.

4.1.2.3 GS-US-281-0102: Phase 1b, Randomized, Double-Blind, Multiple-Dose Ranging Study Evaluating the Safety, Tolerability, Pharmacokinetics, and Antiviral Activity of GS-5816 in Subjects with Chronic Hepatitis C Virus Infection

Objectives:

- To evaluate antiviral activity of GS-5816 against HCV in genotype 1-6 subjects
- To characterize viral dynamics, pharmacokinetics (PK), and pharmacodynamics (PD) of GS-5816

Study Design: This was a Phase 1b double-blind, randomized, placebo-controlled multicenter study of GS-5816 in HCV-infected subjects. Dosing was planned in up to 12 unique dosing cohorts as described below.

- **Cohort 1** (n = 10, genotype 1a): up to GS-5816 150 mg or placebo once daily (QD) fasted for 3 days
- **Cohort 2** (n = 10, genotype 1a): up to GS-5816 150 mg or placebo QD fasted for 3 days
- **Cohort 3** (n = 10, genotype 1a): up to GS-5816 150 mg or placebo QD fasted for 3 days
- **Cohort 4** (n = 10, genotype 1a): up to GS-5816 150 mg or placebo QD fasted for 3 days
- **Cohort 5** (n = 10, genotype 2): up to GS-5816 400 mg or placebo QD fasted for 3 days
- **Cohort 6** (n = 10, genotype 2): up to GS-5816 400 mg or placebo QD fasted for 3 days (note: this cohort was not enrolled)
- **Cohort 7** (n = 10, genotype 3): up to GS-5816 400 mg or placebo QD fasted for 3 days
- **Cohort 8** (n = 10, genotype 4, 5, or 6): up to GS-5816 400 mg QD fasted for 3 days
- **Cohorts 9 to 12** (n = 10, genotype 1a, 1b, 2, or 3, or 4, 5, or 6): up to GS-5816 400 mg or placebo QD fasted for 3 days

Cohorts 1 through 4 were conducted in parallel. The doses to be evaluated in Cohorts 1 through 4 were determined based on the safety and available PK data from Study GS-US-281-0101. Dosing in subsequent cohorts was conducted in parallel and started after review of the safety data through Day 10 and the available HCV ribonucleic acid (RNA) and PK data from Cohorts 1 through 4.

The actual treatments administered are presented in Table 1, all under fasted conditions.

Table 1: GS-US-281-0102: Administration of GS-5816 and Placebo by Ascending Dose and HCV Genotype

Cohort[a]	Number of Subjects	HCV Genotype	Total Daily Dose of GS-5816 (mg)
4	4 GS-5816 2 placebo	1a	5
11	8 GS-5816 2 placebo	1a	25
2	8 GS-5816 2 placebo	1a	50
1	8 GS-5816 2 placebo	1a	100
3	7 GS-5816 2 placebo	1a	150
9	8 GS-5816 2 placebo	1b	150
5	8 GS-5816 2 placebo	2	150
12	7 GS-5816	3	25
10	4 GS-5816 1 placebo	3	50
7	6 GS-5816 2 placebo	3	150
8	2 GS-5816	4	150

a Cohort 6 was not enrolled

Due to the limited number of subjects with genotype 4, 5, or 6 HCV infection in the US population, Cohort 8 did not include placebo treatment.

Patients with creatinine clearance (CLcr) < 70 mL/min or with current or prior history of clinical hepatic decompensation were excluded from the study.

Formulation:
5-mg GS-5816 tablets: Lot number DL1202B1
50-mg GS-5816 tablets: Lot number DL1203B1
Matching placebo tablets: Lot number DL1201B1

PK Sampling: Blood samples will be collected after the first and last (third) dose at the following time-points: 0 (pre-dose), 0.5, 1, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 16 and 24 hours. Additional blood samples will be collected at 48 (Day 5), 72 (Day 6), 96 (Day 7) and 120 hours (Day 8) post the third dose.

Analytical Methods: Concentrations of GS-5816 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 GS-5816 were performed using validated methods by (b) (4).

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

Pharmacokinetics Results: Table 2 presents PK parameters of GS-5816 after single-dose and multiple-dose administration of GS-5816 in subjects with genotype 1a, 1b, 2, 3, or 4 HCV infection. GS-5816 Cmax and AUC increased in an approximately dose-proportional manner between 25 mg and 150 mg. Increases in GS-5816 exposures were generally greater than dose-proportional from 5 mg to 25 mg. Median time to reach maximal GS-5816 concentration (Tmax) ranged between 2.25 to 2.75 hours after the first dose and 1.5 to 3.0 hours after multiple (3) doses. On Day 3, the median half-life (t1/2) across cohorts ranged from approximately 14 to 20 hours, supporting once-daily dosing. With the small number of subjects, it is difficult to determine whether exposures were statistically similar across all genotypes. However, the population PK analysis for GS-5816 with multiple studies also shows that GS-5816 was similar among genotypes. PK Table 3 shows the GS-5826 PK parameters by grouping all genotypes with the same doses.

Table 2: GS-US-281-0102: Plasma GS-5816 Pharmacokinetic Parameters Following Single-Dose and Multiple-Dose Administration in Subjects with Genotype 1 to 4 HCV Infection (PK Analysis Set)

PK Parameter Mean (%CV)	GT 1a 5 mg (N = 4)	GT 1a 25 mg (N = 8)	GT 3 25 mg (N = 7)	GT 1a 50 mg (N = 8)	GT 3 50 mg (N = 4)	GT 1a 100 mg (N = 8)	GT 1a 150 mg (N = 7)	GT 1b 150 mg (N = 7)	GT 2 150 mg (N = 8)	GT 3 150 mg (N = 6)	GT 4 150 mg (N = 2)
Single Dose (Day 1)											
C_{max} (ng/mL)	20.1 (72.9)	94.0 (12.4)	130.1 (61.2)	301.1 (31.1)	214.8 (37.1)	372.8 (59.8)	515.1 (30.3)	557.7 (43.2)	657.3 (56.6)	616.7 (35.7)	515.5 (69.0)
T_{max} (h)[a]	2.50 (1.75, 6.25)	2.25 (2.00, 3.25)	2.50 (2.00, 3.50)	2.50 (2.00, 2.75)	2.75 (2.50, 4.00)	2.25 (2.00, 2.50)	2.50 (2.00, 2.50)	2.50 (2.00, 3.00)	2.50 (2.00, 2.50)	2.50 (2.00, 3.00)	2.50 (2.00, 3.00)
C_{last} (ng/mL)	11.06 (176.2)	6.27 (31.0)	9.62 (86.4)	17.06 (29.9)	14.23 (47.9)	21.83 (53.3)	32.71 (59.6)	82.69 (123.5)	42.58 (40.8)	31.48 (27.4)	38.10 (47.9)
T_{last} (h)[a]	13.00 (10.00, 19.96)	23.92 (23.92, 23.92)	23.83 (23.83, 23.92)	23.92 (23.84, 23.92)	23.83 (23.83, 23.83)	23.83 (23.83, 23.83)	23.83 (23.83, 23.92)	23.92 (23.87, 23.93)	23.83 (23.83, 23.92)	23.83 (23.83, 23.88)	23.83 (23.83, 23.83)
AUC_{inf} (ng•h/mL)	113.8 (62.9)	696.3 (20.7)	1042.5 (84.5)	2114.1 (32.9)	1934.7 (57.3)	2727.3 (59.4)	3965.3 (52.1)	5092.6 (27.8)	4850.9 (43.7)	4333.0 (32.8)	4093.1 (51.5)
CL/F (mL/h)	61312.2 (69.7)	37161.0 (19.1)	37181.8 (64.9)	25976.0 (32.3)	31839.8 (47.0)	49237.5 (51.9)	44528.7 (37.4)	32615.1 (42.3)	36391.8 (41.7)	37452.8 (28.0)	42246.3 (51.5)
$t_{1/2}$ (h)[a]	5.88 (3.92, 9.64)	11.55 (10.00, 15.23)	11.06 (7.99, 18.87)	12.48 (10.51, 13.84)	11.43 (7.33, 30.32)	10.79 (8.34, 13.68)	12.32 (9.60, 13.62)	14.21 (12.41, 15.40)	9.53 (9.07, 12.79)	11.21 (9.05, 12.56)	13.75 (10.79, 16.71)
Multiple Dose (Day 3)											
C_{max} (ng/mL)	16.2 (14.4)	94.7 (26.2)	155.1 (49.1)	345.5 (14.2)	186.3 (14.8)	413.9 (58.9)	690.3 (47.6)	587.0 (60.9)	844.1 (42.2)	601.5 (22.5)	700.0 (22.6)
T_{max} (h)[a]	1.50 (1.00, 2.00)	2.50 (2.00, 3.50)	2.00 (2.00, 3.00)	2.50 (2.25, 3.00)	3.00 (1.75, 3.75)	2.25 (2.00, 2.50)	2.50 (2.00, 3.00)	2.50 (2.00, 3.00)	2.50 (2.00, 3.00)	2.75 (2.50, 4.00)	2.25 (2.00, 2.50)
C_{tau} (ng/mL)	0.6 (115.5)	8.6 (35.7)	13.6[b] (91.9)	24.2 (18.3)	18.8 (14.5)	30.8 (48.9)	58.4 (72.3)	59.5 (58.3)	76.3 (48.4)	47.3 (32.9)	49.0 (3.5)
T_{last} (h)[a]	20.00 (14.00, 24.00)	60.00 (48.00, 72.00)	48.00 (48.00, 72.00)	84.00 (72.00, 108.10)	84.47 (62.70, 98.08)	84.46 (60.00, 96.00)	96.00 (72.00, 120.85)	120.00 (96.00, 120.00)	108.21 (96.00, 122.83)	95.92 (95.83, 120.00)	108.00 (96.00, 120.00)
AUC_{tau} (ng•h/mL)	86.4 (16.6)	680.2 (26.7)	1094.0[b] (58.2)	2223.7 (14.4)	1404.1 (12.9)	2745.3 (53.9)	4866.1 (61.2)	4343.3 (58.5)	6193.2 (41.0)	4381.2 (30.9)	4896.5 (7.9)
CL_{ss}/F (mL/h)	59173.6 (17.7)	39581.6 (31.3)	28471.4[b] (43.7)	22860.2 (13.1)	36068.8 (13.3)	53194.3 (74.5)	39115.3 (46.3)	45326.0 (53.9)	30670.6 (63.6)	37161.2 (31.1)	30731.0 (7.9)
$t_{1/2}$ (h)[a]	13.94 (5.31, 25.33)	16.83 (14.30, 18.62)	15.12[b] (12.12, 19.41)	14.78 (13.17, 22.46)	20.17 (16.59, 22.46)	15.28 (13.77, 18.20)	18.15 (15.88, 22.20)	19.99 (18.22, 21.99)	16.93 (15.28, 21.06)	16.56 (14.03, 18.10)	16.82 (15.30, 18.33)

GT = genotype

a Median (Q1, Q3)

b N = 6

Table 3: GS-US-281-0102: Plasma GS-5816 Pharmacokinetic Parameters Following Single-Dose and Multiple-Dose Administration in Subjects with Genotype 1 to 4 HCV Infection (PK Analysis Set, Pooled Data)

	Mean (%CV)				
PK Parameter	GS-5816 5 mg (N = 4)	GS-5816 25 mg (N = 15)	GS-5816 50 mg (N = 12)	GS-5816 100 mg (N = 8)	GS-5816 150 mg (N = 30)
Single Dose (Day 1)					
C_{max} (ng/mL)	20.1 (72.9)	110.8 (50.5)	272.3 (35.1)	372.8 (59.8)	583.3 (44.2)
T_{max} (h)[a]	2.50 (1.75, 6.25)	2.50 (2.00, 3.50)	2.50 (2.25, 3.00)	2.25 (2.00, 2.50)	2.50 (2.00, 2.50)
C_{last} (ng/mL)	11.06 (176.2)	7.83 (75.0)	16.12 (34.6)	21.83 (53.3)	47.12 (111.3)
T_{last} (h)[a]	13.00 (10.00, 19.96)	23.92 (23.83, 23.92)	23.84 (23.83, 23.92)	23.83 (23.83, 23.83)	23.83 (23.83, 23.92)
AUC_{last} (ng•h/mL)	65.8 (42.8)	656.8 (53.0)	1682.9 (36.0)	2379.0 (62.5)	3812.4 (38.4)
AUC_{inf} (ng•h/mL)	113.8 (62.9)	857.9 (71.4)	2054.3 (39.3)	2727.3 (59.4)	4546.6 (38.6)
CL/F (mL/hr)	61,312.2 (69.7)	37,170.7 (44.6)	27,930.6 (38.3)	49,237.5 (51.9)	38,011.7 (37.9)
$t_{1/2}$ (h)[a]	5.88 (3.92, 9.64)	11.06 (9.70, 15.95)	12.48 (8.87, 15.02)	10.79 (8.34, 13.68)	11.93 (9.30, 14.09)
Multiple Dose (Day 3)					
C_{max} (ng/mL)	16.2 (14.4)	122.9 (50.0)	292.4 (30.4)	413.9 (58.9)	690.1 (44.5)
T_{max} (h)[a]	1.50 (1.00, 2.00)	2.00 (2.00, 3.50)	2.50 (2.25, 3.25)	2.25 (2.00, 2.50)	2.50 (2.00, 3.00)
C_{tau} (ng/mL)	0.6 (115.5)[b]	10.8 (79.1)[c]	22.4 (20.8)	30.8 (48.9)	60.6 (54.9)
T_{last} (h)[a]	20.00 (14.00, 24.00)	48.00 (48.00, 72.00)	84.05 (72.00, 98.08)	84.46 (60.00, 96.00)	96.21 (96.00, 120.02)
AUC_{tau} (ng•h/mL)	86.4 (16.6)	857.5 (54.5)[c]	1950.5 (25.0)	2745.3 (53.9)	5003.0 (47.4)
CL_{ss}/F (mL/hr)	59,173.6 (17.7)	34,820.1 (38.0)[c]	27,263.0 (27.0)	53,194.3 (74.5)	37,362.8 (49.2)
$t_{1/2}$ (h)[a]	13.94 (5.31, 25.33)	16.67 (12.88, 19.41)[c]	17.20 (13.51, 22.46)	15.28 (13.77, 18.20)	18.12 (15.47, 20.96)

Note: Subject 6003-9001 discontinued from the study early and did not have an evaluable PK profile

a Median (Q1, Q3)

b N = 2 of 4 subjects included were BLQ

c N = 14

Mean plasma GS-5816 Ctau on Day 3 following administration of 5 mg was up to 3-fold greater than the predicted protein binding-adjusted mean EC50 value (0.18 ng/mL to 0.73 ng/mL) against genotype 1- 4 HCV replicons, assuming a 52-fold protein binding shift in human plasma compared with cell culture medium, determined by equilibrium dialysis. Mean plasma GS-5816 Ctau on Day 3 following administration of 25 mg was 15 to 59-fold greater than the predicted protein binding-adjusted mean EC50 values against genotype 1- 4 HCV replicons.

In the dose proportionality analysis using the power model (Table 4), AUC and C_{max} values for GS-5816 were approximately dose proportional over the dose range of 5 mg to 150 mg.

Table 4: GS-US-281-0102: Statistical Analysis of Dose Proportionality of GS-5816 Following Single- and Multiple–Dose Administration of GS-5816 (PK Analysis Set)

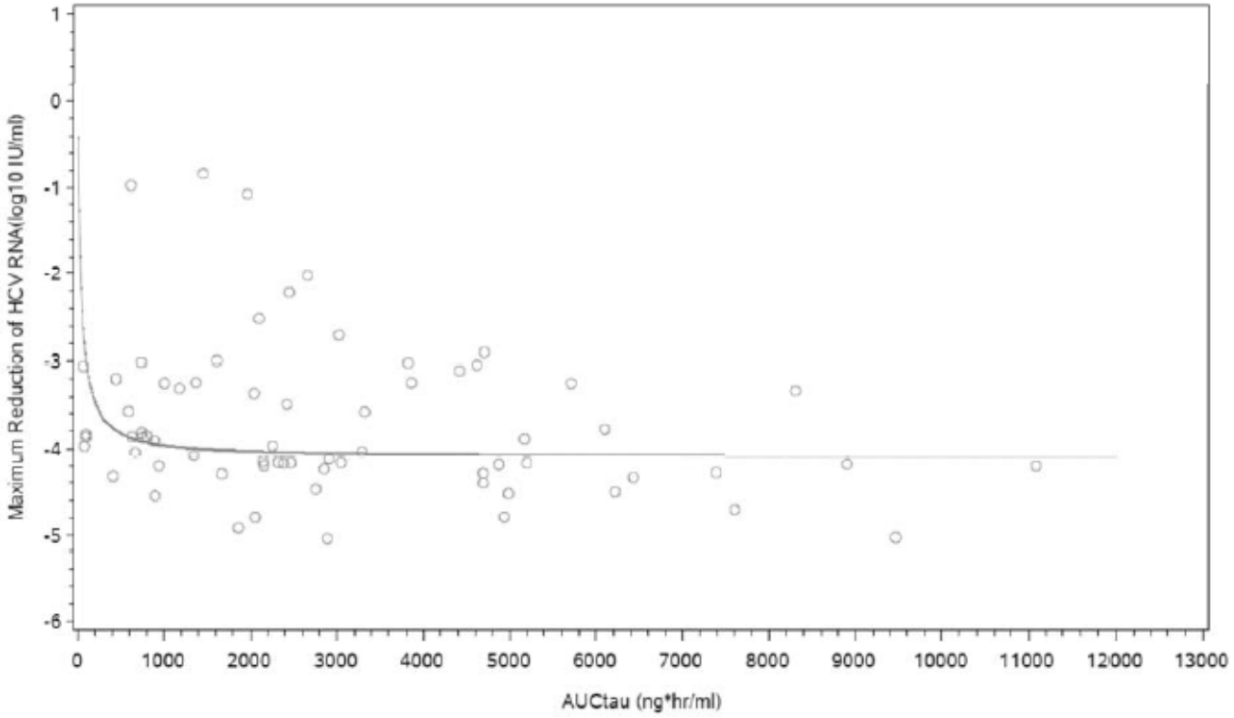
GS-5816 PK Parameter	Day	Degrees of Freedom	Slope of ln (dose)	90% CI Around Slope
AUC_{inf} (ng*hr/mL)	1	67	1.02	0.92, 1.12
C_{max} (ng/mL)	1	67	0.96	0.86, 1.06
AUC_{tau} (ng*hr/mL)	3	66	1.06	0.96, 1.16
C_{max} (ng/mL)	3	67	1.00	0.90, 1.10

Accumulation indices were calculated by comparing the AUC0-24, Cmax, and C24 values following single-dose administration of GS-5816 on Day 1 with the AUCtau, Cmax, and Ctau values, respectively, following multiple-dose administration of GS-5816 for 3 days. Accumulation was minimal at the 5-mg dose. Modest accumulation (1.06 to 1.44) was observed at doses ≥ 25 mg, a finding consistent with the half-life of GS-5816.

Exposure-Response Results:

Similar median maximal antiviral responses (> 3.0 log10 reduction) were observed following GS-5816 doses of 5, 25, 50, 100, or 150 mg. The relationship between GS-5816 exposure and anti-HCV activity was explored using a pharmacologically simple Emax model (Phoenix WinNonlin, v.6.3) and is illustrated in Figure 1. The Emax model indicated that exposures achieved following administration of GS-5816 doses ≥ 5 mg were predicted to provide > 95% of maximal antiviral response in subjects with genotype 1 HCV infection. Based on this model, GS-5816 systemic exposures for subjects with genotype 3 HCV infection were predicted to achieve at least 80% of maximal antiviral response at the ≥ 25-mg dose.

Figure 1: GS-US-281-0102: AUCtau Exposure-Response Relationship of Anti-HCV Activity of GS-5816 Following 3-Day Monotherapy (PK/Efficacy Analysis Set)



Conclusions:

- GS-5816 exhibited near dose-proportional increases in exposure from 25 mg to 150 mg.
- Modest GS-5816 accumulation (< 1.5-fold) was observed with time, consistent with median t1/2 of 14 to 20 hours.
- Mean plasma GS-5816 Ctau on Day 3 was at least 15-fold greater than the predicted protein binding-adjusted mean EC50 value against genotype 1–4 HCV replicons at doses ≥ 25 mg.
- GS-5816 demonstrates robust antiviral activity with median maximal viral load reductions of > 3 log10 IU/mL for all doses (5-150 mg) of GS-5816 across all HCV genotypes.

4.1.2.4 GS-US-281-1054: A Phase 1, Partially-Blinded, Randomized, Placebo- and Positive-Controlled Study to Evaluate the Effect of GS-5816 on the QT/QTc Interval in Healthy Subjects

This study was reviewed by Interdisciplinary Review Team for QT Studies Consultation, dated 4/15/2015. This review only summarizes the PK data from this study to aid the discussion of PK results from other studies.

Summary: This is a randomized, partial-blinded, placebo- and positive-controlled, 4-period, 8-treatment sequence, single-dose crossover study. Forty-eight healthy subjects received GS-5816 100 mg, GS-5816 500 mg, placebo, and moxifloxacin 400 mg. There was a 6-day washout period between treatments. No significant QTc prolongation effect of GS-5816 (100 mg and 500 mg) was detected in this TQT study as shown in Table 1.

Table 1: The Point Estimates and the 90% CIs Corresponding to the Largest Upper Bounds for GS-5816 (100 mg and 500 mg) and the Largest Lower Bound for Moxifloxacin (FDA Analysis)

Treatment	Time (hour)	ΔΔQTcF (ms)	90% CI (ms)
GS-5816 100 mg	0.5	1.8	(-0.4, 4.1)
GS-5816 500 mg	5	1.5	(-0.8, 3.8)
Moxifloxacin 400 mg*	6	11.5	(9.2, 13.8)

* Multiple endpoint adjustment was not applied. The largest lower bound after Bonferroni adjustment for 4 time points is 7.3 ms at 3 hours after dosing.

PK results are presented in Table 2 (GS-5816). GS-5816 exhibited less than dose-proportional increase on AUC (3.7-fold increase with 5-fold increase on dose) and Cmax (3.3-fold increase with 5-fold increase on dose) between 100mg and 500 mg; Following administration of a supratherapeutic dose of GS-5816 (500 mg) to healthy subjects, mean maximum GS-5816 concentration (Cmax) was approximately 3.1-fold that observed in SOF+GS-5816 Phase 2 studies (GS-US-342-0102, GS-US-342-0109, GS-US-337-0122 Cohort 4 [SOF 400 mg + GS-5816 100 mg]). Overall GS-5816 exposure (AUC) was approximately 4.4-fold that observed in SOF+GS-5816 Phase 2 studies. Highest increase (2.4-fold) of GS-5816 due to drug-drug interaction was observed when SOF/GS-5816 was coadministered with boosted ATV. Therefore GS-5816 concentrations at supratherapeutic dose of GS-5816 were above those for the predicted worst case scenario and show that at these concentrations there are no detectable prolongations of the QT-interval.

Table 2: GS-5816 Pharmacokinetic Parameters Following Administration of GS-5816 100 mg or 500 mg

	Mean (%CV)	
GS-5816 PK Parameter	**GS-5816 100 mg (N = 48)**	**GS-5816 500 mg (N = 48)**
$\%AUC_{exp}$	1.97 (59.97)	1.26 (76.05)
AUC_{inf} (h*ng/mL)	3894.8 (58.0)	14280.3 (37.3)
AUC_{last} (h*ng/mL)	3822.5 (57.7)	14079.1 (36.7)
C_{max} (ng/mL)	387.0 (50.9)	1292.4 (27.7)
C_{last} (ng/mL)	2.86 (74.31)	7.77 (93.17)
T_{max} (h)[a]	4.00 (4.00, 4.02)	4.00 (4.00, 4.03)
T_{last} (h)[a]	96.00 (72.00, 96.00)	96.00 (96.00, 96.00)
$t_{1/2}$ (h)[a]	16.02 (14.35, 18.41)	15.59 (14.35, 17.37)
CL/F (mL/h)	39214.5 (87.8)	40190.7 (42.2)

a Data presented as median (Q1, Q3)

4.1.2.5 GS-US-337-0122: A Phase 2, Multicenter, Open-Label Study to Assess the Efficacy and Safety of Sofosbuvir Containing Regimens for the Treatment of Chronic HCV Infection (Cohort 4)

Objectives:

- To evaluate the antiviral efficacy and safety of combination therapy with sofosbuvir (SOF)-containing regimens for the treatment of chronic hepatitis C virus (HCV) infection
- To characterize steady-state pharmacokinetics (PK) of Sofosbuvir (SOF), GS-566500, GS-331007, velpatasvir (VEL) in subjects with treatment-naïve, noncirrhotic subjects with Genotype 3 chronic HCV infection.

Study Design: This is an ongoing Phase 2 multicenter, open-label study in subjects with treatment-naïve, noncirrhotic subjects with Genotype 3 chronic HCV infection. Cohort 4 was comprised of 4 treatment groups to evaluate the safety and efficacy of SOF and one of 2 doses of GS-5816 (25 and 100 mg) with and without RBV. Approximately 100 treatment-naive, noncirrhotic subjects with HCV infection were planned to be enrolled and randomized (1:1:1:1) into one of the following 4 treatments:

- **Cohort 4, Group 1 (SOF+GS-5816 25 mg 8 weeks):** SOF 400 mg + GS-5816 25 mg once daily for 8 weeks
- **Cohort 4, Group 2 (SOF+GS-5816 25 mg+RBV 8 weeks):** SOF 400 mg + GS-5816 25 mg once daily + RBV 1000 or 1200 mg/day divided BID for 8 weeks
- **Cohort 4, Group 3 (SOF+GS-5816 100 mg 8 weeks):** SOF 400 mg + GS-5816 100 mg once daily for 8 weeks
- **Cohort 4, Group 4 (SOF+GS-5816 100 mg+RBV 8 weeks):** SOF 400 mg + GS-5816 100 mg once daily + RBV 1000 or 1200 mg/day divided BID for 8 weeks

SOF and GS-5816 were administered daily without regard to food. RBV was administered with food.

Formulation:
SOF 400-mg tablets: Lot # DC1205B1and DC1209B1
GS-5816 25-mg and 100-mg tablets: Lot # DL1301C1 and DL1301D1, respectively
RBV 200-mg tablets: Lot # A97943Z and AA2773Z

PK Sampling: A single PK blood sample was collected at each on-treatment visit for all subjects. However, sparse PK samples for the VEL 25 mg dose in this Phase 2 study were not analyzed. An intensive PK substudy was performed sometime on or between the Week 2 or 4 on-treatment visit in a subset of subjects. The PK of SOF (and its metabolites GS-566500 and GS-331007), GS-5816, and RBV was assessed. No detailed PK sampling time was provided.

Analytical Methods: Concentrations of SOF, GS-566500, GS-331007, and GS-5816 in plasma samples were determined using fully validated high-performance liquid chromatography-tandem mass spectroscopy (LC/MS/MS) bioanalytical methods. All samples were analyzed within the timeframe supported by frozen stability storage data. The assays for SOF, GS-566500, GS-331007, and GS-5816 were performed and validated by (b) (4) . The standard curve and QC data indicated that the plasma assay method for VEL was precise and accurate.

Pharmacokinetics Results: Table 1 presents PK parameters for SOF, GS-566500, GS-331007, and GS-5816 at steady state following administration of SOF + GS-5816 25 or 100 mg

± RBV for 8 weeks to treatment-naive subjects with genotype 3 HCV infection. One subject who received SOF + GS-5816 100 mg + RBV appeared to have taken a subsequent dose of study drug prior to the postdose 24 hour time point; data presented exclude this subject (1069-77307)

Table 1. GS-US-337-0122: SOF, GS-566500, GS-331007, and GS-5816 Plasma Pharmacokinetic Parameters Measured at Week 2 or 4 in Cohort 4 (PK Substudy Analysis Set, Source: Applicant's Study Report)

	Mean (%CV)			
	Group 1	Group 2	Group 3	Group 4
Analyte PK Parameter	SOF + GS-5816 25 mg 8 Weeks	SOF + GS-5816 25 mg + RBV 8 Weeks	SOF + GS-5816 100 mg 8 Weeks	SOF + GS-5816 100 mg + RBV 8 Weeks
SOF	N = 5	N = 4	N = 5	N = 6[f]
AUC_{tau} (h•ng/mL)	1692.0 (62.7)[b]	1672.2 (53.0)	2825.2 (41.2)	2890.0 (59.6)[d]
C_{max} (ng/mL)	911.6 (98.9)	1172.0 (82.4)	1347.2 (38.0)	1692.5 (62.5)
C_{tau} (ng/mL)	—	—	—	—
T_{max} (h)[a]	4.00 (2.00, 4.00)	1.25 (0.50, 2.00)	1.00 (0.98, 1.00)	1.50 (0.50, 2.00)
$t_{1/2}$ (h)[a]	0.48 (0.40, 0.70)[b]	0.50 (0.45, 2.91)	0.63 (0.53, 0.65)	0.73 (0.57, 0.84)[d]
GS-566500	N = 5	N = 4	N = 5	N = 6[f]
AUC_{tau} (h•ng/mL)	2742.3 (74.3)	2088.5 (28.0)	3363.1 (38.7)	3586.9 (14.0)
C_{max} (ng/mL)	576.0 (80.3)	528.3 (22.8)	571.2 (20.0)	710.0 (17.7)
C_{tau} (ng/mL)	—	—	361.0 (—)[e]	—
T_{max} (h)[a]	4.00 (4.00, 4.00)	1.56 (1.06, 3.00)	2.00 (1.98, 2.00)	3.03 (2.00, 4.00)
$t_{1/2}$ (h)[a]	2.29 (2.23, 2.29)	2.22 (2.16, 2.30)	2.46 (2.23, 2.50)	2.33 (2.26, 2.37)
GS-331007	N = 5	N = 4	N = 5	N = 6[f]
AUC_{tau} (h•ng/mL)	11728.3 (22.9)	7587.5 (19.0)	12432.7 (20.4)	11819.5 (20.7)
C_{max} (ng/mL)	966.0 (23.9)	672.8 (19.5)	825.4 (14.0)	823.2 (23.8)
C_{tau} (ng/mL)	282.2 (30.6)	184.0 (31.8)	417.4 (51.0)	323.5 (21.9)
T_{max} (h)[a]	4.00 (4.00, 4.00)	4.00 (3.00, 4.00)	4.00 (2.00, 4.05)	4.00 (4.00, 4.00)
$t_{1/2}$ (h)[a]	14.20 (13.51, 14.73)	15.84 (13.15, 21.37)	18.49 (17.74, 19.77)[b]	19.42 (17.75, 21.35)
GS-5816	N = 5	N = 4	N = 5	N = 6[f]
AUC_{tau} (h•ng/mL)	488.2 (57.6)	303.8 (107.3)	4059.7 (22.5)	3823.3 (51.7)
C_{max} (ng/mL)	62.4 (50.1)	49.2 (97.3)	503.2 (29.9)	532.7 (50.2)
C_{tau} (ng/mL)	7.6 (70.7)	3.2 (58.0)[c]	63.2 (45.0)	46.7 (64.9)
T_{max} (h)[a]	4.00 (4.00, 4.00)	2.00 (1.25, 3.00)	3.98 (2.00, 4.00)	4.00 (2.00, 4.00)
$t_{1/2}$ (h)[a]	10.38 (9.45, 10.79)	9.67 (8.14, 10.01)	10.56 (9.66, 10.86)	8.26 (8.03, 10.68)

a Median (Q1, Q3)
b N = 4
c N = 3
d N = 5
e N = 1
f N = 6; summary data excludes Subject 1069-77307.

Modestly higher SOF and GS-566500 exposures (approximately 65%) were observed when coadministered with GS-5816 100 mg with or without RBV compared with coadministered with GS-5816 25 mg. In general, plasma exposure of GS-331007, the primary circulating metabolite of SOF, was similar regardless of GS-5816 dose, though mean GS-331007 exposure in subjects who received SOF + GS-5816 25 mg + RBV was lower than the other groups and may be attributable to the low subject number (N = 4). The results indicated that the effect of GS-5816 on SOF and GS-566500 is dose dependent. The observation of no difference in GS-331007 AUC following coadministration of SOF with different doses of GS-5816 is consistent with the result of no effect of GS-5816 on GS-331007 exposure observed in Study GS-US-281-0101. Plasma exposure of SOF, GS-566500, and GS-331007, when administered with GS-5816 100 mg, were similar to those observed when SOF was administered with LDV (Studies GS-US-337-0102 and GS-US-337-0109). GS-5816 exposure increased greater than dose-proportional between GS-5816 25 mg and 100 mg, with an approximately 10-fold exposure increase when GS-5816 dose was increased from 25 mg to 100 mg, with or without RBV.

Efficacy Results: No exposure-response relationship has been performed for this individual study.

Table 2 presents the proportion of subjects with SVR12 following treatment with SOF + GS-5816 25 or 100 mg ± RBV for 8 weeks in treatment-naive subjects with genotype 3 HCV infection. All subjects who received SOF + GS-5816 25 mg for 8 weeks achieved SVR12 (100.0%, 27 of 27 subjects). A total of 21 of 24 subjects (87.5%) who received SOF + GS-5816 25 mg + RBV for 8 weeks achieved SVR12. A total of 26 of 27 subjects (96.3%) who received SOF + GS-5816 100 mg for 8 weeks achieved SVR12. All subjects who received SOF + GS-5816 100 mg + RBV for 8 weeks achieved SVR12 (100.0%, 26 of 26 subjects).

Table 9-32. GS-US-337-0122: Proportion of Subjects With SVR12 Following Treatment With SOF + GS-5816 25 or 100 mg ± RBV for 8 Weeks in Treatment-Naive Subjects with Genotype 3 HCV Infection (Cohort 4) (Full Analysis Set, Source: Applicant's Study Report)

	Cohort 4			
	Group 1	Group 2	Group 3	Group 4
	Treatment Naive, Genotype 3			
	SOF+GS-5816 25 mg 8 Weeks (N = 27)	SOF+GS-5816 25 mg + RBV 8 Weeks (N = 24)	SOF+GS-5816 100 mg 8 Weeks (N = 27)	SOF+GS-5816 100 mg + RBV 8 Weeks (N = 26)
SVR12	27/27 (100.0%)	21/24 (87.5%)	26/27 (96.3%)	26/26 (100.0%)
95% CI	87.2% to 100.0%	67.6% to 97.3%	81.0% to 99.9%	86.8% to 100.0%

HCV RNA analyzed using COBAS AmpliPrep/COBAS TaqMan HCV Quantitative Test, version 2.0 assay with limit of quantitation 15 IU/mL.
SVR12 was sustained virologic response (HCV RNA < LLOQ) 12 weeks after stopping study treatment.
A missing SVR12 value was imputed as a success if it was bracketed by values that are termed successes (i.e., '< LLOQ TND' or '< LLOQ detected'), otherwise, the missing SVR12 value was imputed as a failure. TND = target not detected. The exact 95% CI for the proportion within treatment group was based on the Clopper-Pearson method.

Conclusions:

- Treatment with SOF + GS-5816 25 or 100 mg ± RBV for 8 weeks in treatment-naïve subjects with Genotype 3 HCV infection without cirrhosis resulted in a high SVR12 rate in all treatment groups. There was no trend in SVR12 with respect to GS-5816 dose or contribution of RBV.
- Administration of SOF + GS-5816 100 mg ± RBV resulted in an approximately 65% higher SOF and GS-566500 exposures compared with administration of SOF + GS-5816 25 mg ± RBV. GS-331007 exposure was similar regardless of GS-5816 dose.
- GS-5816 exposure was more than dose-proportional between 25 mg and 100 mg.

4.1.2.6 GS-US-342-0102: A Phase 2, Multicenter, Randomized, Open-Label Study to Investigate the Safety and Efficacy of Sofosbuvir + GS-5816 for 12 Weeks in Treatment-Naive Subjects with Chronic HCV Infection

Objectives:

- To determine the antiviral efficacy and safety of combination treatment with sofosbuvir (SOF) + GS-5816 with or without ribavirin (RBV)
- To characterize the steady-state pharmacokinetics (PK) of study drugs

Study Design: This is a Phase 2, multicenter, randomized, open-label study. Approximately 340 subjects were randomized into 1 of 14 treatment groups as described below.

Approximately 50 subjects with genotype 1 HCV infection were randomized 1:1 to one of the following 2 treatment groups:

- Group 1: SOF 400 mg + GS-5816 25 mg once daily for 12 weeks
- Group 2: SOF 400 mg + GS-5816 100 mg once daily for 12 weeks

Approximately 50 subjects with genotype 3 HCV infection were randomized 1:1 to one of the following 2 treatment groups:

- Group 3: SOF 400 mg + GS-5816 25 mg once daily for 12 weeks
- Group 4: SOF 400 mg + GS-5816 100 mg once daily for 12 weeks

Approximately 40 subjects with genotype 2, 4, 5, or 6 HCV infection were randomized 1:1 to one of the following 2 treatment groups:

- Group 5: SOF 400 mg + GS-5816 25 mg once daily for 12 weeks
- Group 6: SOF 400 mg + GS-5816 100 mg once daily for 12 weeks

Approximately 100 subjects with genotype 1 HCV infection were randomized 1:1:1:1 to one of the following 4 treatment groups:

- Group 7: SOF 400 mg + GS-5816 25 mg once daily for 8 weeks
- Group 8: SOF 400 mg + GS-5816 25 mg once daily + RBV (1000 or 1200 mg/day divided twice daily [BID]) for 8 weeks
- Group 9: SOF 400 mg + GS-5816 100 mg once daily for 8 weeks
- Group 10: SOF 400 mg + GS-5816 100 mg once daily + RBV (1000 or 1200 mg/day divided BID) for 8 weeks

Approximately 100 subjects with genotype 2 HCV infection were randomized 1:1:1:1 to one of the following 4 treatment groups:

- Group 11: SOF 400 mg + GS-5816 25 mg once daily for 8 weeks

- Group 12: SOF 400 mg + GS-5816 25 mg once daily + RBV (1000 or 1200 mg/day divided BID) for 8 weeks
- Group 13: SOF 400 mg + GS-5816 100 mg once daily for 8 weeks
- Group 14: SOF 400 mg + GS-5816 100 mg once daily + RBV (1000 or 1200 mg/day divided BID) for 8 weeks

Randomization was stratified by HCV genotype (genotype 1a or 1b for Groups 1, 2, and 7 through 10; genotype 2, 4, 5, or 6 for Groups 5 and 6). For stratification, mixed genotype 1a/1b or genotype 1 was considered genotype 1a.

SOF and GS-5816 were administered daily without regard to food. RBV was administered with food.

Formulation:
SOF 400-mg tablets: Lot # DC1205B1
GS-5816 25-mg and 100-mg tablets: Lot # DL1301C1 and DL1301D1, respectively
RBV 200-mg tablets: Lot # AA2773Z

PK Sampling: A single PK blood sample was collected from all subjects at each on-treatment visit. However, sparse PK samples for the VEL 25 mg dose in this Phase 2 study was not analyzed. For a subset of subjects who consented to participate in the optional PK substudy, intensive serial PK blood samples were collected at the on-treatment Week 2 or 4 visit at predose, 0.25, 0.5, 1, 2, 3, 4, 8, 12, and 24 hours postdose.

Analytical Methods: Concentrations of SOF, GS-566500, GS-331007, and GS-5816 in plasma samples were determined using fully validated high-performance liquid chromatography-tandem mass spectroscopy (HPLC/MS/MS) bioanalytical methods. All samples were analyzed within the timeframe supported by frozen stability storage data. The assays for SOF, GS-566500, GS-331007, and GS-5816 were all performed and validated by (b) (4) .The standard curve and QC data indicated that the plasma assay method for VEL was precise and accurate.

Pharmacokinetics Results: A total of 37 subjects participated in the PK substudy; 19 were administered SOF + GS-5816 25 mg ± RBV and 18 were administered SOF + GS-5816 100 mg ± RBV. The applicant summarized the steady-state PK for SOF, GS-566500, GS-331007, and GS-5816 pooled by GS-5816 dose in the PK substudy without regard to RBV (Table 1). Approximately 50% higher SOF and GS-566500 AUC and similar Cmax were observed when coadministered with GS-5816 100 mg compared with when coadministered with GS-5816 25 mg. Plasma exposure of GS-331007, the primary circulating metabolite of SOF, was similar regardless of GS-5816 dose. GS-5816 exposure increased greater than dose proportional between 25 mg and 100 mg, with a 6- to 8-fold higher exposure for GS-5816 100 mg.

Table 1. GS-US-342-0102: SOF, GS-566500, GS-331007, and GS-5816 Multiple-Dose PK Parameters Following Administration of SOF + GS-5816 25 mg or 100 mg ± RBV (PK Substudy Analysis Set) (Source: Applicant's study report)

PK Parameter	Mean (% CV)	
	SOF 400 mg + GS-5816 25 mg ± RBV (N = 19)	SOF 400 mg + GS-5816 100 mg ± RBV (N = 18)
SOF		
AUC_{tau} (h•ng/mL)	1250.7 (41.4)	1928.9 (67.3)
C_{max} (ng/mL)	1086.8 (60.0)	1184.9 (50.0)
C_{tau} (ng/mL)	BLQ	BLQ
T_{max} (h)	1.00 (0.50, 1.17)	1.00 (0.50, 1.00)
$t_{1/2}$ (h)	0.47 (0.38, 0.57)	0.54 (0.41, 0.63)
GS-566500		
AUC_{tau} (h•ng/mL)	1941.4 (41.5)	2848.0 (58.4)
C_{max} (ng/mL)	433.5 (31.8)	565.1 (27.2)
C_{tau} (ng/mL)	BLQ	BLQ
T_{max} (h)	2.00 (1.00, 2.00)	2.00 (1.98, 2.00)
$t_{1/2}$ (h)	2.21 (2.07, 2.30)[a]	2.38 (2.17, 2.54)
GS-331007		
AUC_{tau} (h•ng/mL)	11307.5 (25.4)	12710.4 (28.2)
C_{max} (ng/mL)	1092.3 (35.7)	976.3 (33.1)
C_{tau} (ng/mL)	259.3 (46.2)[a]	346.8 (51.4)[b]
T_{max} (h)	3.00 (2.00, 4.00)	3.00 (2.98, 4.00)
$t_{1/2}$ (h)	14.37 (10.05, 16.84)[a]	16.27 (14.06, 20.37)[b]
GS-5816		
AUC_{tau} (h•ng/mL)	424.6 (69.6)	3248.4 (85.8)
C_{max} (ng/mL)	68.8 (78.0)	428.5 (83.9)
C_{tau} (ng/mL)	8.3 (144.6)[a]	51.6 (116.5)[b]
T_{max} (h)	2.00 (1.00, 3.00)	3.00 (2.00, 3.02)
$t_{1/2}$ (h)	13.15 (9.81, 16.01)[a]	9.76 (7.64, 12.17)[b]

BLQ = below the limit of quantitation
a N = 18
b N = 17
Data are mean (%CV) except T_{max} and $t_{1/2}$, which are median (Q1, Q3)

Reviewer Comments:
-The population PK analyses indicated that ribavirin use had no effect on the exposures of SOF and VEL, but has an effect on GS-331007 exposures. RBV use decreased GS-331007 AUC, Cmax and Cmin by 20%, 25% and 18%, respectively. Therefore, the reviewer reanalyzed the data for subjects who did not take RBV, as shown in Table 2. The exposure ratios between SOF +GS-5816 100 mg vs SOF +GS-5816 25 mg were similar to Table 1 when subjects with RBV use were excluded, for SOF, GS-566500, or GS-331007. For GS-5816, the exposures still show a more than dose proportional increase between GS-5816 25 mg and 100 mg, but with only 4- to 6-fold higher exposure for GS-5816 100 mg vs. 25 mg, which is less than observed when data from SOF + GS-5816 +RBV arms were included.

Table 2. GS-US-342-0102: SOF, GS-566500, GS-331007, and GS-5816 Multiple-Dose PK Parameters Following Administration of SOF + GS-5816 25 mg or 100 mg without RBV (PK Substudy Analysis Set)

PK Parameter	Mean (% CV)	
	SOF 400 mg + GS-5816 25 mg (N = 14)	SOF 400 mg + GS-5816 100 mg (N = 9)
SOF		
AUCtau (h•ng/mL)	1355(509)	2137(1399)
Cmax (ng/mL)	1223(664)	1096(491)
Ctau (ng/mL)	BLQ	BLQ
GS-566500		
AUCtau (h•ng/mL)	2122(811)	3278(2258)
Cmax (ng/mL)	474(124)	554(187)
Ctau (ng/mL)	BLQ	BLQ
GS-331007		
AUCtau (h•ng/mL)	11900(2673)	14161(3565)
Cmax (ng/mL)	1162(391)	1124(352)
Ctau (ng/mL)	274(120)	393(224)
GS-5816		
AUCtau (h•ng/mL)	487(298)	2834(1874)
Cmax (ng/mL)	79.0(54.5)	353(245)
Ctau (ng/mL)	9.7(13.3)	57(69)

Efficacy Results: Table 3 presents the proportion of subjects with SVR12 by HCV genotype for subjects with genotype 1, 2, or 3 HCV infection in the 12-week treatment groups. Across the 12-week treatment groups, the proportions of subjects with genotype 1, 2, or 3 HCV infection who achieved SVR12 ranged from 90.9% to 100.0%.

Table 3. GS-US-342-0102: SVR12 (12-Week Treatment Groups; Genotypes 1, 2, or 3) (Full Analysis Set) (Source: Applicant's study report)

	Genotype 1		Genotype 2		Genotype 3	
	Group 1	Group 2	Group 5	Group 6	Group 3	Group 4
	SOF 400 mg + GS-5816 25 mg 12 Weeks (N = 27)	SOF 400 mg + GS-5816 100 mg 12 Weeks (N = 28)	SOF 400 mg + GS-5816 25 mg 12 Weeks (N = 11)	SOF 400 mg + GS-5816 100 mg 12 Weeks (N = 10)	SOF 400 mg + GS-5816 25 mg 12 Weeks (N = 27)	SOF 400 mg + GS-5816 100 mg 12 Weeks (N = 27)
SVR12	26/27 (96.3%)	28/28 (100.0%)	10/11 (90.9%)	10/10 (100.0%)	25/27 (92.6%)	25/27 (92.6%)
95% CI	81.0% to 99.9%	87.7% to 100.0%	58.7% to 99.8%	69.2% to 100.0%	75.7% to 99.1%	75.7% to 99.1%

Table 4 presents the proportion of subjects with SVR12 by HCV genotype for subjects with genotype 4, 5, or 6 HCV infection in the 12-week treatment groups. A total of 23 of 24 subjects with genotype 4, 5, or 6 HCV infection achieved SVR12. Across the 12-week treatment groups, the proportions of subjects with genotype 4, 5, or 6 HCV infection who achieved SVR12 ranged from 85.7% to 100.0%.

Table 4. GS-US-342-0102: SVR12 (12-Week Treatment Groups; Genotypes 4, 5, or 6) (Full Analysis Set) (Source: Applicant's study report)

	Genotype 4		**Genotype 5**	**Genotype 6**	
	Group 5	**Group 6**	**Group 5**	**Group 5**	**Group 6**
	SOF 400 mg + GS-5816 25 mg 12 Weeks (N = 7)	**SOF 400 mg + GS-5816 100 mg 12 Weeks (N = 7)**	**SOF 400 mg + GS-5816 25 mg 12 Weeks (N = 1)**	**SOF 400 mg + GS-5816 25 mg 12 Weeks (N = 4)**	**SOF 400 mg + GS-5816 100 mg 12 Weeks (N = 5)**
SVR12	**7/7 (100.0%)**	**6/7 (85.7%)**	**1/1 (100.0%)**	**4/4 (100.0%)**	**5/5 (100.0%)**
95% CI	**59.0% to 100.0%**	**42.1% to 99.6%**	**2.5% to 100.0%**	**39.8% to 100.0%**	**47.8% to 100.0%**

Table 5 presents the proportion of subjects with SVR12 by HCV genotype for subjects with genotype 1 or 2 HCV infection in the 8-week treatment groups. A total of 190 of 223 subjects with genotype 1 or 2 HCV infection achieved SVR12 across all treatment groups. Within individual treatment groups, proportions of subjects with genotype 1 HCV infection who achieved SVR12 ranged from 83.3% to 86.7% in the SOF + GS-5816 25 mg ± RBV 8-week treatment groups and 80.6% to 89.7% in the SOF + GS-5816 100 mg ± RBV 8-week treatment groups. Treatment with SOF + GS-5816 25 mg or 100 mg ± RBV for 8 weeks resulted in lower SVR12 rates than treatment with SOF + GS-5816 25 mg or 100 mg for 12 weeks in treatment-naive subjects with genotype 1 or 2 HCV infection without cirrhosis.

Table 5. GS-US-342-0102: SVR12 (8-Week Treatment Groups; Genotypes 1 or 2) (Full Analysis Set) (Source: Applicant's study report)

	Genotype 1				**Genotype 2**			
	Group 7	**Group 8**	**Group 9**	**Group 10**	**Group 11**	**Group 12**	**Group 13**	**Group 14**
	SOF 400 mg + GS-5816 25 mg 8 Weeks (N = 30)	**SOF 400 mg + GS-5816 25 mg + RBV 8 Weeks (N = 30)**	**SOF 400 mg + GS-5816 100 mg 8 Weeks (N = 29)**	**SOF 400 mg + GS-5816 100 mg + RBV 8 Weeks (N = 31)**	**SOF 400 mg + GS-5816 25 mg 8 Weeks (N = 26)**	**SOF 400 mg + GS-5816 25 mg + RBV 8 Weeks (N = 25)**	**SOF 400 mg + GS-5816 100 mg 8 Weeks (N = 26)**	**SOF 400 mg + GS-5816 100 mg + RBV 8 Weeks (N = 26)**
SVR12	26/30 (86.7%)	25/30 (83.3%)	26/29 (89.7%)	25/31 (80.6%)	20/26 (76.9%)	22/25 (88.0%)	23/26 (88.5%)	23/26 (88.5%)
95% CI	69.3% to 96.2%	65.3% to 94.4%	72.6% to 97.8%	62.5% to 92.5%	56.4% to 91.0%	68.8% to 97.5%	69.8% to 97.6%	69.8% to 97.6%

Conclusions:

- Administration of SOF + GS-5816 100 mg ± RBV resulted in approximately 50% higher SOF and GS-566500 exposures and 4- to 6-fold higher GS-5816 exposures compared

with administration of SOF + GS-5816 25 mg ± RBV. GS-331007 exposure was similar regardless of GS-5816 dose.
- Treatment with SOF + GS-5816 25 mg or 100 mg for 12 weeks resulted in high SVR12 rates in treatment-naive subjects with genotype 1 to 6 HCV infection without cirrhosis.
- Treatment with SOF + GS-5816 25 mg or 100 mg ± RBV for 8 weeks resulted in lower SVR12 rates than treatment with SOF + GS-5816 25 mg or 100 mg for 12 weeks in treatment-naive subjects with genotype 1 or 2 HCV infection without cirrhosis.
- The addition of RBV to an 8-week regimen in treatment-naive patients with GT 1 or 2 did not affect the overall SVR12 rates.

4.1.2.7 GS-US-342-0109: A Phase 2, Multicenter, Randomized, Open-Label Study to Investigate the Safety and Efficacy of Sofosbuvir + GS-5816 for 12 Weeks in Treatment Experienced Subjects with Chronic HCV Infection

Objectives:
- To determine the antiviral efficacy and safety of combination treatment with sofosbuvir (SOF) + GS-5816 with or without ribavirin (RBV)
- To characterize the steady-state pharmacokinetics (PK) of study drugs

Study Design: This is a Phase 2, multicenter, randomized, open-label study. A total of 323 treatment-experienced subjects with chronic HCV infection were randomized into 1 of 12 treatment groups as described below. Subjects were treated with SOF+GS-5816 (25 mg or 100 mg) with or with RBV for 12 weeks.

- A total of 107 noncirrhotic subjects with genotype 3 HCV infection were randomized 1:1:1:1 to 1 of the following 4 treatment groups:
  Group 1: SOF 400 mg + GS-5816 25 mg once daily for 12 weeks
  Group 2: SOF 400 mg + GS-5816 25 mg once daily + RBV (1000 or 1200 mg/day divided twice daily [BID]) for 12 weeks
  Group 3: SOF 400 mg + GS-5816 100 mg once daily for 12 weeks
  Group 4: SOF 400 mg + GS-5816 100 mg once daily + RBV (1000 or 1200 mg/day divided BID) for 12 weeks

- A total of 104 cirrhotic subjects with genotype 3 HCV infection were randomized 1:1:1:1 to 1 of the following 4 treatment groups:
  Group 5: SOF 400 mg + GS-5816 25 mg once daily for 12 weeks
  Group 6: SOF 400 mg + GS-5816 25 mg once daily + RBV (1000 or 1200 mg/day divided BID) for 12 weeks
  Group 7: SOF 400 mg + GS-5816 100 mg once daily for 12 weeks
  Group 8: SOF 400 mg + GS-5816 100 mg once daily + RBV (1000 or 1200 mg/day divided BID) for 12 weeks

- A total of 112 subjects with genotype 1 HCV infection were randomized 1:1:1:1 to 1 of the following 4 treatment groups:
  Group 9: SOF 400 mg + GS-5816 25 mg once daily for 12 weeks
  Group 10: SOF 400 mg + GS-5816 25 mg once daily + RBV (1000 or 1200 mg/day divided BID) for 12 weeks
  Group 11: SOF 400 mg + GS-5816 100 mg once daily for 12 weeks
  Group 12: SOF 400 mg + GS-5816 100 mg once daily + RBV (1000 or 1200 mg/day divided BID) for 12 weeks

Randomization into Groups 9 through 12 was stratified by HCV genotype (genotype 1a or 1b) and cirrhosis (presence or absence). For stratification, mixed genotype 1a/1b or genotype 1 was considered genotype 1a. Approximately 50% of the subjects with genotype 1 HCV infection were to have compensated cirrhosis.

SOF and GS-5816 were administered daily without regard to food. RBV was administered with food.

Formulation:
SOF 400-mg tablets: Lot # DC1211B1, DC1207B1, DC1209B1
GS-5816 25-mg and 100-mg tablets: Lot # DL1301C1, DL1301C1-A, DL1301D1, DL1301D1-A, and DL1301D1-B
RBV 200-mg tablets: Lot # A97943Z and AA2773Z

PK Sampling: A single PK blood sample was collected from all subjects at each on-treatment visit. However, sparse PK samples for the VEL 25 mg dose in this Phase 2 study was not analyzed. For a subset of subjects who consented to participate in the optional PK substudy, intensive serial PK blood samples were collected at the on-treatment Week 2 or 4 visit at predose, 0.25, 0.5, 1, 2, 3, 4, 8, 12, and 24 hours postdose.

Analytical Methods: Concentrations of SOF, GS-566500, GS-331007, and GS-5816 in plasma samples were determined using fully validated high-performance liquid chromatography-tandem mass spectroscopy (LC/MS/MS) bioanalytical methods. All samples were analyzed within the timeframe supported by frozen stability storage data. The assays for SOF, GS-566500, GS-331007, and GS-5816 were all performed and validated by (b) (4) .The standard curve and QC data indicated that the plasma assay method for VEL was precise and accurate.

Pharmacokinetics Results: A total of 34 subjects participated in the PK substudy; 14 were administered SOF + GS-5816 25 mg ± RBV and 20 were administered SOF + GS-5816 100 mg ± RBV. The applicant summarized the steady-state PK for SOF, GS-566500, GS-331007, and GS-5816 pooled by GS-5816 dose in the PK substudy without regard to RBV (Table 1). Modestly higher SOF (approximately 20%) and GS-566500 (approximately 26%) overall exposures (AUC) were observed when coadministered with GS-5816 100 mg compared with coadministration with GS-5816 25 mg. Plasma exposure of GS-331007, the primary circulating metabolite of SOF, was similar regardless of GS-5816 dose. GS-5816 exposure was not dose proportional between GS-5816 25 mg and 100 mg, with a 6- to 8-fold exposure range observed (AUC, Cmax, and Ctau).

Table 1. GS-US-342-0109: SOF, GS-566500, GS-331007, and GS-5816 Multiple-Dose PK Parameters Following Administration of SOF + GS-5816 25 mg or 100 mg ± RBV (PK Substudy Analysis Set) (Source: Applicant's study report)

PK Parameter	Mean (% CV)	
	SOF 400 mg + GS-5816 25 mg ± RBV (N = 14)	SOF 400 mg + GS-5816 100 mg ± RBV (N = 20)
SOF		
AUC_{tau} (h•ng/mL)	1430.2 (31.6)[a]	1711.4 (51.6)[b]
C_{max} (ng/mL)	1188.8 (44.8)	1370.3 (60.2)
C_{tau} (ng/mL)	BLQ	BLQ
T_{max} (h)	2.00 (0.50, 2.00)	1.00 (0.51, 1.52)
$t_{1/2}$ (h)	0.52 (0.45, 0.61)[a]	0.50 (0.42, 0.61)[b]
GS-566500		
AUC_{tau} (h•ng/mL)	2332.0 (16.2)[a]	2926.9 (29.9)[c]
C_{max} (ng/mL)	553.7 (17.1)	655.6 (38.5)
C_{tau} (ng/mL)	BLQ	BLQ
T_{max} (h)	2.00 (1.00, 3.00)	2.00 (1.00, 3.01)
$t_{1/2}$ (h)	2.18 (2.09, 2.28)[a]	2.31 (2.21, 2.61)[c]
GS-331007		
AUC_{tau} (h•ng/mL)	10799.4 (35.0)	11562.0 (30.2)[c]
C_{max} (ng/mL)	878.6 (36.1)	908.3 (39.4)
C_{tau} (ng/mL)	251.3 (44.9)	290.5 (33.0)[c]
T_{max} (h)	4.00 (2.00, 4.00)	3.06 (3.00, 4.00)
$t_{1/2}$ (h)	13.66 (11.16, 16.34)	17.52 (14.58, 23.20)[c]
GS-5816		
AUC_{tau} (h•ng/mL)	407.9 (60.2)	2791.3 (55.8)[c]
C_{max} (ng/mL)	63.7 (56.6)	371.4 (53.1)
C_{tau} (ng/mL)	4.5 (56.0)	34.7 (56.6)[c]
T_{max} (h)	2.00 (1.00, 3.00)	2.05 (2.00, 3.01)
$t_{1/2}$ (h)	9.19 (6.90, 10.80)	9.13 (7.77, 9.96)[c]

a N = 13
b N = 16
c N = 19
Data are mean (%CV) except T_{max} and $t_{½}$, which are median (Q1, Q3)

The population PK analyses indicated that ribavirin use had no effect on the exposures of SOF and VEL, but has an effect on GS-331007 exposures. Ribavirin use decreased GS-331007 AUC, Cmax and Cmin by 20%, 25% and 18%, respectively. Therefore, the reviewer reanalyzed the data for subjects who did not take RBV, as shown in Table 2. The exposure ratios between SOF +GS-5816 100 mg vs SOF +GS-5816 25 mg were similar to Table 1 when subjects with

RBV use were excluded, for SOF, GS-566500, or GS-331007. For GS-5816, the exposures show near dose proportional between GS-5816 25 mg and 100 mg, with only approximately 4-fold exposure higher for GS-5816 100 mg vs. 25 mg, which is less than observed when data from SOF + GS-5816 +RBV arms were included.

Table 2. GS-US-342-0109: SOF, GS-566500, GS-331007, and GS-5816 Multiple-Dose PK Parameters Following Administration of SOF + GS-5816 25 mg or 100 mg without RBV (PK Substudy Analysis Set) (Source: Applicant's Study Report)

PK Parameter	Mean (% CV)	
	SOF 400 mg + GS-5816 25 mg (N = 5)	SOF 400 mg + GS-5816 100 mg (N = 11)
SOF		
AUCtau (h•ng/mL)	1051(302)	1415(330)
Cmax (ng/mL)	884(572)	1347(370)
Ctau (ng/mL)	BLQ	BLQ
GS-566500		
AUCtau (h•ng/mL)	2113(252)	2511(629)
Cmax (ng/mL)	574(148)	538(125)
Ctau (ng/mL)	BLQ	BLQ
GS-331007		
AUCtau (h•ng/mL)	11255(2841)	12052(4372)
Cmax (ng/mL)	1051(189)	951(345)
Ctau (ng/mL)	232(96)	294(113)
GS-5816		
AUCtau (h•ng/mL)	479(247)	2053(893)
Cmax (ng/mL)	72(41)	285(123)
Ctau (ng/mL)	4.7(1.6)	27(14)

Efficacy Results: Tables 1 and 2 present the proportion of subjects with SVR12 for subjects with genotypes 1 and 3 HCV infection by treatment group, respectively. The data show that for geonotype 1 subjects, SVR12 rate are high (> 96%) across all 4 treatment arms. For noncirrhotic subjects with genotype 3, SVR rates were 100% for SOF +GS-5816 100 mg ± RBV, while SVR rates were lower (85% to 96%) for SOF +GS-5816 25 mg ± RBV, indicating a dose-response relationship for GS-5816 following coadministration of SOF +GS-5816 ± RBV For noncirrhotic subjects with genotype 3. This dose-response relationship was also observed for cirrhotic subjects with genotype 3. The SVR12 rate is relatively lower for subjects with cirrhosis.

Table 3. GS-US-342-0109: SVR12 (Genotype 1 Treatment Groups) (Full Analysis Set)
(Source: Applicant's Study Report)

	SOF 400 mg + GS-5816 25 mg (N = 26)	SOF 400 mg + GS-5816 25 mg + RBV (N = 28)	SOF 400 mg + GS-5816 100 mg (N = 27)	SOF 400 mg + GS-5816 100 mg + RBV (N = 26)
SVR12	27/27 (100.0%)	28/29 (96.6%)	27/27 (100.0%)	27/28 (96.4%)
95% CI	87.2% to 100.0%	82.2% to 99.9%	87.2% to 100.0%	81.7% to 99.9%

Table 4. GS-US-342-0109: SVR12 (Genotype 3 Treatment Groups) (Full Analysis Set)
(Source: Applicant's Study Report)

	Genotype 3 Noncirrhotic Subjects				Genotype 3 Cirrhotic Subjects			
	SOF 400 mg + GS-5816 25mg (N = 26)	SOF 400 mg + GS-5816 25mg + RBV (N = 28)	SOF 400 mg + GS-5816 100 mg (N = 27)	SOF 400 mg + GS-5816 100 mg + RBV (N = 26)	SOF 400 mg + GS-5816 25 mg (N = 26)	SOF 400 mg + GS-5816 25 mg + RBV (N = 25)	SOF 400 mg + GS-5816 100 mg (N = 26)	SOF 400 mg + GS-5816 100 mg RBV (N = 26)
SVR12	22/26 (84.6%)	27/28 (96.4%)	27/27 (100.0%)	26/26 (100.0%)	15/26 (57.7%)	21/25 (84.0%)	23/26 (88.5%)	25/26 (96.2%)
95% CI	65.1% to 95.6%	81.7% to 99.9%	87.2% to 100.0%	86.8% to 100.0%	36.9% to 76.6%	63.9% to 95.5%	69.8% to 97.6%	80.4% to 99.9%

Conclusions:

- Administration of SOF + GS-5816 100 mg ± RBV resulted in modestly higher SOF (~20%) and GS-566500 (~26%) exposures compared with administration of SOF + GS-5816 25 mg ± RBV. GS-331007 exposure was similar regardless of GS-5816 dose. GS-5816 exposure was near dose-proportional between GS-5816 25 mg and GS-5816 100 mg.
- Treatment with SOF + GS-5816 100 mg with or without RBV for 12 weeks resulted in high SVR12 rates in treatment-experienced subjects with genotype 1 or 3 HCV infection with or without cirrhosis. There were fewer virologic failures in treatment-experienced subjects with genotype 3 HCV infection who received SOF + GS-5816 100 mg ± RBV compared with those who received SOF + GS-5816 25 mg ± RBV.
- The addition of RBV did not affect the overall SVR12 rate.
- SOF in combination with GS-5816 100 mg for 12 weeks is the regimen that should be studied in Phase 3 trials.

4.1.3 Intrinsic Factors (by Abhay Joshi)

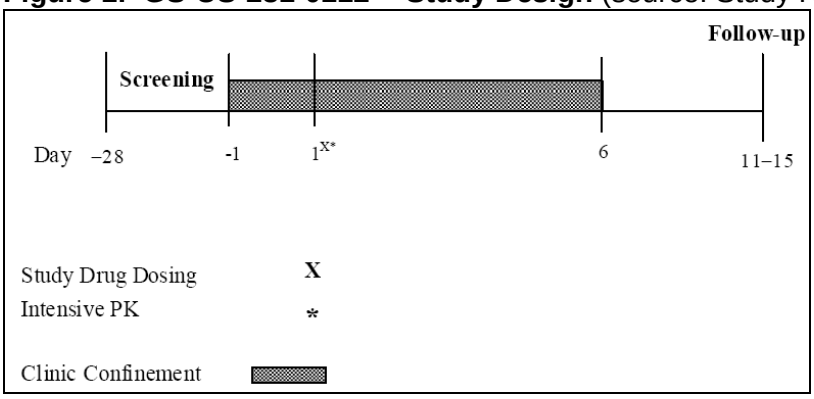
4.1.3.1 GS-US-281-0112: A Phase 1 Open-Label, Parallel-Group, Single-Dose Study to Evaluate the Pharmacokinetics of GS-5816 in Subjects with Normal Hepatic Function and Moderate or Severe Hepatic Impairment

Study Rationale: Results from the nonclinical and the mass balance study (GS-US-281-1055) indicated that GS-5816 (Velpatasvir) is eliminated predominantly via hepatobiliary route; as unchanged GS-5816 as well as metabolites. Additionally, very little unchanged GS-5816 or metabolites are excreted renally (0.4%). Therefore, this study was conducted to assess the effect of hepatic impairment on GS-5816 pharmacokinetics (PK).

Primary Objective(s): To evaluate the single-dose PK of GS-5816 in subjects with normal hepatic function, moderate hepatic impairment, and severe hepatic impairment

Study Design: Out of total 33 enrolled subjects, 10 subjects were enrolled in Cohort 1 (moderate hepatic impairment) and 10 subjects were enrolled in Cohort 2 (severe hepatic impairment). Severity of the subject's hepatic impairment (moderate or severe) was determined based on the Child-Pugh-Turcotte classification. Once a hepatic impaired subject completed through Day 6, a healthy match (matched for age (± 10 years), sex, and BMI (± 15%)) was enrolled into the control group, within the Cohort 1 or 2. Given that the protocol allowed a subject with normal hepatic function to be a matched control subject for both Cohort 1 and 2 (moderate and severe hepatic impairment), out of 13 subjects enrolled in the control group, 7 subjects with normal hepatic function were each matched as a respective control subject for both Cohort 1 and 2. While, the remaining 6 subjects were each matched as a control subject, either for Cohort 1 or Cohort 2 (3 subjects/cohort). In the morning on Day 1, following a light breakfast, subjects received 100 mg (2 × 50-mg tablets) of GS-5816 and were under Clinical Confinement for 5 days for pharmacokinetic sampling (Figure 1). Dosing of subjects with severe hepatic impairment in Cohort 2 began after a review of safety and available PK data from subjects with moderate hepatic impairment in Cohort 1.

Figure 1: GS-US-281-0112 -- Study Design (source: Study Report Figure 7-1)



Formulation: 50 mg Tablet (Lot # DL1203B1)

Pharmacokinetic Sampling: PK sampling was performed at 0 (pre-dose ≤ 5 min), 0.5, 1, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 18, 24, 48, 72, 96, and 120 hours postdose. In addition, at 2.5 and 3.5 hours postdose, additional blood samples were collected for plasma protein binding evaluation.

Analytical methods: Quantification of GS-5816 was performed by the validated high-performance liquid chromatography-tandem mass spectroscopy (HPLC/MS/MS) methods at (b) (4). Assay validation parameters are summarized in Table 1.

Table 1: Bioanalytical assay validation parameters for GS-5816 (source: Study Report Table 7-3)

Parameter	GS-5816
Linear Range (ng/mL)	1 to 1,000
Lower Limit of Quantitation (ng/mL)	1
Interassay Precision Range (%CV)	1.7 to 4.9
Interassay Accuracy Range (%RE)	-1.5 to 4.1
Stability in Frozen Matrix (days)	174 days at -70°C and 161 days at -20°C

CV = coefficient of variation; RE = relative error

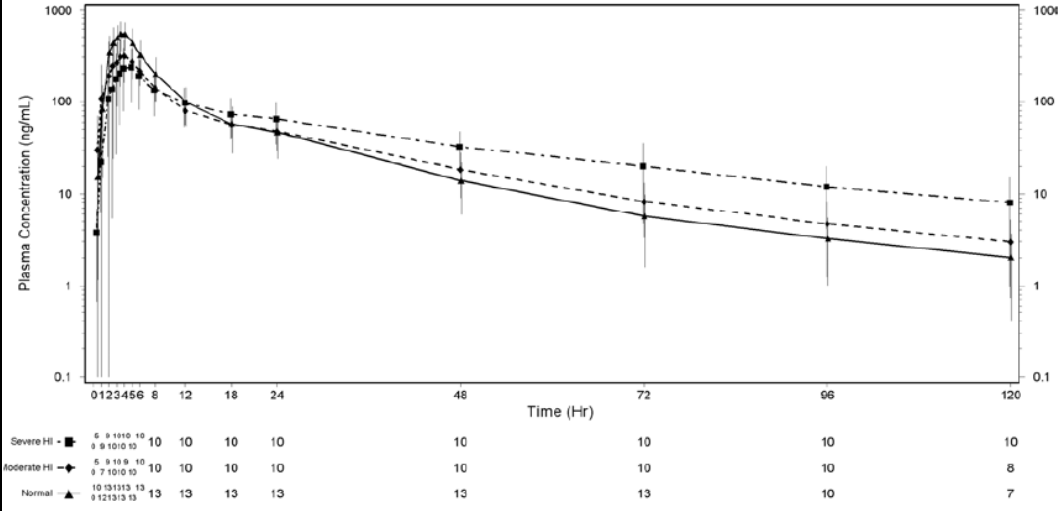
PK assessment was performed by Noncompartmental analysis (NCA) by utilizing Phoenix WinNonlin® Professional Version 6.3 (Pharsight Corporation, Mountain View, CA, USA). The primary PK parameters were AUC_{last}, AUC_{inf}, and C_{max} of GS-5816. Following the parameter estimation, mean GS-5816 plasma PK parameters and 90% confidence intervals (CIs) for the % Geometric Least-Squares Mean (GLSM) ratios were derived and compared (Table 3).

Results:

Pharmacokinetics of GS-5816

In subjects with hepatic impairment (moderate and severe), mean C_{max} values were lower than in subjects with normal hepatic function (Figure 2). GS-5816 elimination rate was lower in subjects with moderate hepatic impairment compared to control subjects and was even lower in subjects with severe hepatic impairment (Figure 2).

Figure 2: Mean (SD) GS-5816 plasma concentration-time profiles following administration of a single 100-mg dose in subjects with normal hepatic function, moderate hepatic impairment, and severe hepatic impairment (source: Study Report Figure 10-1)



Note: Plasma concentration below limit of quantitation was treated as 0 for summary purpose and was treated as missing for log-transformed data.

Note: HI = hepatic impairment

The other PK parameters that were estimated in this study included T_{max}, C_{last}, T_{last}, λ_z, $\%AUC_{exp}$, $t_{1/2}$, V/F, and CL/F (Table 2). GS-5816 plasma exposure parameter estimates (AUC_{inf} and AUC_{last}) in subjects with hepatic impairment (moderate and severe) were similar to the estimates in subjects with normal hepatic function. Compared to control subjects, observed C_{max} of GS-5816 was approximately 41% and 53% lower in subjects with moderate and severe hepatic impairment, respectively. Median terminal $t_{1/2}$ was prolonged in subjects with moderate hepatic impairment (~23 h) and severe hepatic impairment (~31 h), compared to control subjects (~18 h). The sponsor indicates that in subjects with hepatic impairment, the simultaneous reduction in bioavailability and systemic clearance of GS-5816 contributed to the observed similar exposures; regardless of a subject's hepatic impairment status.

Table 2: GS-5816 plasma pharmacokinetic parameters following administration of a single 100-mg dose in subjects with moderate or severe hepatic impairment and subjects with normal hepatic function (source: Study Report Table 10-1)

	Mean (%CV)		
GS-5816 PK Parameter	Normal Hepatic Function (N = 13)	Moderate Hepatic Impairment (N = 10)	Severe Hepatic Impairment (N = 10)
AUC_{exp} (%)	1.24 (64.24)	2.41 (87.82)	8.84 (83.58)
AUC_{last} (ng•h/mL)	4880.0 (40.7)	4001.6 (37.9)	4897.3 (47.8)
AUC_{inf} (ng•h/mL)	4937.4 (40.7)	4104.6 (37.9)	5403.7 (50.3)
C_{max} (ng/mL)	559.7 (34.4)	343.8 (49.0)	268.4 (54.5)
T_{max} (h)[a]	3.50 (3.50, 4.00)	4.00 (3.00, 5.00)	4.02 (4.00, 5.00)
C_{last} (ng/mL)	1.95 (60.90)	2.67 (77.28)	8.07 (87.99)
T_{last} (h)[a]	120.00 (96.00, 120.00)	120.00 (120.00, 120.00)	120.00 (120.00, 120.00)
$t_{1/2}$ (h)[a]	18.03 (17.27, 21.36)	22.58 (18.90, 26.88)	30.98 (25.19, 52.29)
V_z/F (mL)	678,639.0 (56.4)	912,169.5 (35.9)	1,280,939.1 (74.2)
Weight normalized V_z/F (mL/kg)	8568.2 (57.0)	11,351.4 (29.3)	14,142.4 (56.8)
CL/F (mL/h)	25,588.1 (70.1)	27,488.1 (34.8)	24,380.8 (59.9)
Weight normalized CL/F (mL/h/kg)	329.9 (73.6)	342.4 (28.1)	278.8 (42.9)

a Median (Q1, Q3)

Table 3: Statistical comparisons of GS-5816 plasma pharmacokinetic parameters following administration of a single 100-mg dose in subjects with moderate or severe hepatic impairment and subjects with normal hepatic function(source: Study Report Table 10-2)

GS-5816 PK Parameter	Geometric Least-Squares Mean (GLSM)		%GLSM Ratio (90% CI) Hepatic Impairment/ Normal Function
	Hepatic Impairment	Normal Hepatic Function	
Moderate Hepatic Impairment, N	10	10	
AUC_{last} (ng•h/mL)	3763.77	4588.70	82.02 (56.80, 118.45)
AUC_{inf} (ng•h/mL)	3857.61	4646.00	83.03 (57.53, 119.83)
C_{max} (ng/mL)	311.71	524.71	59.41 (39.78, 88.71)
Severe Hepatic Impairment, N	10	10	
AUC_{last} (ng•h/mL)	4313.85	4119.12	104.73 (68.71, 159.63)
AUC_{inf} (ng•h/mL)	4746. 85	4177.17	113.64 (74.72, 172.82)
C_{max} (ng/mL)	224.78	476.36	47.19 (29.32, 75.96)

Protein Binding: Protein binding was assessed at T_{max} or at the time closest to T_{max}. In comparison to control subjects, the mean unbound drug fraction estimates were 1.4 times and 2.2 times higher in subjects with moderate and severe hepatic impairment, respectively (Table 4).

Table 4: Plasma protein binding of GS-5816 following administration of a single 100-mg dose in subjects with moderate or severe hepatic impairment and subjects with normal hepatic function

	Mean (SD)		Ratio
	Moderate Hepatic Impairment	**Normal Hepatic Function**	
Percent Free	0.74% (0.166%)	0.54 % (0.116%)	1.37
Percent Bound	99.26% (0.184%)	99.47% (0.111%)	-
	Severe Hepatic Impairment	**Normal Hepatic Function**	
Percent Free	1.21% (0.395%)	0.54 % (0.116%)	2.24
Percent Bound	98.78% (0.402%)	99.47% (0.111%)	-

Reviewer's notes: *This study (GS-US-281-0112) was conducted in non-HCV-infected subjects with normal hepatic function, moderate hepatic impairment, and severe hepatic impairment. Overall, the extent of the systemic exposure to GS-5816 was found to be similar regardless of a subject's hepatic impairment status. One of the reasons for the observed similarity could be the decrease in GS-5816 C_{max} with the simultaneous significant prolongation of GS-5816 elimination half-life. Based on these results, the sponsor indicates that both GS-5816 bioavailability and systemic clearance may be reduced in subjects with hepatic impairment.*

However, the integrated population pharmacokinetic (PopPK) analysis of the GS-5816 PK data from 11 clinical studies (Study Report 15-0001), indicates that HCV infected subjects with decompensated cirrhosis (CPT-B or CPT-C) had higher drug clearance and, median estimated

AUC of patients with decompensated cirrhosis was 27% lower than the hepatic normal or subjects with compensated cirrhosis.

Additionally, protein binding results from the non-HCV-infected subjects (GS-US-281-0112) shows that in comparison to control subjects, mean unbound drug fraction is 1.4 times and 2.2 times higher in subjects with moderate and severe hepatic impairment, respectively. Therefore, higher clearance estimates could partly be a consequence of the reduced protein binding.

Overall, although these results indicate that hepatic impairment may differentially affect GS-5816 exposure in healthy vs. HCV-infected subjects, the lower exposures in HCV-infected subjects are not likely to be clinically relevant, based on the efficacy results of the phase 3 trials.

Conclusions:

- For GS-5816, dose modification is not warranted for patients with any degree of hepatic impairment
- Overall, GS-5816 is well-tolerated by subjects with moderate or severe hepatic impairment

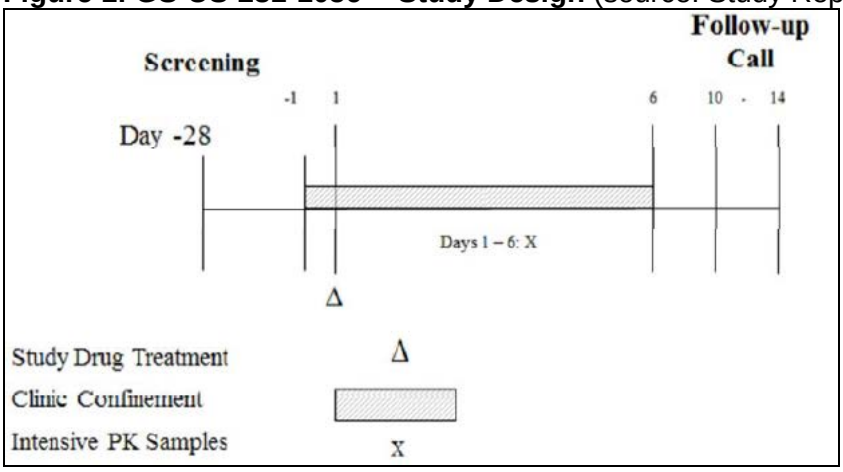
4.1.3.2 GS-US-281-1056: A Phase 1, Open-Label, Parallel-Group, Single-Dose Study to Evaluate the Pharmacokinetics of GS-5816 in Subjects with Normal Renal Function and Severe Renal Impairment

Study Rationale: In the Mass balance study (GS-US-281-1055), following a single dose of radiolabeled [^{14}C] GS-5816, approximately 0.4% of the dose was present in urine as parent and metabolites. Given that renal excretion of GS-5816 is a minor pathway for elimination, the sponsor has conducted this reduced (design) PK study to assess the effect of renal impairment on GS-5816 PK. Additionally, in vitro study results indicated that GS-5816 is highly protein bound (>99.5%); therefore, this study also included the assessment of GS-5816 protein binding in subjects with renal impairment.

Primary Objective(s): To evaluate the single-dose PK of GS-5816 in subjects with severe renal impairment and matched healthy control subjects. The primary endpoints were the PK parameters AUC_{last}, AUC_{inf}, and C_{max} of GS-5816.

Study Design: In this single dose study, 19 eligible subjects were enrolled in 2 groups (10 subjects in Group 1 and 9 subjects in Group 2). Subjects with calculated creatinine clearance (CLcr) < 30 mL/min (using Cockcroft-Gault formula) were enrolled in the severe renal impairment group (Group 1). Once a renally impaired subject completed through Day 6 of the study, a healthy match (matched for age (± 10 years), sex, and BMI (± 15%)) was enrolled into the control group (Group 2). However, because of the extended difficulty in identifying a healthy match for one subject with severe renal impairment, only 9 subjects were enrolled in the control group. Pharmacokinetic sampling was performed following a single oral dose of GS-5816 100 mg (2 × 50-mg tablets) with a light breakfast on Day 1 (Figure 1), until 120 hours postdose.

Figure 1: GS-US-281-1056 -- Study Design (source: Study Report Figure 7-1)



Formulation: 50 mg Tablet (Lot # DL1203B1)

Pharmacokinetic Sampling: Blood samples were collected at predose (≤ 5 min), 0.5, 1, 2, 2.5, 3, 3.5, 4, 6, 8, 12, 18, 24, 48, 72, 96, and 120 hours postdose. At the 2 and 3 hour postdose time points, an additional blood sample was collected for the plasma protein binding evaluation.

Urine PK samples were collected predose and at the following intervals: 0 to 6, 6 to 12, 12 to 24, 24 to 48, 48 to 72, 72 to 96, and 96 to 120 hours postdose.

Analytical methods: Quantification of GS-5816 was performed using validated high-performance liquid chromatography-tandem mass spectroscopy (HPLC/MS/MS) methods at (b) (4). Assay validation parameters are summarized in Table 1. Neither assay information nor results are provided from the urine sample analysis.

Table 1: Bioanalytical assay validation parameters for GS-5816 (source: Study Report Table 7-3)

Parameter	GS-5816
Linear range (ng/mL)	1 to 1000
Lower limit of quantitation (ng/mL)	1
Interday precision range (%CV)	1.6 to 3.8
Interday accuracy range (%RE)	-2.0 to 4.5
Stability in frozen matrix (days)	161 at -20°C and 570 at -70°C

PK assessment was performed by Noncompartmental analysis (NCA) by utilizing Phoenix WinNonlin® software. The primary PK parameters estimated were AUC_{last}, AUC_{inf}, and C_{max} of GS-5816. Following the parameter estimation, mean GS-5816 plasma PK parameters and 90% confidence intervals (CIs) for the % Geometric Least-Squares Mean (GLSM) were derived and compared (Table 4).

Results:

Baseline creatinine clearance values for the enrolled subjects are summarized in Table 2.

Table 2: GS-US-281-1056: Baseline creatinine clearance values (source: Study Report Table 8-3)

Characteristic	Severe Renal Impairment (N = 10)	Normal Renal Function (N = 9)	Overall (N = 19)
Creatinine Clearance (mL/min)			
Mean (SD)	17.902 (5.4493)	101.265 (11.3284)	57.390 (43.5966)
Median	17.705	97.234	27.041
Q1, Q3	12.723, 22.301	91.944, 109.476	14.994, 97.234
Min, Max	12.146, 27.041	90.749, 120.430	12.146, 120.430

Pharmacokinetics of GS-5816

The mean GS-5816 plasma concentration- time profile was higher in subjects with severe renal impairment when compared to subjects with normal renal function (Figure 2).

Figure 2: Mean (SD) GS-5816 plasma concentration-time profiles for subjects with severe renal impairment and normal renal function (source: Study Report Figure 10-1)

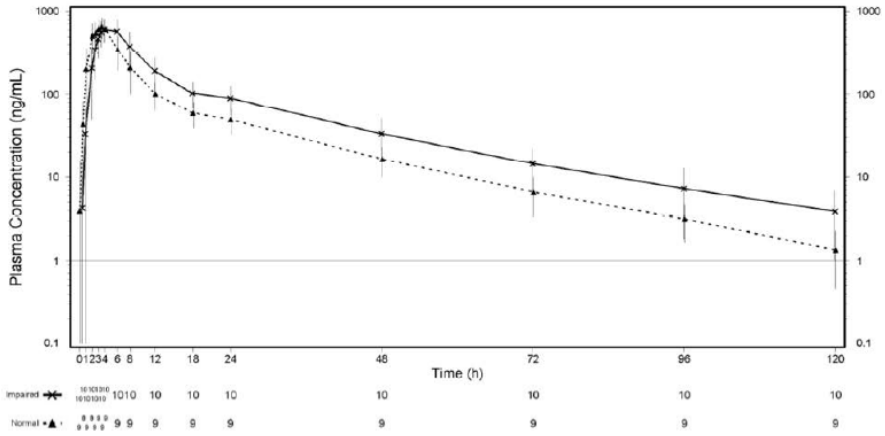


Table 3: GS-5816 plasma PK parameters following administration of GS-5816 in subjects with severe renal impairment and normal renal function (source: Study Report Table 10-1)

GS-5816 PK Parameter	Mean (%CV)	
	Severe Renal Impairment (N = 10)	Normal Renal Function (N = 9)
$\%AUC_{exp}$ (%)	1.50 (62.69)	0.96 (21.01)
AUC_{last} (ng•h/mL)	7971.7 (31.8)	5597.8 (31.2)
AUC_{inf} (ng•h/mL)	8108.3 (32.4)	5651.6 (31.2)
C_{max} (ng/mL)	732.4 (24.1)	702.7 (28.1)
T_{max} (h)[a]	4.00 (3.50, 6.00)	3.00 (2.50, 3.50)
C_{last} (ng/mL)	3.91 (73.44)	1.91 (22.73)
T_{last} (h)[a]	120.00 (120.00, 120.00)	120.00 (96.00, 120.00)
$t_{1/2}$ (h)[a]	21.53 (19.50, 25.76)	20.54 (16.22, 21.38)
V_z/F (mL)	428650.2 (33.5)	521018.9 (25.5)
Weight Normalized V_z/F (mL/kg)	5999.5 (27.9)	6629.2 (28.0)
CL/F (mL/h)	13715.0 (36.6)	19042.5 (26.0)
Weight Normalized CL/F (mL/h/kg)	194.2 (34.8)	240.2 (25.2)

a Median (Q1, Q3)

Table 4: Statistical evaluations of GS-5816 plasma pk parameters following administration of GS-5816 in subjects with severe renal impairment and normal renal function (source: Study Report Table 10-2)

GS-5816 PK Parameter	GLSM		%GLSM Ratio (Impaired/Normal)	90% CI
	Severe Renal Impairment (N = 9)	Normal Renal Function (N = 9)		
C_{max} (ng/mL)	752.5	678.8	110.9	90.8, 135.4
AUC_{inf} (ng•h/mL)	8148.2	5435.6	149.9	117.0, 192.1
AUC_{last} (ng•h/mL)	8023.5	5383.3	149.1	116.6, 190.5

Matched pairs only were used in the comparison analyses.

The other PK parameters that were estimated in this study included T_{max}, C_{last}, T_{last}, $\%AUC_{exp}$, $t_{1/2}$, V/F, and CL/F (Table 3). In subjects with severe renal impairment, mean GS-5816 plasma exposure (AUC) was approximately 43% higher compared to subjects with normal renal function. However, reported mean estimates for C_{max} and median $t_{1/2}$ were similar between the groups. Median time to reach C_{max} (T_{max}) was longer in subjects with severe renal impairment (4 hours) than in subjects with normal renal function (3 hours).

Protein Binding: Protein binding was assessed at T_{max} or at the time closest to T_{max}. Protein binding estimates were similar in subjects with severe renal impairment and with normal renal function (Table 5).

Table 5: Plasma protein binding of GS-5816 following administration of a single 100-mg dose in subjects severe renal impairment and normal renal function

	Mean (%CV)		Ratio
	Severe Renal Impairment	**Normal Renal Function**	
Percent Free	0.29% (4.27%)	0.29 % (4.13%)	1
Percent Bound	99.71% (0.01%)	99.71% (0.01%)	-

Conclusions:

- GS-5816 PK was not clinically significantly altered in subjects with severe renal impairment compared to PK in subjects with normal renal function.
- No dose adjustment of GS-5816 is necessary when administered to subjects with mild, moderate, or severe renal impairment.
- Single doses of GS-5816 were generally well-tolerated in subjects, regardless of renal function.

4.1.4 Extrinsic Factors

4.1.4.1 GS-US-281-0115: A Phase 1 Study to Evaluate Transporter and Cytochrome P (CYP) 450-Mediated Drug-Drug Interactions between Velpatasvir and Probe Drugs

Objectives:

- To evaluate the effect of velpatasvir (VEL) on organic anion transporting polypeptide (OATP)/breast cancer resistance protein (BCRP), and P-glycoprotein (P-gp) substrates using phenotypic probes
- To evaluate the effect of cytochrome P450 (CYP) 3A (CYP3A)/CYP2C8/P-gp inducers or inhibitors on the pharmacokinetics (PK) of VEL
- To evaluate the effect of selective OATP1B1/1B3 inhibitors and mixed OATP/P-gp/ multidrug resistance protein 2 (MRP2) inhibitors on the PK of VEL
- To evaluate the effect of potent selective CYP3A or CYP2C8 inhibitors on the PK of VEL

Study Design: This was a randomized, cross-over, open-label, single and multiple-dose, multiple-cohort study designed to evaluate transporter and CYP-mediated drug-drug interactions between VEL and probe drugs. Eligible subjects were assigned to one of 5 cohorts and then randomized to a treatment sequence within their respective cohort. An overview of the study design for Cohorts 1 to 5 is provided below.

Cohort 1 (OATP substrate pravastatin and OATP/BCRP substrate rosuvastatin): Subjects (18) were randomized to one of 2 sequences and received Treatments A and B with a 5-day washout interval in between.

- Treatment A: a single dose of pravastatin 40 mg, followed by a 3-day washout period, and a single dose of rosuvastatin 10 mg
- Treatment B: VEL 100 mg administered every day for 11 days, with a single dose of pravastatin 40 mg administered on the fifth day, and a single dose of rosuvastatin 10 mg administered on the ninth day

Cohort 2 (P-gp substrate digoxin): Subjects (22) were randomized to one of 2 sequences and received Treatments C and D with a 10-day washout interval in between.

- Treatment C: single dose of digoxin 0.25 mg

- Treatment D: VEL 100 mg administered every day for 4 days with a single dose of digoxin 0.25 mg on the first day

Cohort 3 (CYP3A/CYP2C8/P-gp inducer rifampin): Subjects (12) were randomized to one of 2 sequences and received Treatments E and F with a 10-day washout interval in between.

- Treatment E: single dose of VEL 100 mg
- Treatment F: rifampin 600 mg administered every day for 7 days followed by a single dose of VEL 100 mg on the eighth day

Cohort 4 (CYP3A/CYP2C8/P-gp inhibitor ketoconazole): Subjects (12) were randomized to one of 2 sequences and received Treatments G and H with a 10-day washout interval in between.

- Treatment G: single dose of VEL 100 mg
- Treatment H: ketoconazole 200 mg administered twice a day for 4 days with a single dose of VEL 100 mg on the first day. The last dose of ketoconazole was administered in the evening on Day 15 for sequence GH and on Day 4 for sequence HG

Cohort 5 (mixed OATP/P-gp/MRP2 inhibitor cyclosporine and OATP1B1/1B3 inhibitor rifampin): Subjects (12) were randomized to one of 4 sequences and received Treatments I, J, K, and L with 7-day washout intervals in between each treatment.

- Treatment I: single dose of VEL 100 mg
- Treatment J: single dose of cyclosporine 600 mg
- Treatment K: single doses of cyclosporine 600 mg plus VEL 100 mg
- Treatment L: single doses of rifampin 600 mg plus VEL 100 mg

Formulation:
Velpatasvir 50-mg tablet: Lot# DL1203B1,
pravastatin 40-mg tablet: Lot# 1H58005A,
rosuvastatin 10-mg tablet Lot# BF0123,
digoxin 0.25-mg tablet Lot# 013513,
rifampin 300-mg capsule Lot# 130860,
ketoconazole 200-mg tablet Lot# 124446,
cyclosporine 100-mg capsule Lot# F4154

PK Sampling: Blood samples for PK analyses were collected for each cohort on the days and time points specified in Table 1.

Table 1: Pharmacokinetic Sampling Time Points

Cohort	Sequence	Time Points
1	AB	Days 1, 5, 15, and 19: 0 (predose), 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 18, 24, 36, 48, and 72 hours postdose
1	BA	Days 5, 9, 17, and 21: 0 (predose), 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 18, 24, 36, 48, and 72 hours postdose
2	CD	Days 1 and 12: 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
2	DC	Days 1 and 15: 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
3	EF	Days 1 and 19: 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
3	FE	Days 8 and 19: 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
4	GH	Days 1 and 12: 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
4	HG	Days 1 and 15: 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
5	All sequences	Days 1, 9, 17, and 25: 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

Analytical Methods: Concentrations of VEL, pravastatin, rosuvastatin, digoxin, ketoconazole and rifampin in human plasma and cyclosporine in human whole blood 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 VEL were performed using validated methods by (b) (4). The assays for pravastatin, rosuvastatin, digoxin, ketoconazole, rifampin, and cyclosporine were performed and validated by (b) (4).

The standard curve and QC data indicated that the plasma assay methods for VEL, pravastatin, rosuvastatin, digoxin, ketoconazole and rifampin and the whole blood assay method for cyclosporine were precise and accurate.

Pharmacokinetic Results:

The effect of VEL on OATP, BCRP, and P-gp substrates using phenotypic probes pravastatin (OATP substrate), rosuvastatin (OATP/BCRP substrate), digoxin (P-gp substrate), and CsA (P-gp/CYP3A substrate) has been evaluated. The statistical comparisons of PK parameters AUClast, AUCinf, and Cmax for pravastatin, rosuvastatin, digoxin, and CsA when administered alone and coadministered with VEL are presented in the table below.

Table 2: The effect of Velpatasvir on the PK of Probe Substrates

PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	Reference	Test Treatment	
Pravastatin PK: Pravastatin + VEL (test) versus Pravastatin (reference) (N = 18)			
AUClast	181.09	243.58	134.51 (117.30, 154.25)
AUCinf (h*ng/mL)	183.59	247.26	134.68 (117.52, 154.35)
Cmax (ng/mL)	81.22	103.92	127.94 (107.61, 152.12)
Rosuvastatin PK: Rosuvastatin + VEL (test) versus Rosuvastatin (reference) (N = 18)			
AUClast	54.10	149.42	276.20 (252.26, 302.42)
AUCinf (h*ng/mL)	57.13	153.77	269.15 (246.31, 294.11)
Cmax (ng/mL)	5.70	14.86	260.57 (232.28, 292.30)
Digoxin PK: Digoxin + VEL (test) versus Digoxin (reference) (N = 21)			
AUClast	9187.94	14,690.33	159.89 (140.68, 181.72)
AUCinf (h*pg/mL)	15,880.69 (N = 20)	21,335.82 (N = 20)	134.35 (112.78, 160.05)
Cmax (pg/mL)	1103.43	2077.33	188.26 (170.55, 207.81)
Cyclosporine PK: Cyclosporine + VEL (test) versus Cyclosporine (reference) (N = 12)			
AUClast	11,708.02	10,369.34	88.57 (78.18, 100.34)
AUCinf (h*ng/mL)	12,726.49	11,245.24	88.36 (77.90, 100.23)
Cmax (ng/mL)	1905.21	1745.42	91.61 (82.20, 102.10)

Pravastatin AUC and Cmax were 35% and 28% higher, respectively, following coadministration with VEL, relative to pravastatin administration alone. The magnitude of this interaction is comparable to that observed with verapamil and is likely due to the first pass inhibition of hepatic drug transporter OATP1B1 by VEL, and does not require pravastatin dose modification.

Reviewer's note: The results may not apply to other statins which are more sensitive to OATP1B1 transport, such as simvastatin acid, pitavastatin, etc.

Rosuvastatin AUC and Cmax were approximately 2.8-and 2.6-fold higher, respectively, following coadministration with VEL, relative to rosuvastatin administration alone. The magnitude of this interaction is comparable to that observed with atazanavir (ATV) boosted with ritonavir (ATV/r) and lopinavir (LPV)/r and is likely due to first pass inhibition of intestinal drug transporter BCRP as well as hepatic drug transporter OATP by VEL. In accordance with the prescribing information on the use of rosuvastatin with ATV/r and LPV/r, rosuvastatin dose should be limited to 10 mg once daily during concomitant use with VEL. Monitoring for signs and symptoms of muscle weakness or myopathy, including rhabdomyolysis, during concomitant use of rosuvastatin with VEL may be warranted.

Reviewer's note: Although the warning for rosuvastatin may not be extrapolated to other statins, an additional study should be conducted for atorvastatin, a substrate of OATP, P-gp and BCRP. In addition, drugs that are substrates of multiple transporters that are inhibited by VEL and with narrow therapeutic index should be avoided for coadministration with VEL (e.g., topotecan, a substrate for P-gp and BCRP).

Digoxin AUClast, AUCinf, and Cmax were 60%, 34%, and 88% higher, respectively, following coadministration with VEL, relative to digoxin administration alone. The increase in digoxin exposure is likely due to pre-systemic inhibition of P-gp. This result does not preclude the use of

P-gp substrates with VEL, but for digoxin, a drug with a narrow therapeutic range, the following precaution similar to the Lanoxin® (digoxin) US Package Insert is recommended: "Measure serum digoxin concentrations before initiating SOF/VEL. Reduce digoxin concentrations by decreasing the dose by approximately 15-30% or by modifying the dosing frequency and continue monitoring.".

Cyclosporine AUC and Cmax were not significantly altered following coadministration with VEL relative to CsA administered alone. Cyclosporine is both a substrate of P-gp and CYP3A. In vitro data suggest that VEL does not induce or inhibit CYP3A, and while VEL demonstrated to at least weakly inhibit P-gp, simultaneous administration of VEL with CsA did not significantly impact the PK of CsA. As only a minor decrease in CsA exposure was observed when simultaneously administered with VEL, no CsA dose adjustment is necessary during concomitant use with VEL.

Drug interaction potential between VEL and inducers and inhibitors of relevant CYP enzymes and drug transport proteins was evaluated. The statistical comparisons of VEL PK parameters AUClast, AUCinf, and Cmax when administered with rifampin, ketoconazole, or CsA or VEL alone are presented in the table below.

Table 3: The effect of Probe inhibitors on Velpatasvir PK

PK Parameter	Geometric Least-Squares Mean		%GLSM Ratio (90% CI) Test/Reference
	Reference	Test Treatment	
VEL PK: VEL + Multiple-Dose Rifampin (test) versus VEL (reference) (N = 12)			
AUC_{last} (h*ng/mL)	4845.7	879.4	18.15 (15.09, 21.82)
AUC_{inf} (h*ng/mL)	4905.4	907.2	18.49 (15.41, 22.20)
C_{max} (ng/mL)	658.3	192.0	29.17 (23.08, 36.86)
VEL PK: VEL + Ketoconazole (test) versus VEL (reference) (N = 12)			
AUC_{last} (h*ng/mL)	4574.4	7659.9	167.45 (131.21, 213.70)
AUC_{inf} (h*ng/mL)	4647.4	7962.8	171.34 (134.70, 217.94)
C_{max} (ng/mL)	543.3	702.3	129.27 (101.76, 164.22)
VEL PK: VEL + Single-Dose Rifampin (test) versus VEL (reference) (N = 12)			
AUC_{last} (h*ng/mL)	4153.0	6108.6	147.09 (117.52, 184.10)
AUC_{inf} (h*ng/mL)	4219.4	6166.1	146.14 (116.93, 182.64)
C_{max} (ng/mL)	528.8	676.7	127.98 (104.93, 156.10)
VEL PK: VEL + Cyclosporine (test) versus VEL (reference) (N = 12)			
AUC_{last} (h*ng/mL)	4153.0	8425.9	202.89 (151.33, 272.00)
AUC_{inf} (h*ng/mL)	4219.4	8553.5	202.72 (151.46, 271.32)
C_{max} (ng/mL)	528.8	826.3	156.27 (121.69, 200.67)

Substantial reductions in VEL AUC (approximately 82%) and Cmax (approximately 71%) were observed following administration with multiple-dose rifampin, a strong inducer of CYP3A4/2C8 and P-gp compared with VEL administered alone. As such, VEL should not be administered with strong inducers of CYP3A4/2C8 and P-gp.

VEL AUC and Cmax were approximately 70% and 29% higher, respectively, following coadministration with ketoconazole, a strong inhibitor of CYP3A4/2C8 and P-gp, as compared with VEL administered alone. As VEL concentrations increased only modestly following

concomitant administration with ketoconazole, in addition to a favorable clinical safety profile to date, inhibitors of CYP3A4/2C8 and P-gp can be administered with VEL.

Administration of single-dose rifampin, an inhibitor of OATP, with VEL resulted in an approximate 47% and 28% increase in VEL AUC and Cmax, respectively, as compared to administration of VEL alone, demonstrating that VEL is a weak substrate of OATP. As VEL concentrations increased only modestly following concomitant administration with single-dose rifampin, an OATP inhibitor, inhibitors of OATP can be administered with VEL.

VEL AUC and Cmax were approximately 2-fold and 56% higher, respectively, following coadministration with CsA, a strong inhibitor of OATP/P-gp/MRP2, as compared to VEL administered alone. As VEL concentrations increased only modestly following concomitant administration with CsA, in addition to a favorable clinical safety profile to date, inhibitors of OATP/P-gp/MRP2 may be administered with VEL without dose adjustment.

Conclusion:

- Administration of VEL resulted in weak inhibition of OATP and P-gp and moderate inhibition of BCRP, as assessed by probe substrates, pravastatin, digoxin, and rosuvastatin, respectively.
- Pravastatin may be administered without dose adjustment during concomitant use with VEL.
- Rosuvastatin can be used at doses no greater than 10 mg during concomitant use with VEL.
- Monitoring for signs and symptoms of muscle weakness or myopathy, including rhabdomyolysis, during concomitant use of rosuvastatin with VEL may be warranted.
- An additional study may be required to assess the interaction between SOF/VEL and atorvastatin.
- Drugs that are substrates of multiple transporters that are inhibited by VEL and with a narrow therapeutic index need to be avoided with coadministration with VEL (e.g., topotecan, a substrate for P-gp and BCRP).
- Therapeutic monitoring and an initial decrease in dose of digoxin is recommended during concomitant use with VEL.
- Cyclosporine and VEL may be coadministered without dose adjustment.
- VEL may be administered with inhibitors of OATP, P-gp, MRP2, and CYP3A4/2C8 without dose adjustment.
- VEL should not be administered with strong inducers of CYP3A4/2C8 and P-gp.

4.1.4.2 GS-US-281-0119: Phase 1 Study to Evaluate the Relative Bioavailability and Pharmacokinetics of Velpatasvir upon Co-administration with a Representative Proton-Pump Inhibitor or an H2-Receptor Antagonist

Objectives: To evaluate the relative bioavailability and PK of Velpatasvir (VEL) upon coadministration with a representative proton-pump inhibitor (PPI) or H2-receptor antagonist (H2RA)

Study Design: This was a Phase 1, randomized, open-label, single-center, single-dose/ multiple-dose study. Healthy subjects were randomized to receive the following treatments, according to their assigned treatment sequences (ABCD, BDAC, CADB, or DCBA):

- **VEL alone (Treatment A):** a single dose of VEL 100 mg administered in the morning under fasted conditions

- **Simultaneous H2RA+VEL (Treatment B):** a single dose of VEL 100 mg administered simultaneously with a single dose of famotidine 20 mg in the morning under fasted conditions
- **12-Hour H2RA Stagger +VEL (Treatment C):** a single dose of famotidine 20 mg administered in the evening with a standardized meal, 12 hours before a single dose of VEL 100 mg administered in the morning under fasted conditions
- **Simultaneous PPI+VEL (Treatment D):** omeprazole 20 mg administered once daily in the morning for 6 days under fasted conditions plus a single dose of VEL 100 mg administered in the morning simultaneously with omeprazole on the sixth dosing day

Eligible subjects were healthy males and non-pregnant, non-lactating females, who were 18 to 45 years of age (inclusive), had a BMI of 19 to 30 kg/m^2 (inclusive), and had no significant medical history. A total of 24 subjects (6 subjects per treatment sequence) were randomized, all received doses of study drug, and completed the study.

Formulation:
VEL 50-mg tablets: Lot# DL1203B1;
Famotidine 20-mg tablets: Lot# LL12124;
Omeprazole 20-mg capsules: Lot# H013300

PK Sampling: Intensive PK sampling occurred relative to VEL dosing on Days 1, 8, 16, and 28 at the following time points: 0 (predose), 0.5, 1, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 18, 24, 48, and 72 hours postdose.

Analytical Methods: Concentrations of VEL in human plasma 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 VEL were all performed and validated by (b) (4) .

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

Pharmacokinetic Results: The statistical comparisons of VEL PK parameters between VEL administered alone and simultaneously/staggered with famotidine are presented in the table below.

Table 1: The Effect of Famotidine on the PK of Velpatasvir

VEL PK Parameter	GLSM		%GLSM Ratio (90% CI)
	Simultaneous VEL+ Famotidine (N = 24)	VEL Alone (N = 24)	VEL+Famotidine/ VEL
AUC_{last} (h*ng/mL)	4107.21	4510.66	91.06 (70.47, 117.65)
AUC_{inf} (h*ng/mL)	4208.99	4617.80	91.15 (70.92, 117.14)
C_{max} (ng/mL)	516.34	601.73	85.81 (67.55, 109.01)
	Staggered VEL+ Famotidine (N = 24)	VEL Alone (N = 24)	VEL+Famotidine/ VEL
AUC_{last} (h*ng/mL)	4155.16	4510.66	92.12 (71.29, 119.03)
AUC_{inf} (h*ng/mL)	4265.25	4617.80	92.37 (71.87, 117.14)
C_{max} (ng/mL)	540.58	601.73	89.84 (70.72, 114.12)

There was no clinically significant effect of either simultaneous or staggered administration of 20 mg famotidine on the PK of VEL.

The statistical comparisons of VEL PK parameters AUClast, AUCinf, and Cmax between VEL administered alone or simultaneously with omeprazole are presented in the table below.

Table 2: The Effect of Omeprazole on the PK of Velpatasvir

VEL PK Parameter	GLSM		%GLSM Ratio (90% CI)
	Simultaneous VEL+ Omeprazole (N = 24)	VEL Alone (N = 24)	VEL+Omeprazole/ VEL
AUC_{last} (h*ng/mL)	2100.72	4510.66	46.57 (36.04, 60.18)
AUC_{inf} (h*ng/mL)	2185.78	4617.80	47.33 (36.83, 60.83)
C_{max} (ng/mL)	270.88	601.73	45.02 (35.44, 57.19)

Simultaneous administration of a representative PPI, omeprazole under fasted conditions, resulted in > 50% decrease in the relative bioavailability of VEL compared with VEL administration alone, indicating that VEL should not be administered with PPIs under fasted conditions as exposure of VEL is considerably decreased in the presence of omeprazole.

Conclusion:

- Simultaneous or staggered administration of famotidine 20 mg with VEL under fasted conditions did not significantly alter the overall bioavailability of VEL as measured by AUC.
- VEL should not be administered with PPIs under fasted conditions as VEL exposure was considerably decreased in the presence of omeprazole.

4.1.4.3 GS-US-342-1346: A Phase 1 Study to Evaluate the Relative Bioavailability and Pharmacokinetics of Sofosbuvir/Velpatasvir Fixed-Dose Combination upon Administration with a Representative H2-Receptor Antagonist or Proton Pump Inhibitor

Objectives: To evaluate the relative bioavailability and PK of Sofosbuvir/Velpatasvir (SOF/VEL) upon coadministration with a representative H2- receptor antagonist (H2RA) or proton pump inhibitor (PPI)

Study Design:
This was a Phase 1, randomized, open-label, single-center, single-dose/multiple-dose study in healthy subjects to evaluate the relative bioavailability and PK of SOF/VEL upon coadministration with a representative H2RA (famotidine) or PPI (omeprazole). Subjects were randomized to receive the following 5 treatments, according to their assigned treatment sequences (ABECD, BCADE, CDBEA, DECAB, EADBC, DCEBA, EDACB, AEBDC, BACED, or CBDAE):

- **Reference (Treatment A):** A single dose of SOF/VEL (400/100 mg) was administered in the morning under fasted conditions.

- **Simultaneous H2RA Administration (Treatment B):** A single dose of SOF/VEL (400/100 mg) was administered simultaneously with a single dose of famotidine 40 mg in the morning under fasted conditions.

- **12-Hour H2RA Stagger (Treatment C):** A single dose of famotidine 40 mg administered in the evening with a standardized meal, 12 hours before a single dose of SOF/VEL (400/100 mg) administered in the morning under fasted conditions.

- **Simultaneous PPI Administration (Treatment D):** Omeprazole 20 mg was administered once daily in the morning for 6 days under fasted conditions. A single dose of SOF/VEL (400/100 mg) was administered in the morning simultaneously with omeprazole on the sixth day of dosing.

- **12-Hour PPI Stagger (Treatment E):** Omeprazole 20 mg was administered once daily in the evening for 6 days 1 hour prior to a standardized meal and 12 hours before a single dose of SOF/VEL (400/100 mg) was administered in the morning under fasted conditions following the sixth day of omeprazole dosing.

Eligible subjects were males and non-pregnant, non-lactating females, who were 18 to 45 years of age (inclusive), had a BMI of 19 to 30 kg/m2 (inclusive), and had no significant medical history. A total of 60 subjects (6 subjects per treatment sequence) were randomized into the study, received all doses of study drug, and completed the study.

Formulation:
SOF/VEL 400/100-mg tablets: Lot# DU1301B1;
Famotidine 40-mg tablets: Lot# 1305005807;
Omeprazole 20-mg capsules: Lot# 1303239

PK Sampling: Intensive PK sampling occurred relative to dosing of SOF/VEL at the following time points: predose (< 5 minutes), 0.5, 1, 2, 3, 4, 6, 8, 12, 18, 24, 48, and 72 hours post dose.

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

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

Pharmacokinetic Results:
Table 1 summarizes single-dose SOF, GS-566500, GS-331007, and VEL exposures following administration of SOF/VEL alone or with famotidine or omeprazole administered simultaneously or staggered by 12 hours.

Table 1: SOF, GS-566500, GS-331007, and VEL Plasma Pharmacokinetic Parameters Following Single-Dose Administration of SOF/GS-5816 Alone or with Famotidine or Omeprazole (Coadministered or 12-Hour Stagger) (PK Analysis Set)

PK Parameter	SOF/VEL Alone (N = 60)	SOF/VEL + Simultaneous Famotidine (N = 60)	SOF/VEL + Staggered Famotidine (N = 60)	SOF/VEL + Simultaneous Omeprazole (N = 60)	SOF/VEL + Staggered Omeprazole (N = 60)
SOF					
AUClast (ng•h/mL)	1706.2 (36.1)	1461.2 (41.7)	1388.1 (36.4)	1298.0 (41.7)	1069.4 (48.7)
AUCinf (ng•h/mL)	1735.1 (35.4)[a]	1472.3 (41.5)	1403.6 (35.9)	1311.3 (41.6)	1089.4 (48.1)[a]
Cmax (ng/mL)	1571.5 (40.6)	1484.9 (44.8)	1211.2 (40.3)	1121.2 (48.7)	939.7 (54.4)
GS-566500					
AUClast (ng•h/mL)	1757.5 (31.1)	1595.6 (32.8)	1487.5 (33.4)	1379.7 (40.5)	1142.8 (48.6)
AUCinf (ng•h/mL)	1822.0 (30.2)	1654.5 (31.9)	1551.1 (32.1)	1460.0 (37.1)[a]	1226.5 (44.6)[a]
Cmax (ng/mL)	442.3 (29.7)	413.8 (33.0)	371.8 (35.1)	349.9 (40.4)	280.3 (46.5)
GS-331007					
AUClast (ng•h/mL)	10831.8 (21.4)	10328.3 (24.1)	11497.7 (21.5)	11146.9 (21.7)	10992.8 (23.0)
AUCinf (ng•h/mL)	12146.1 (21.3)	11561.7 (24.1)	12671.3 (22.1)	12231.5 (23.5)	11930.3 (24.0)
Cmax (ng/mL)	843.9 (30.7)	724.1 (38.1)	1008.6 (27.9)	1009.3 (34.7)	1069.2 (31.2)
VEL					
AUClast (ng•h/mL)	3905.2 (45.5)	3280.4 (51.9)	3393.7 (53.0)	2999.7 (67.1)	2172.9 (73.5)
AUCinf (ng•h/mL)	4007.7 (45.5)	3367.4 (52.1)	3478.5 (52.8)	3080.3 (66.8)	2270.7 (73.6)
Cmax (ng/mL)	476.6 (43.0)	397.8 (49.2)	425.8 (51.3)	360.5 (65.5)	263.8 (71.2)

[a] N = 59

The differences in primary PK parameters of SOF, GS-566500, GS-331007, and VEL following administration of SOF/VEL alone and with famotidine or omeprazole administered simultaneously or staggered by 12 hours are summarized in the table below.

Table 2: Summary of Differences in Pharmacokinetic Parameters Following Administration of SOF/VEL Alone or Coadministered with Famotidine or Omeprazole

Acid Reducing Agent	SOF PK Parameters			GS-566500 PK Parameters		
	AUC_{last}	AUC_{inf}	C_{max}	AUC_{last}	AUC_{inf}	C_{max}
Famotidine Simultaneous	↓17%	↓17%	↔	↔	↔	↔
Famotidine Staggered	↓20%	↓20%	↓23%	↓16%	↓16%	↓17%
Omeprazole Simultaneous	↓29%	↓29%	↓34%	↓30%	↓22%	↓27%
Omeprazole Staggered	↓44%	↓44%	↓45%	↓43%	↓37%	↓43%
Acid Reducing Agent	**GS-331007 PK Parameters**			**VEL PK Parameters**		
	AUC_{last}	AUC_{inf}	C_{max}	AUC_{last}	AUC_{inf}	C_{max}
Famotidine Simultaneous	↔	↔	↓16%	↓20%	↓19%	↓20%
Famotidine Staggered	↔	↔	↑20%	↓15%	↓15%	↓13%
Omeprazole Simultaneous	↔	↔	↑18%	↓37%	↓36%	↓37%
Omeprazole Staggered	↔	↔	↑26%	↓56%	↓55%	↓57%

Ninety percent CIs of the %GLSM ratios were within (↔), extended above (↑), or extended below (↓) the equivalence boundaries of 80% to 125%.

Administration of SOF/VEL with famotidine 40 mg (simultaneously or staggered by 12 hours) did not clinically significantly alter AUC or Cmax of SOF, GS-566500, GS-331007, or VEL.

Administration of SOF/VEL with omeprazole 20 mg (simultaneous or staggered by 12 hours) under fasted conditions resulted in decreased SOF, GS-566500, and VEL exposures, and no alteration in the AUC of GS-331007.

Conclusion:

- SOF/VEL may be administered with famotidine up to 40 mg or equivalent.
- SOF/VEL should not be administered with PPIs under fasted conditions.

4.1.4.4 GS-US-342-1709: A Phase 1 Study to Evaluate the Pharmacokinetics of Sofosbuvir/Velpatasvir Fixed-Dose Combination (FDC) upon Administration with a Representative Proton Pump Inhibitor

Objectives: To evaluate the relative bioavailability and PK of Sofosbuvir/Velpatasvir (SOF/VEL) FDC upon co-administration with a representative PPI and food.

Study Design: This study included up to 4 cohorts (Cohorts 1 to 4) that comprised a total of 8 treatment sequences with 2 treatment sequences per cohort (ie, AB and BA [Cohort 1], CD and DC [Cohort 2], EF and FE [Cohort 3], and GH and HG [Cohort 4]).

Cohort 1			
Period	**1**		**2**
	Treatment	**Washout**	**Treatment**
Sequence AB (N = 20)	A (Day 1)	Days 2-7	B (Days 8-13)
Sequence BA (N = 20)	B (Days 1-6)	Days 7-12	A (Day 13)
Cohort 2			
Period	**1**		**2**
	Treatment	**Washout**	**Treatment**
Sequence CD (N = 20)	C (Day 1)	Days 2-7	D (Days 8-13)
Sequence DC (N = 20)	D (Days 1-6)	Days 7-12	C (Day 13)
Cohort 3 (Not Conducted)			
Period	**1**		**2**
	Treatment	**Washout**	**Treatment**
Sequence EF (N = 20)	E (Day 1)	Days 2-7	F (Days 8-13)
Sequence FE (N = 20)	F (Days 1-6)	Days 7-12	E (Day 13)
Cohort 4			
Period	**1**		**2**
	Treatment	**Washout**	**Treatment**
Sequence GH (N = 20)	G (Day 1)	Days 2-7	H (Days 8-13)
Sequence HG (N = 20)	H (Days 1-6)	Days 7-12	G (Day 13)

Based on available safety and PK data from Cohort 1, and at the discretion of the sponsor, adaptive cohorts (Cohorts 2 and 4) were initiated. Adaptive Cohort 3 was not conducted as Cohort 1 results did not support the initiation of this cohort.

Treatments A (Cohort 1), C (Cohort 2), E (Cohort 3; not conducted), and G (Cohort 4): A single dose of the SOF/VEL 400/100-mg FDC tablet was administered orally in the AM under fasted conditions.

Treatment B (Cohort 1, 2-hour PPI stagger): A single omeprazole 20-mg capsule once daily was administered orally in the AM for 6 days in the fasted state. On Day 6, a single dose of the SOF/VEL 400/100-mg FDC tablet was administered orally with food 2 hours after the omeprazole dose.

Treatment D (Cohort 2, 4-hour PPI stagger): A single omeprazole 20-mg capsule once daily was administered orally on an empty stomach 1 hour before lunch for 6 days. On Day 6, a single dose of the SOF/VEL 400/100-mg FDC tablet was administered orally with food 4 hours before the omeprazole dose.

Treatment H (Cohort 4, 4-hour PPI stagger): A single omeprazole 40-mg capsule once daily was administered orally on an empty stomach 1 hour before lunch for 6 days. On Day 6, a single dose of SOF/VEL 400/100-mg FDC tablet was administered orally with food 4 hours before the omeprazole dose.

Formulation: The lot numbers for the omeprazole capsule were K000981 (20 mg) and K002680 (40 mg) and the lot number for the SOF/ VEL FDC tablet was DU1404B1.

PK Sampling: Intensive PK samplings for SOF, GS-566500, GS-331007, and VEL were collected relative to the morning dose of SOF/VEL at the following time points: predose (< 5 min), 0.5, 1, 2, 3, 4, 6, 8, 12, 18, 24, 48, and 72 hours postdose.

Analytical methods: Concentrations of SOF, GS-566500, GS-331007, and VEL in plasma samples were determined using 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 were performed by (b) (4) .

The standard curve and QC data indicated that the plasma assay method for SOF, GS-566500, GS-331007, and VEL were precise and accurate.

Pharmacokinetic Results:

Table 1 summarizes single-dose SOF, GS-566500, GS-331007, and VEL exposures following administration of SOF/VEL alone or with omeprazole.

A total of 3 of 40 subjects (7.5%) from Cohort 2 prematurely discontinued study drug. Subjects 9192-2021 and 9192-2035 (Cohort 2, Sequence DC) were withdrawn by the investigator after completing 6 days of treatment. Therefore, no PK data were collected for Treatment C. Subject 9192-2006 (Cohort 2, Sequence CD) withdrew due to AEs after completing 11 days of treatment. Therefore, no PK data were collected for Treatment D.

Table 1: SOF, GS-566500, GS-331007, and VEL Plasma Pharmacokinetic Parameters Following Single-Dose Administration of SOF/VEL Alone or with Omeprazole (PK Analysis Set)

PK Parameter	SOF/VEL 2 hours After OME 20 mg (Cohort 1)		SOF/VEL 4 hours Before OME 20 mg (Cohort 2)		SOF/VEL 4 hours Before OME 40 mg (Cohort 4)	
	Test (Fed, N = 40)	Reference (Fasted, N = 40)	Test (Fed, N = 39)	Reference (Fasted, N = 38)	Test (Fed, N = 40)	Reference (Fasted, N = 40)
SOF						
AUClast (ng•h/mL)	1532.9 (38.5)	1514.2 (46.0)	1850.7 (37.7)	1969.9 (77.0)	1459.8 (63.1)	1660.1 (43.8)
AUCinf (ng•h/mL)	1568.1 (37.7)	1525.8 (45.6)	1874.1 (37.0)	1982.5 (76.7)	1504.0 (61.5)	1674.2 (43.6)
Cmax (ng/mL)	947.7 (51.0)	1181.4 (57.4)	1307.1 (47.2)	1598.5 (41.0)	968.3 (70.8)	1336.1 (42.1)
GS-566500						
AUClast (ng•h/mL)	1917.1 (28.8)	1790.7 (39.2)	2031.7 (21.0)	1908.3 (28.1)	1818.8 (31.8)	1875.4 (36.1)
AUCinf (ng•h/mL)	1989.2 (28.2)	1847.9 (38.3)	2101.5 (20.8)	1964.7 (27.6)	1887.7 (31.2)	1936.8 (35.5)
Cmax (ng/mL)	423.0 (29.1)	426.5 (39.3)	487.4 (22.6)	471.7 (25.4)	417.7 (30.6)	450.8 (34.7)
GS-331007						
AUClast (ng•h/mL)	11403.2 (18.3)	11794.1 (21.6)	11961.7 (33.0)	12267.9 (29.3)	12278.0 (20.9)	12635.2 (22.1)
AUCinf (ng•h/mL)	13011.5 (19.5)	13202.0 (22.8)	13604.7 (33.9)	13594.0 (30.6)	13812.1 (20.7)	14145.8 (23.5)
Cmax (ng/mL)	855.6 (21.9)	918.2 (26.2)	816.8 (31.3)	887.7 (26.1)	953.3 (25.4)	941.6 (26.0)
VEL						
AUClast (ng•h/mL)	2449.7 (35.3)	4427.8 (50.4)	3360.8 (45.4)	4692.2 (53.8)	1792.9 (49.2)	4327.1 (50.8)
AUCinf (ng•h/mL)	2524.4 (35.6)	4550.8 (50.5)	3447.9 (45.1)	4815.2 (54.0)	1846.5 (48.4)	4436.3 (51.4)
Cmax (ng/mL)	239.5 (37.0)	511.1 (49.3)	342.5 (45.9)	503.1 (43.9)	187.8 (52.6)	481.4 (49.3)

OME = omeprazole

The differences in primary PK parameters of SOF, GS-566500, GS-331007, and VEL following administration of SOF/VEL alone and with omeprazole are summarized in the table below.

Table 2: Summary of Differences in Pharmacokinetic Parameters Following Administration of SOF/VEL Alone or Coadministered with Omeprazole

Dosing Scheme	SOF PK Parameters			GS-566500 PK Parameters			GS-331007 PK Parameters			GS-5816 PK Parameters		
	AUC_{las}	AUC_{inf}	C_{max}	AUC_{las}	AUC_{inf}	C_{ma}	AUC_{las}	AUC_{inf}	C_{ma}	AUC_{las}	AUC_{inf}	C_{max}
SOF/VEL 2 hours After OME 20 mg	↔	↑8	↓16%	↑12	↑12	↔	↔	↔	↔	↓38%	↓38%	↓48%
SOF/ VEL 4 hours Before OME 20 mg	↔	↔	↓21%	↔	↔	↔	↔	↔	↔	↓26%	↓26%	↓33%
SOF/ VEL 4 hours Before OME 40 mg	↔	↔	↓30%	↔	↔	↔	↔	↔	↔	↓53%	↓53%	↓56%

OME = omeprazole
The 90% CIs of the %GLSM ratios were within (↔), extended above (↑), or extended below (↓) the equivalence boundary of 80%.

Administration of SOF/VEL with food and omeprazole (regardless of timing or dose of omeprazole) resulted in similar overall AUC of SOF or its metabolites GS-566500 and GS-331007, as compared to when SOF/VEL was administered alone under fasted conditions, . A small decrease in SOF Cmax (16% to 30%) was observed following administration of SOF/VEL with food and omeprazole.

Administration of SOF/VEL with food and omeprazole resulted in a decrease in VEL exposure. The smallest decrease in VEL exposure (AUC: 26%, Cmax: 33%) was observed following administration of SOF/VEL with food 4 hours before omeprazole 20 mg. A slightly larger decrease in VEL exposure (AUC: 38%, Cmax: 48%) was observed when SOF/VEL was administered with food 2 hours after omeprazole 20 mg. The largest decline in VEL exposure (AUC: 53%, Cmax: 56%) was observed following SOF/VEL administration with food 4 hours before omeprazole 40 mg.

Reviewer's note: the reference SOF/VEL was given under fasted conditions. Therefore, the reduced omeprazole effects could be partially due to the food effect on VEL (increased VEL concentration by up to 34%) rather than reduced omeprazole effect by food.

Conclusion:

- The overall exposure of SOF and its metabolites were similar when SOF/GS-5816 was administered with food and with omeprazole.
- Staggering timing of omeprazole 20-mg administration modestly reduced GS-5816 exposure but not SOF or GS-331007 exposure when administered with food.

- Omeprazole 40 mg significantly reduced GS-5816 exposure regardless of dosing interval or coadministration of SOF/GS-5816 with food.

4.1.4.5 GS-US-342-1167: A Phase 1 Study to Evaluate the Pharmacokinetic Drug-Drug Interactions between Sofosbuvir/GS-5816 Fixed-Dose Combination (FDC) Tablet and Antiretrovirals Efavirenz/Emtricitabine/Tenofovir Disoproxil Fumarate (EFV/FTC/TDF; Atripla®), Emtricitabine/Rilpivirine/Tenofovir Disoproxil Fumarate (FTC/RPV/TDF; Complera®), Dolutegravir (DTG; Tivicay®), or Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Alafemamide Fumarate (EVG/COBI/FTC/TAF) in Healthy Subjects

Objectives:

- To evaluate the pharmacokinetics (PK) of sofosbuvir (SOF) and metabolites GS-566500 and GS-331007, and GS-5816 upon administration of SOF/GS-5816 fixed-dose combination (FDC) with Atripla® (ATR; efavirenz/emtricitabine/tenofovir disoproxil fumarate [EFV/FTC/TDF]), Complera® (emtricitabine/rilpivirine/tenofovir disoproxil fumarate [FTC/RPV/TDF]), Tivicay® (dolutegravir [DTG]), or elvitegravir/cobicistat/emtricitabine/tenofovir alafemamide fumarate (EVG/COBI/FTC/TAF).
- To evaluate the pharmacokinetics of COBI, DTG, EFV, EVG, FTC, RPV, TAF, and/or tenofovir (TFV) upon administration of Atripla, Complera, Tivicay, or EVG/COBI/FTC/TAF with SOF/GS-5816 FDC.

Study Design: This Phase 1, open-label, multiple-dose, 4-cohort study evaluated the drug-drug interaction potential between SOF/GS-5816 and EFV/FTC/TDF, FTC/RPV/TDF, DTG, or EVG/COBI/FTC/TAF in healthy subjects. The screening visit was performed within 28 days prior to study drug dosing. Following completion of screening and baseline (Day –1) procedures, eligible subjects were enrolled into one of 4 cohorts and received the study treatments. Subjects were confined to the clinic at the study center beginning on Day -1 and remained there until the morning of Day 29 (Cohort 1) or Day 33 (Cohorts 2 through 4). A follow-up phone call was performed 7 to 10 days after the last dose of study drug. Eligible subjects were enrolled into one of the following 4 cohorts:

Cohort 1:
Subjects were randomized to one of 2 treatment sequences (AC or BC) and received 2 of the 3 treatments described below with no washout between treatments:

- Treatment A: SOF/GS-5816 (1 × 400-mg/100-mg tablet, once daily) administered in the morning under fasted conditions for 14 days
- Treatment B: EFV/FTC/TDF (1 × EFV 600-mg/FTC 200-mg/TDF 300-mg tablet, once daily) administered in the morning under fasted conditions for 14 days
- Treatment C: SOF/GS-5816 (1 × 400-mg/100-mg tablet, once daily) plus EFV/FTC/TDF (1 × EFV 600-mg/FTC 200-mg/TDF 300-mg tablet, once daily) administered in the morning under fasted conditions for 14 days.

Cohort 2:
Subjects were randomized to one of 6 treatment sequences (DEF, EFD, FDE, FED, DFE, or EDF) and received the 3 treatments described below with a 4-day washout between each treatment:

- Treatment D: SOF/GS-5816 (1 × 400-mg/100-mg tablet, once daily) administered in the morning with a moderate fat meal for 8 days
- Treatment E: FTC/RPV/TDF (1 × FTC 200-mg/RPV 25-mg/TDF 300-mg tablet, once daily) administered in the morning with a moderate fat meal for 8 days
- Treatment F: SOF/GS-5816 (1 × 400-mg/100-mg tablet, once daily) plus FTC/RPV/TDF (1 × FTC 200-mg/RPV 25-mg/TDF 300-mg tablet, once daily) administered in the morning with a moderate fat meal for 8 days

Cohort 3:
Subjects were randomized to one of 6 treatment sequences (GHI, HIG, IGH, IHG, GIH, or HGI) and received the 3 treatments described below with a 4-day washout between each treatment:

- Treatment G: SOF/GS-5816 (1 × 400-mg/100-mg tablet, once daily) administered in the morning with a moderate fat meal for 8 days
- Treatment H: DTG (1 × DTG 50-mg tablet, once daily) administered in the morning with a moderate fat meal for 8 days
- Treatment I: SOF/GS-5816 (1 × 400-mg/100-mg tablet, once daily) plus DTG (1 × DTG 50-mg tablet, once daily) administered in the morning with a moderate fat meal for 8 days

Cohort 4:
Subjects were randomized to one of 6 treatment sequences (JKL, KLJ, LJK, LKJ, JLK, or KJL) and received the 3 treatments described below with a 4-day washout between each treatment:

- Treatment J: SOF/GS-5816 (1 × 400-mg/100-mg tablet, once daily) administered in the morning with a moderate fat meal for 8 days
- Treatment K: EVG/COBI/FTC/TAF (1 × EVG 150-mg/COBI 150-mg/FTC 200-mg/TAF 10-mg tablet, once daily) administered in the morning with a moderate fat meal for 8 days
- Treatment L: SOF/GS-5816 (1 × 400-mg/100-mg tablet, once daily) plus EVG/COBI/FTC/TAF (1 × EVG 150-mg/COBI 150-mg/FTC 200-mg/TAF 10-mg tablet, once daily) administered in the morning with a moderate fat meal for 8 days

Formulation: SOF/GS-5816: SOF 400-mg/GS-5816 100-mg tablets, Lot # DU1301B1; FTC/RPV/TDF: FTC 200-mg/RPV 25-mg/TDF 300-mg tablets, Lot # 001973; EFV/FTC/TDF: EFV 600-mg/FTC 200-mg/TDF 300-mg tablets, Lot # 000475; DTG: 50-mg tablets, Lot #3ZP6428; EVG/COBI/FTC/TAF: EVG 150-mg/COBI 150-mg/FTC 200-mg/TAF 10-mg tablets, Lot # CP1209B1.

PK Sampling:

Cohort 1:
Intensive PK sampling occurred on Days 14 and 28 at the following time points: predose (≤ 5 minutes), 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, 20, and 24 hours postdose. The evening prior to days of intensive PK collection, subjects underwent an overnight fast (no food or liquids, except water, for at least 10 hours).

Cohorts 2-4:
Intensive PK sampling occurred on Days 8, 20, and 32 at the following time points: predose (≤ 5 minutes), 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, 20, and 24 hours postdose. The

evening prior to days of intensive PK collection, subjects underwent an overnight fast (no food or liquids, except water, for at least 10 hours).

Analytical methods: Concentrations of SOF, GS-566500, GS-331007, FTC, TAF, TFV, EFV, RPV, DTG, EVG, COBI, and GS-5816 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, FTC, TAF, TFV, EFV, RPV, DTG, EVG, and COBI were performed and validated by (b) (4). The assays for GS-5816 were performed and validated by (b) (4)

The standard curve and QC data indicated that the plasma assay methods for of SOF, GS-566500, GS-331007, FTC, TAF, TFV, EFV, RPV, DTG, EVG, COBI, and GS-5816 were precise and accurate.

Results:

Effect of EFV/FTC/TDF, FTC/RPV/TDF, DTG, or EVG/COBI/FTC/TAF on Sofosbuvir, GS-566500, GS-331007, and GS-5816 PK

Tables 1 to 4 summarize the effect of EFV/FTC/TDF, FTC/RPV/TDF, DTG, or EVG/COBI/FTC/TAF on Sofosbuvir PK, GS-566500 PK, GS-331007 PK, and GS-5816 PK, respectively.

Table 1: Effect of EFV/FTC/TDF, FTC/RPV/TDF, DTG, or EVG/COBI/FTC/TAF on SOF PK

SOF PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	SOF/GS-5816 (Reference, N = 14)	**SOF/GS-5816 + EFV/FTC/TDF (Test, N = 14)**	
AUCtau (ng•h/mL)	1989.8	1935.2	97.26 (83.19, 113.70)
Cmax (ng/mL)	1555.2	2148.2	138.13 (114.37, 166.82)
	SOF/GS-5816 (Reference, N = 24)	**SOF/GS-5816 + FTC/RPV/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	2444.9	2845.3	116.38 (109.49, 123.70)
Cmax (ng/mL)	1674.7	1831.1	109.34 (95.37, 125.35)
	SOF/GS-5816 (Reference, N = 24)	**SOF/GS-5816 + DTG (Test, N = 24)**	
AUCtau (ng•h/mL)	2279.4	2088.7	91.63 (84.60, 99.26)
Cmax (ng/mL)	1446.5	1276.0	88.22 (79.78, 97.54)
	SOF/GS-5816 (Reference, N = 23)	**SOF/GS-5816 + EVG/COBI/FTC/TAF (Test, N = 24)**	
AUCtau (ng•h/mL)	2601.3	3565.3	137.06 (123.53, 152.07)
Cmax (ng/mL)	1720.9	2116.0	122.96 (106.59, 141.84)

CI = confidence interval; GLSM = geometric least-squares (mean)

No significant effect has been observed on SOF AUC following coadministration of SOF/GS-5816 with EFV/FTC/TDF or on SOF AUC and Cmax following coadministration of SOF/GS-5816 with FTC/RPV/TDF. A modest increase in SOF Cmax (approximately 38%) was observed when administered with EFV/FTC/TDF, which is not considered clinically significant. Concomitant administration of SOF/GS-5816 with EVG/COBI/FTC/TAF resulted in an approximate 37% increase in SOF overall exposure (AUC). Of note, SOF plasma exposures are approximately 2-fold higher in the presence of GS-5816 (Study GS-US-281-0101), a finding attributed to the inhibitory effects of GS-5816 on efflux transporters P-gp and/or BCRP for which SOF is a substrate. Administration of SOF/GS-5816 with another P-gp and BCRP inhibitor (ie, COBI) resulted in only incremental additional inhibition of these drug transporters. The magnitude of increase in SOF plasma exposures caused by EVG/COBI/FTC/TAF was small and was not deemed clinically relevant.

Table 2: Effect of EFV/FTC/TDF, FTC/RPV/TDF, DTG, or EVG/COBI/FTC/TAF on GS-566500 PK

GS-566500 PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	SOF/GS-5816 (Reference, N = 14)	**SOF/GS-5816 + EFV/FTC/TDF (Test, N = 14)**	
AUCtau (ng•h/mL)	1985.4	1691.5	85.19 (75.71, 95.87)
Cmax (ng/mL)	485.1	432.9	89.24 (79.91,99.66)
	SOF/GS-5816 (Reference, N = 24)	**SOF/GS-5816 + FTC/RPV/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	2544.3	2457.0	96.57 (92.33, 101.00)
Cmax (ng/mL)	579.7	556.2	95.95 (91.15, 101.01)
	SOF/GS-5816 (Reference, N = 24)	**SOF/GS-5816 + DTG (Test, N = 24)**	
AUCtau (ng•h/mL)	2200.1	2183.6	99.25 (95.22, 103.46)
Cmax (ng/mL)	467.5	453.3	96.97 (91.55, 102.72)
	SOF/GS-5816 (Reference, N = 23)	**SOF/GS-5816 + EVG/COBI/FTC/TAF (Test, N = 24)**	
AUCtau (ng•h/mL)	2360.2	2855.1	120.97 (114.74, 127.53)
Cmax (ng/mL)	523.3	600.0	114.65 (106.78, 123.10)

CI = confidence interval; GLSM = geometric least-squares (mean)

Consistent with the lack of an effect of EFV/FTC/TDF, FTC/RPV/TDF, or DTG on the PK of the prodrug SOF, the 90% CIs for the %GLSM ratios for GS-566500 PK parameters were contained within the protocol defined lack of PK alteration boundaries of 70% to 143% indicating the lack of effect of EFV/FTC/TDF, FTC/RPV/TDF, or DTG on GS-566500 PK. While a modest increase in SOF overall exposure (AUC) was observed following administration with EVG/COBI/FTC/TAF compared to SOF/GS-5816 alone, no alteration in GS-566500 PK was observed.

Table 3: Effect of EFV/FTC/TDF, FTC/RPV/TDF, DTG, or EVG/COBI/FTC/TAF on GS-331007 PK

GS-331007 PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	SOF/GS-5816 (Reference, N = 14)	**SOF/GS-5816 + EFV/FTC/TDF (Test, N = 14)**	
AUCtau (ng•h/mL)	11,177.8	10,108.2	90.43 (85.38, 95.78)
Cmax (ng/mL)	1161.3	1002.2	86.30 (80.29, 92.76)
Ctau (ng/mL)	224.4	225.7	100.57 (94.81, 106.68)
	SOF/GS-5816 (Reference, N = 24)	**SOF/GS-5816 + FTC/RPV/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	10,803.7	11,221.0	103.86 (100.40, 107.45)
Cmax (ng/mL)	917.1	877.3	95.66 (90.40, 101.22)
Ctau (ng/mL)	290.5	325.0	111.85 (106.69, 117.27)
	SOF/GS-5816 (Reference, N = 24)	**SOF/GS-5816 + DTG (Test, N = 24)**	
AUCtau (ng•h/mL)	10861.0	10749.5	98.97 (96.64, 101.36)
Cmax (ng/mL)	956.5	965.2	100.90 (92.69, 109.84)
Ctau (ng/mL)	277.9	276.1	99.35 (97.32, 101.43)
	SOF/GS-5816 (Reference, N = 23)	**SOF/GS-5816 + EVG/COBI/FTC/TAF (Test, N = 24)**	
AUCtau (ng•h/mL)	11,657.3	17,234.8	147.85 (143.31, 152.52)
Cmax (ng/mL)	942.2	1215.6	129.01 (124.81, 133.36)
Ctau (ng/mL)	327.5	517.7	158.07 (151.63, 164.78)

CI = confidence interval; GLSM = geometric least-squares (mean)

There was no effect of EFV/FTC/TDF, FTC/RPV/TDF, or DTG on GS-331007 PK. Coadministration of SOF/GS-5816 with EVG/COBI/FTC/TAF increased GS-331007 AUCtau, Cmax, and Ctau by approximately 48%, 29%, and 58%, respectively.

Table 4: Effect of EFV/FTC/TDF, FTC/RPV/TDF, DTG, or EVG/COBI/FTC/TAF on GS-5816 PK

GS-5816 PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	SOF/GS-5816 (Reference, N = 14)	**SOF/GS-5816 + EFV/FTC/TDF (Test, N = 14)**	
AUCtau (ng•h/mL)	4941.3	2337.9	47.31 (39.01, 57.38)
Cmax (ng/mL)	705.3	370.9	52.59 (43.17, 64.07)
Ctau (ng/mL)	65.5	28.3	43.18 (35.97, 51.84)
	SOF/GS-5816 (Reference, N = 24)	**SOF/GS-5816 + FTC/RPV/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	4574.6	4536.2	99.16 (88.27, 111.39)
Cmax (ng/mL)	655.0	631.4	96.40 (84.78, 109.61)
Ctau (ng/mL)	58.9	60.1	101.93 (90.55, 114.73)
	SOF/GS-5816 (Reference, N = 24)	**SOF/GS-5816 + DTG (Test, N = 24)**	
AUCtau (ng•h/mL)	4194.2	3807.4	90.78 (84.21, 97.86)
Cmax (ng/mL)	562.2	527.0	93.75 (86.15, 102.01)
Ctau (ng/mL)	62.2	54.8	88.01 (82.32, 94.09)
	SOF/GS-5816 (Reference, N = 23)	**SOF/GS-5816 + EVG/COBI/FTC/TAF (Test, N = 24)**	
AUCtau (ng•h/mL)	**4543.1**	**6808.4**	149.86 (135.13, 166.20)
Cmax (ng/mL)	565.4	736.8	130.30 (117.30, 144.74)
Ctau (ng/mL)	73.3	117.3	160.05 (144.05, 177.83)

CI = confidence interval; GLSM = geometric least-squares (mean)

GS-5816 AUCtau, Cmax, and Ctau were approximately 53%, 47%, and 57% lower following administration of SOF/GS-5816 + EFV/FTC/TDF compared with SOF/GS-5816 alone, indicating an impact of EFV/FTC/TDF on GS-5816 PK. The mechanism for the interaction may be related to the inductive effects of EFV on CYP enzymes and drug transporters (ie, P-gp).

Effect of Sofosbuvir/GS-5816 on Efavirenz (EFV), Rilpivirine (RPV), Dolutegravir (DTG), Elvitegravir (EVG), Cobicistat (COBI), Emtricitabine (FTC), Tenofovir (TFV), and Tenofovir Alafenamide Fumarate (TAF)

The effects of SOF/GS-5816 on EFV, RPV, DTG, EVG, COBI, FTC, TFV, and TAF were summarized in Tables 5 to 12.

Table 5: Statistical Comparisons of EFV Plasma Pharmacokinetic Parameter Estimates Following Administration of Multiple Doses of EFV/FTC/TDF or SOF/GS-5816 + EFV/FTC/TDF (Cohort 1, EFV PK Analysis Set)

Efavirenz PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	EFV/FTC/TDF (Reference, N = 15)	SOF/GS-5816 + EFV/FTC/TDF (Test, N = 15)	
AUCtau (ng•h/mL)	46,755.7	39,930.0	85.40 (80.13, 91.02)
Cmax (ng/mL)	2895.3	2346.0	81.03 (73.82, 88.93)
Ctau (ng/mL)	1396.5	1255.5	89.91 (84.89, 95.22)

CI = confidence interval; GLSM = geometric least-squares (mean)

EFV exposures were approximately 10% to 19% lower following administration of SOF/GS-5816 + EFV/FTC/TDF. Because EFV significantly reduces the exposures of GS-5816 (Table 4), SOF/GS-5816 should not be coadministered with EFV containing regimens.

Table 6: Statistical Comparisons of RPV Plasma Pharmacokinetic Parameter Estimates Following Administration of Multiple Doses of FTC/RPV/TDF or SOF/GS-5816 + FTC/RPV/TDF (Cohort 2, RPV PK Analysis Set)

Rilpivirine PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	FTC/RPV/TDF (Reference, N = 24)	SOF/GS-5816 + FTC/RPV/TDF (Test, N = 24)	
AUCtau (ng•h/mL)	2695.9	2561.0	95.00 (89.95, 100.33)
Cmax (ng/mL)	189.8	176.7	93.07 (88.18, 98.23)
Ctau (ng/mL)	91.6	88.0	96.11 (89.67, 103.01)

CI = confidence interval; GLSM = geometric least-squares (mean)

Table 7: Statistical Comparisons of DTG Plasma Pharmacokinetic Parameter Estimates Following Administration of Multiple Doses of DTG or SOF/GS-5816 + DTG (Cohort 3, DTG PK Analysis Set)

Dolutegravir PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	DTG (Reference, N = 24)	SOF/GS-5816 + DTG (Test, N = 24)	
AUCtau (ng•h/mL)	66,076.1	70,324.5	106.43 (100.68, 112.50)
Cmax (ng/mL)	4839.5	5134.1	106.09 (101.04, 111.39)
Ctau (ng/mL)	1607.6	1669.2	103.84 (97.74, 110.31)

CI = confidence interval; GLSM = geometric least-squares (mean)

Table 8: Statistical Comparisons of EVG Plasma Pharmacokinetic Parameter Estimates Following Administration of Multiple Doses of EVG/COBI/FTC/TAF or SOF/GS-5816 + EVG/COBI/FTC/TAF (Cohort 4, EVG PK Analysis Set)

Elvitegravir PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	EVG/COBI/FTC/TAF (Reference, N = 23)	SOF/GS-5816 + EVG/COBI/FTC/TAF (Test, N = 24)	
AUCtau (ng•h/mL)	25,213.8	23,700.3	94.00 (88.05, 100.35)
Cmax (ng/mL)	2166.3	1876.6	86.62 (80.12, 93.66)
Ctau (ng/mL)	489.5	528.2	107.92 (97.01, 120.07)

CI = confidence interval; GLSM = geometric least-squares (mean)

SOF/GS-5816 had no effect on the PK of RPV, DTG or EVG.

Table 9: Statistical Comparisons of COBI Plasma Pharmacokinetic Parameter Estimates Following Administration of Multiple Doses of EVG/COBI/FTC/TAF or SOF/GS-5816 + EVG/COBI/FTC/TAF (Cohort 4, COBI PK Analysis Set)

Cobicistat PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	EVG/COBI/FTC/TAF (Reference, N = 23)	SOF/GS-5816 + EVG/COBI/FTC/TAF (Test, N = 24)	
AUCtau (ng•h/mL)	11,197.5	14,597.3	130.36 (122.97, 138.20)
Cmax (ng/mL)	1607.1	1857.0	115.55 (108.70, 122.83)
Ctau (ng/mL)	27.2	55.4	203.45 (166.78, 248.17)

CI = confidence interval; GLSM = geometric least-squares (mean)

COBI AUCtau, Cmax, and Ctau were approximately 30%, 16%, and 103% higher, respectively, following administration of SOF/GS-5816 + EVG/COBI/FTC/TAF compared with EVG/COBI/FTC/TAF alone, indicating an impact of SOF/GS-5816 on COBI PK. While COBI Ctau increased approximately 2-fold, the impact on overall exposure (AUCtau) was minor due to the relatively short COBI $t_{1/2}$. The increase in COBI overall exposure when EVG/COBI/FTC/TAF is administered with SOF/GS-5816 is not considered clinically relevant.

Table 10: Statistical Comparisons of TAF Plasma Pharmacokinetic Parameter Estimates Following Administration of Multiple Doses of EVG/COBI/FTC/TAF or SOF/GS-5816 + EVG/COBI/FTC/TAF (Cohort 4, TAF PK Analysis Set)

TAF PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	EVG/COBI/FTC/TAF (Reference, N = 23)	SOF/GS-5816 + EVG/COBI/FTC/TAF (Test, N = 24)	
AUCtau (ng•h/mL)	331.7	289.9	87.39 (81.06, 94.22)
Cmax (ng/mL)	307.6	244.8	79.57 (67.68, 93.54)

CI = confidence interval; GLSM = geometric least-squares (mean)

Concomitant administration of EVG/COBI/FTC/TAF with SOF/GS-5816 did not alter overall TAF PK. A modest decrease in TAF Cmax (approximately 20%) was observed when administered with SOF/GS-5816. As the overall exposure (AUC) of TAF was unchanged, 20% decrease in Cmax observed following administration of SOF/GS-5816 with EVG/COBI/FTC/TAF in this study is not considered clinically relevant.

Table 11: Statistical Summary of FTC PK

FTC PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	EFV/FTC/TDF (Reference, N = 15)	**SOF/GS-5816 + EFV/FTC/TDF (Test, N = 15)**	
AUCtau (ng•h/mL)	9240.7	9857.9	106.68 (99.86, 113.96)
Cmax (ng/mL)	1733.0	1860.9	107.38 (97.71, 118.01)
Ctau (ng/mL)	60.4	66.3	109.71 (96.61, 124.59)
	FTC/RPV/TDF (Reference, N = 24)	**SOF/GS-5816 + FTC/RPV/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	9774.1	9719.1	99.44 (96.72, 102.23)
Cmax (ng/mL)	1934.8	1833.3	94.75 (89.64, 100.15)
Ctau (ng/mL)	74.3	77.8	104.69 (98.79, 110.93)
	EVG/COBI/FTC/TAF (Reference, N = 23)	**SOF/GS-5816 + EVG/COBI/FTC/TAF (Test, N = 24)**	
AUCtau (ng•h/mL)	11,679.1	11,812.1	101.14 (98.08, 104.29)
Cmax (ng/mL)	2122.8	2161.2	101.81 (97.39, 106.44)
Ctau (ng/mL)	94.0	95.6	101.71 (96.80, 106.88)

CI = confidence interval; GLSM = geometric least-squares (mean)

SOF/GS-5816 had no effect on the PK of FTC.

Table 12: Statistical Summary of TFV PK

TFV PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	EFV/FTC/TDF (Reference, N = 15)	**SOF/GS-5816 + EFV/FTC/TDF (Test, N = 15)**	
AUCtau (ng•h/mL)	2111.9	3817.3	180.75 (168.16, 194.29)
Cmax (ng/mL)	282.5	499.5	176.83 (153.12, 204.22)
Ctau (ng/mL)	39.5	87.2	220.71 (200.23, 243.30)
	FTC/RPV/TDF (Reference, N = 24)	**SOF/GS-5816 + FTC/RPV/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	3088.7	4317.8	139.79 (133.97, 145.87)
Cmax (ng/mL)	350.5	503.2	143.57 (132.73, 155.30)
Ctau (ng/mL)	57.1	104.9	183.86 (175.74, 192.36)
	EVG/COBI/FTC/TAF (Reference, N = 23)	**SOF/GS-5816 + EVG/COBI/FTC/TAF (Test, N = 24)**	
AUCtau (ng•h/mL)	284.5	346.1	121.63 (118.09, 125.28)
Cmax (ng/mL)	17.1	20.4	119.68 (115.53, 123.98)
Ctau (ng/mL)	10.2	12.6	123.44 (119.27, 127.75)

CI = confidence interval; GLSM = geometric least-squares (mean)

Modest increases in TFV AUCtau, Cmax, and Ctau of approximately 81%, 77%, and 121%, respectively, were observed following coadministration of SOF/GS-5816 + EFV/FTC/TDF compared to EFV/FTC/TDF alone. Similarly, increases in TFV AUCtau, Cmax, and Ctau of approximately 40%, 44%, and 84%, respectively, were observed following coadministration of SOF/GS-5816 + FTC/RPV/TDF compared to FTC/RPV/TDF alone. Since administration of food increases TFV AUC and Cmax, TFV exposures when administered as a component of FTC/RPV/TDF were higher than TFV exposures when administered as a component of EFV/FTC/TDF.

Overall, similar absolute TFV plasma exposures were achieved following treatment with SOF/GS-5816 + EFV/FTC/TDF or SOF/GS-5816 + FTC/RPV/TDF. The effect of SOF/GS-5816 on TFV exposures following coadministration of SOF/GS-5816 with EFV/FTC/TDF or FTC/RPV/TDF was similar to the effect of ledipasvir (LDV)/SOF on TFV exposures. Therefore, similar to the Harvoni® US Package Insert, the following statement, monitor for tenofovir-associated adverse reactions in patients receiving SOF/VEL concomitantly with a regimen containing tenofovir DF without a HIV protease inhibitor/ritonavir or cobicistat, is recommended.

Small increases in TFV PK primary PK parameters (AUCtau, Cmax, and Ctau) of approximately 1.2-fold were observed following coadministration of SOF/GS-5816 + EVG/COBI/FTC/TAF compared to EVG/COBI/FTC/TAF alone. The magnitudes of the increases are not considered clinically significant.

Conclusion

- Based on the safety and PK data, SOF/GS-5816 may be coadministered with FTC/RPV/TDF, DTG, FTC/TDF, or EVG/COBI/FTC/TAF without dose adjustment to any of the agents. However, monitor for tenofovir-associated adverse reactions in patients receiving SOF/VEL concomitantly with a regimen containing tenofovir DF without a HIV protease inhibitor/ritonavir or cobicistat is recommended.
- Based on a decrease in GS-5816 exposure, SOF/GS-5816 should not be coadministered with EFV/FTC/TDF or EFV as part of other ARV regimens.

4.1.4.6 GS-US-342-1326: A Phase 1 Study to Evaluate the Pharmacokinetic Drug-Drug Interaction Potential between Sofosbuvir/GS-5816 (SOF/GS-5816) Fixed-Dose Combination (FDC) Tablet and HIV Antiretroviral Regimens Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Disoproxil Fumarate (EVG/COBI/FTC/TDF), Ritonavir-boosted Darunavir (DRV plus RTV) plus Emtricitabine/Tenofovir Disoproxil Fumarate (FTC/TDF), Ritonavir-boosted Atazanavir (ATV plus RTV) plus FTC/TDF, Ritonavir-boosted Lopinavir (LPV/RTV) plus FTC/TDF, or Raltegravir (RAL) plus FTC/TDF

Objectives:

- To evaluate the pharmacokinetics (PK) of sofosbuvir (SOF), its metabolites GS-566500 and GS-331007, and GS-5816 upon administration of SOF/GS-5816 with elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (EVG/COBI/FTC/TDF), darunavir (DRV) + ritonavir (RTV) + FTC/TDF, atazanavir (ATV) + RTV + FTC/TDF, lopinavir (LPV)/RTV + FTC/TDF, or raltegravir (RAL) + FTC/TDF
- To evaluate the PK of EVG, COBI, FTC, tenofovir (TFV), DRV, RTV, ATV, LPV, and RAL upon administration of SOF/GS-5816 with EVG/COBI/FTC/TDF, DRV+RTV+FTC/TDF, ATV+RTV+FTC/TDF, LPV/RTV+FTC/TDF, or RAL+FTC/TDF

Study Design:

This is a Phase 1, randomized, open label, 5-cohort, multiple-dose study in healthy subjects. A total of 132 subjects were randomized to 1 of 6 sequences and received the 3 treatments as presented below. Subjects were confined to the clinic at the study center beginning on Day −1 and remained there until the morning of Day 31 (Cohorts 1 to 4) or Day 22 (Cohort 5).

Cohort 1:

Treatment A: SOF/GS-5816 (1 × 400/100 mg tablet), once daily, administered with food in the morning

Treatment B: EVG/COBI/FTC/TDF (1 × 150/150/200/300 mg tablet), once daily, administered with food in the morning

Treatment C: SOF/GS-5816 (1 × 400/100 mg tablet) plus EVG/COBI/FTC/TDF (1 × 150/150/200/300 mg tablet), once daily, administered with food in the morning

Treatment Sequence	Days 1-10	Days 11-20	Days 21-30
I	A	B	C
II	A	C	B
III	B	C	A
IV	B	A	C
V	C	B	A
VI	C	A	B

Cohort 2:

Treatment A: SOF/GS-5816 (1 × 400/100 mg tablet), once daily, administered with food in the morning

Treatment D: DRV (1 × 800 mg tablet) plus RTV (1 × 100 mg tablet) plus FTC/TDF (1 x 200/300 mg tablet), once daily, administered with food in the morning

Treatment E: SOF/GS-5816 (1 × 400/100 mg tablet) plus DRV (1 × 800 mg tablet) plus RTV (1 × 100 mg tablet) plus FTC/TDF (1 × 200/300 mg tablet), once daily, administered with food in the morning

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

Cohort 3:

Treatment A: SOF/GS-5816 (1 × 400/100 mg tablet), once daily, administered with food in the morning

Treatment F: ATV (1 × 300 mg capsule) plus RTV (1 × 100 mg tablet) plus FTC/TDF (1 × 200/300 mg tablet), once daily, administered with food in the morning

Treatment G: SOF/GS-5816 (1 × 400/100 mg tablet) plus ATV (1 × 300 mg capsule) plus RTV (1 × 100 mg tablet) plus FTC/TDF (1 × 200/300 mg tablet), once daily, administered with food in the morning

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

Cohort 4:

Treatment A: SOF/GS-5816 (1 × 400/100 mg tablet), once daily, administered with food in the morning

Treatment H: LPV/RTV (4 × 200/50 mg tablets) plus FTC/TDF (1 × 200/300 mg tablet), once daily, administered with food in the morning

Treatment I: SOF/GS-5816 (1 × 400/100 mg tablet) plus LPV/RTV (4 × 200/50 mg tablets) plus FTC/TDF (1 × 200/300 mg tablet), once daily, administered with food in the morning

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

Cohort 5:

Treatment A: SOF/GS-5816 (1 × 400/100 mg tablet), once daily, administered with food in the morning

Treatment J: RAL (1 × 400 mg tablets, twice daily [BID], approximately 12 hours apart) administered with food, plus FTC/TDF (1 × 200/300 mg tablet, once daily) administered with food in the morning

Treatment K: SOF/GS-5816 (1 × 400/100 mg tablet) plus FTC/TDF (1 × 200/300 mg tablet), once daily, administered with food in the morning, plus RAL (1 × 400 mg tablets, BID, approximately 12 hours apart) administered with food

Treatment Sequence	Days 1-7	Days 8-14	Days 15-21
I	A	J	K
II	A	K	J
III	J	K	A
IV	J	A	K
V	K	J	A
VI	K	A	J

Formulation: SOF/GS-5816: SOF 400-mg/GS-5816 100-mg tablets, Lot # DU1403B1 and DU1404B1; EVG/COBI/FTC/TDF 150/150/200/300-mg tablets: Lot # 002055; DRV 800-mg tablets: Lot # 14HG291 and 14JG400; RTV 100-mg tablets: Lot # 1021437, 1022511, and 1021438; FTC/TDF 200/300-mg tablets: Lot # 0021080; ATV 300-mg capsules: Lot # 4D80987A; LPV/RTV 200/50-mg tablets: Lot # 1020324; RAL 400-mg tablets: Lot # K010811

PK Sampling: In Cohorts 1 to 4, serial blood samples for PK analyses were collected on Days 10, 20, and 30 at the following time points. In Cohort 5, serial blood samples were collected on Days 7, 14, and 21 at the following time points: Predose (≤ 5 min), 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 16, 20, and 24 hours postdose.

Analytical methods: Concentrations of GS-5816, SOF, GS-566500, GS-331007, FTC, TFV, EVG, COBI, DRV, ATV, LPV, RTV, and RAL in human plasma samples were determined using fully validated high-performance liquid chromatography-tandem mass spectroscopy bioanalytical methods. All samples were analyzed in the timeframe supported by frozen stability storage data. The assays for GS-5816, SOF, GS-566500, GS-331007, FTC, TFV, EVG, COBI, DRV, ATV, LPV, RTV, and RAL were all performed and validated by (b) (4) .
The standard curve and QC data indicated that the plasma assay methods for of GS-5816, SOF, GS-566500, GS-331007, FTC, TFV, EVG, COBI, DRV, ATV, LPV, RTV, and RAL were precise and accurate.

Results:
There were 5 subjects who prematurely discontinued from the study; 4 subjects withdrew consent and 1 subject was withdrawn due to pregnancy.

Effect of EVG/COBI/FTC/TDF, DRV+RTV+FTC/TDF, ATV+RTV+FTC/TDF, LPV/RTV+FTC/TDF, or RAL+FTC/TDF on SOF, GS-566500, GS-331007, and GS-5816 Pharmacokinetics

Tables 1 to 4 summarize the effect of EVG/COBI/FTC/TDF, DRV+RTV+FTC/TDF, ATV+RTV+FTC/TDF, LPV/RTV+FTC/TDF, or RAL+FTC/TDF on SOF, GS-566500, GS-331007, and GS-5816 PK, respectively. There were 5 subjects who prematurely discontinued from the study; 4 subjects withdrew consent and 1 subject was withdrawn due to pregnancy. Those subjects may not have PK for some of the periods.

No alteration in the exposure (AUCtau, Cmax, or Ctau [as applicable]) of SOF, GS-566500, GS-331007, or GS-5816 was observed following administration of SOF/GS-5816 with RAL+FTC/TDF. Up to 35% increase in the overall exposure of SOF, GS-566500, GS-331007, or GS-5816 was observed when SOF/GS-5816 was administered with EVG/COBI/FTC/TDF. These changes are not considered clinically significant. Administration of SOF/GS-5816 with DRV+RTV+FTC/TDF or LPV/RTV+FTC/TDF resulted in a modest decrease in the overall exposure of SOF (~28% and ~29%, respectively) with no significant alteration in the overall exposure of GS-566500, GS-331007, or GS-5816.

Administration of SOF/GS-5816 with ATV+RTV+FTC/TDF resulted in an increase in GS-5816 AUCtau (~142%), Cmax (~55%), and Ctau (~301%) and up to 32% increase in the overall exposures of SOF and its metabolites (GS-566500 and GS-331007). The changes of the exposures of SOF and its metabolites are not considered clinically significant. In addition, the preliminary data from 20 subjects receiving ATV/r and 86 not on ATV/r in ASTRAL-5, a HIV/HCV coinfection trial, show that safety were comparable between patients receiving ATV/r and patients not on ATV/r. In addition, in Phase 3 trials (ASTRAL-1, -2, -3, and -4), the following P-gp inhibitors have been used as concomitant medications: azithromycin, carvedilol, clarithromycin, erythromycin, felodipine, fluvoxamine, ketoconazole, quercetin, and verapamil. A total of 36 subjects used a P-gp inhibitor chronically (>14 days) and 33 subjects reported short term (≤ 14 days) use. No new safety observation has been identified by the Medical Officer. Therefore, the totality of the data does not suggest a dose adjustment when SOF/VEL is coadministered with boosted ATV.

Table 1: Effect of EVG/COBI/FTC/TDF, DRV+RTV+FTC/TDF, ATV+RTV+FTC/TDF, LPV/RTV+FTC/TDF, or RAL+FTC/TDF on SOF PK

SOF PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	SOF/GS-5816 (Reference, N = 24)	**SOF/GS-5816 + EVG/COBI/FTC/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	2168.76	2697.80	124.39 (113.04, 136.89)
Cmax (ng/mL)	1581.74	1597.05	100.97 (85.49, 119.24)
	SOF/GS-5816 (Reference, N = 29)	**SOF/GS-5816 + DRV+RTV+FTC/TDF (Test, N = 29)**	
AUCtau (ng•h/mL)	2463.31	1784.58	72.45 (65.89, 79.66)
Cmax (ng/mL)	1632.73	1010.98	61.92 (54.02, 70.98)
	SOF/GS-5816 (Reference, N = 24)	**SOF/GS-5816 + ATV+RTV+FTC/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	2581.84	3152.79	122.11 (111.87, 133.30)
Cmax (ng/mL)	1409.76	1578.63	111.98 (97.12, 129.12)
	SOF/GS-5816 (Reference, N = 24)	**SOF/GS-5816 + LPV/RTV+FTC/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	2148.36	1526.71	71.06 (64.39, 78.44)
Cmax (ng/mL)	1543.19	905.80	58.70 (48.77, 70.65)
	SOF/GS-5816 (Reference, N = 30)	**SOF/GS-5816 + RAL+FTC/TDF (Test, N = 30)**	
AUCtau (ng•h/mL)	2387.28	2771.37	116.09 (107.46, 125.41)
Cmax (ng/mL)	1301.44	1421.51	109.23 (97.35, 122.55)

CI = confidence interval; GLSM = geometric least-squares mean

Table 2: Effect of EVG/COBI/FTC/TDF, DRV+RTV+FTC/TDF, ATV+RTV+FTC/TDF, LPV/RTV+FTC/TDF, or RAL+FTC/TDF on GS-566500 PK

GS-566500 PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	SOF/GS-5816 (Reference, N = 24)	**SOF/GS-5816 + EVG/COBI/FTC/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	2229.12	2383.22	106.91 (100.55, 113.68)
Cmax (ng/mL)	526.65	533.43	101.29 (93.14, 110.15)
	SOF/GS-5816 (Reference, N = 29)	**SOF/GS-5816 + DRV+RTV+FTC/TDF (Test, N = 29)**	
AUCtau (ng•h/mL)	2435.30	2510.40	103.08 (96.90, 109.67)
Cmax (ng/mL)	545.02	547.43	100.44 (92.33, 109.27)
	SOF/GS-5816 (Reference, N = 24)	**SOF/GS-5816 + ATV+RTV+FTC/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	2535.28	2959.90	116.75 (110.53, 123.32)
Cmax (ng/mL)	516.89	624.76	120.87 (110.67, 132.01)
	SOF/GS-5816 (Reference, N = 23)	**SOF/GS-5816 + LPV/RTV+FTC/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	2340.04	1992.94	85.17 (78.93, 91.90)
Cmax (ng/mL)	552.15	433.92	78.59 (69.65, 88.67)
	SOF/GS-5816 (Reference, N = 30)	**SOF/GS-5816 + RAL+FTC/TDF (Test, N = 30)**	
AUCtau (ng•h/mL)	2626.09	2589.05	98.59 (93.71, 103.72)
Cmax (ng/mL)	553.79	515.90	93.16 (87.79, 98.85)

CI = confidence interval; GLSM = geometric least-squares mean

Table 3: Effect of EVG/COBI/FTC/TDF, DRV+RTV+FTC/TDF, ATV+RTV+FTC/TDF, LPV/RTV+FTC/TDF, or RAL+FTC/TDF on GS-331007 PK

GS-331007 PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	SOF/GS-5816 (Reference, N = 24)	**SOF/GS-5816 + EVG/COBI/FTC/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	10730.48	14477.21	134.92 (130.10, 139.91)
Cmax (ng/mL)	915.78	1031.00	112.58 (107.15, 118.29)
Ctau (ng/mL)	311.99	451.69	144.78 (137.93, 151.97)
	SOF/GS-5816 (Reference, N = 29)	**SOF/GS-5816 + DRV+RTV+FTC/TDF (Test, N = 29)**	
AUCtau (ng•h/mL)	10887.57	12285.18	112.84 (108.31, 117.56)
Cmax (ng/mL)	931.52	964.72	103.56 (99.38, 107.93)
Ctau (ng/mL)	309.14	347.88	112.53 (106.49, 118.92)
	SOF/GS-5816 (Reference, N = 24)	**SOF/GS-5816 + ATV+RTV+FTC/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	10985.57	14487.65	131.88 (127.43, 136.48)
Cmax (ng/mL)	895.46	1079.68	120.57 (112.30, 129.45)
Ctau (ng/mL)	316.01	450.28	142.49 (136.67, 148.56)
	SOF/GS-5816 (Reference, N = 23)	**SOF/GS-5816 + LPV/RTV+FTC/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	11011.45	12673.84	115.10 (109.23, 121.28)
Cmax (ng/mL)	980.44	992.60	101.24 (97.68, 104.93)
Ctau (ng/mL)	301.69	348.08	115.38 (106.51, 124.98)
	SOF/GS-5816 (Reference, N = 30)	**SOF/GS-5816 + RAL+FTC/TDF (Test, N = 30)**	
AUCtau (ng•h/mL)	10692.64	11021.51	103.08 (99.97, 106.28)
Cmax (ng/mL)	885.55	837.20	94.54 (91.44, 97.74)
Ctau (ng/mL)	291.94	316.20	108.31 (103.55, 113.29)

CI = confidence interval; GLSM = geometric least-squares mean

Table 4: Effect of EVG/COBI/FTC/TDF, DRV+RTV+FTC/TDF, ATV+RTV+FTC/TDF, LPV/RTV+FTC/TDF, or RAL+FTC/TDF on GS-5816 PK

GS-5816 PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	SOF/GS-5816 (Reference, N = 24)	**SOF/GS-5816 + EVG/COBI/FTC/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	4653.22	5548.79	119.25 (106.50, 133.52)
Cmax (ng/mL)	600.47	631.73	105.21 (92.63, 119.49)
Ctau (ng/mL)	66.32	90.95	137.15 (121.98, 154.20)
	SOF/GS-5816 (Reference, N = 29)	**SOF/GS-5816 + DRV+RTV+FTC/TDF (Test, N = 29)**	
AUCtau (ng•h/mL)	5456.94	4592.88	84.17 (72.42, 97.81)
Cmax (ng/mL)	681.94	517.12	75.83 (64.95, 88.54)
Ctau (ng/mL)	78.30	79.24	101.20 (86.56, 118.32)
	SOF/GS-5816 (Reference, N = 24)	**SOF/GS-5816 + ATV+RTV+FTC/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	4300.54	10428.04	242.48 (223.04, 263.62)
Cmax (ng/mL)	608.61	943.55	155.04 (140.79, 170.72)
Ctau (ng/mL)	62.06	248.78	400.86 (357.04, 450.07)
	SOF/GS-5816 (Reference, N = 23)	**SOF/GS-5816 + LPV/RTV+FTC/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	4963.76	5050.12	101.74 (88.78, 116.59)
Cmax (ng/mL)	644.96	449.86	69.75 (58.92, 82.57)
Ctau (ng/mL)	67.10	109.17	162.69 (142.88, 185.24)
	SOF/GS-5816 (Reference, N = 30)	**SOF/GS-5816 + RAL+FTC/TDF (Test, N = 30)**	
AUCtau (ng•h/mL)	4985.96	4888.62	98.05 (87.74, 109.56)
Cmax (ng/mL)	656.38	638.37	97.26 (87.43, 108.19)
Ctau (ng/mL)	68.19	65.93	96.69 (87.07, 107.38)

CI = confidence interval; GLSM = geometric least-squares mean

Effect of SOF/GS-5816 on EVG, COBI, FTC, TFV, DRV, RTV, ATV, LPV, and RAL Pharmacokinetics

As shown in Tables 5 to 13, the overall exposure (AUCtau) and maximal exposure (Cmax) of EVG, COBI, DRV, ATV, LPV, RTV, and FTC were not altered when administered with SOF/GS-5816, as the 90% CIs for the %GLSM ratios for AUCtau were within the protocol-predefined lack of PK alteration boundaries of 70% to 143%. An increase in the Ctau of ATV (~39%) and RTV (~29%) when administered as part of ATV+RTV+FTC/TDF, and COBI (~71%) when administered as part of EVG/COBI/FTC/TDF was observed following coadministration with SOF/GS-5816. Decrease in the Ctau of RAL (~21%) was observed following coadministration with SOF/GS-5816 with no change in overall or maximal RAL exposure. These changes are not considered clinically significant.

Modest increases in TFV AUCtau (range: 22% to 40%), Cmax (range: 36% to 55%), and Ctau (range: 45% to 70%) were observed following administration of EVG/COBI/FTC/TDF, DRV+RTV+FTC/TDF, ATV+RTV+FTC/TDF, LPV/RTV+FTC/TDF, or RAL+FTC/TDF with SOF/GS-5816. The safety of higher TFV exposures when TDF containing regimen is coadministered with SOF/VEL for the duration of treatment is being evaluated in ASTRAL-5 (HIV/HCV coinfection trial).

Table 5: Statistical Comparisons of EVG Plasma PK Parameter Estimates Following Administration of Multiple Doses of EVG/COBI/FTC/TDF or SOF/GS-5816 + EVG/COBI/FTC/TDF

EVG PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	EVG/COBI/FTC/TDF (Reference, N = 24)	SOF/GS-5816 + EVG/COBI/FTC/TDF (Test, N = 24)	
AUCtau (ng•h/mL)	19001.12	17672.06	93.01 (87.43, 98.94)
Cmax (ng/mL)	1585.07	1466.66	92.53 (85.75, 99.85)
Ctau (ng/mL)	365.24	353.58	96.81 (90.55, 103.50)

CI = confidence interval; GLSM = geometric least-squares mean

Table 6: Statistical Comparisons of COBI Plasma PK Parameter Estimates Following Administration of Multiple Doses of EVG/COBI/FTC/TDF or SOF/GS-5816 + EVG/COBI/FTC/TDF

COBI PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	EVG/COBI/FTC/TDF (Reference, N = 24)	SOF/GS-5816 + EVG/COBI/FTC/TDF (Test, N = 24)	
AUCtau (ng•h/mL)	11609.30	14277.40	122.98 (116.86, 129.42)
Cmax (ng/mL)	1591.94	1768.84	111.11 (105.83, 116.66)
Ctau (ng/mL)	30.72	52.54	171.02 (153.67, 190.32)

CI = confidence interval; GLSM = geometric least-squares mean

Table 7: Effect of SOF/GS-5816 on FTC Pharmacokinetics

FTC PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	EVG/COBI/FTC/TDF (Reference, N = 24)	**SOF/GS-5816 + EVG/COBI/FTC/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	10472.68	10540.77	100.65 (97.85, 103.53)
Cmax (ng/mL)	1712.44	1752.94	102.36 (97.29, 107.71)
Ctau (ng/mL)	87.64	92.93	106.04 (100.95, 111.39)
	DRV+RTV+FTC/TDF (Reference, N = 30)	**SOF/GS-5816 + DRV+RTV+FTC/TDF (Test, N = 29)**	
AUCtau (ng•h/mL)	8465.62	8906.72	105.21 (102.46, 108.04)
Cmax (ng/mL)	1560.32	1631.65	104.57 (100.83, 108.45)
Ctau (ng/mL)	68.83	71.34	103.65 (98.32, 109.27)
	ATV+RTV+FTC/TDF (Reference, N = 24)	**SOF/GS-5816 + ATV+RTV+FTC/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	9651.05	9811.70	101.66 (99.11, 104.28)
Cmax (ng/mL)	1551.90	1561.71	100.63 (95.76, 105.75)
Ctau (ng/mL)	80.65	85.73	106.30 (101.96, 110.83)
	LPV/RTV+FTC/TDF (Reference, N = 24)	**SOF/GS-5816 + LPV/RTV+FTC/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	7853.07	7841.60	99.85 (94.07, 106.00)
Cmax (ng/mL)	1337.25	1359.77	101.68 (92.73, 111.51)
Ctau (ng/mL)	64.48	62.84	97.46 (91.25, 104.10)
	RAL+FTC/TDF (Reference, N = 30)	**SOF/GS-5816 + RAL+FTC/TDF (Test, N = 30)**	
AUCtau (ng•h/mL)	9030.64	9453.26	104.68 (102.86, 106.53)
Cmax (ng/mL)	1401.61	1510.96	107.80 (103.85, 111.90)
Ctau (ng/mL)	72.26	73.97	102.37 (97.35, 107.65)

CI = confidence interval; GLSM = geometric least-squares mean

Table 8: Effect of SOF/GS-5816 on TFV Pharmacokinetics

TFV PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	EVG/COBI/FTC/TDF (Reference, N = 24)	**SOF/GS-5816 + EVG/COBI/FTC/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	3547.66	4795.00	135.16 (128.76, 141.87)
Cmax (ng/mL)	401.94	544.81	135.54 (124.69, 147.35)
Ctau (ng/mL)	67.44	97.77	144.97 (139.02, 151.18)
	DRV+RTV+FTC/TDF (Reference, N = 30)	**SOF/GS-5816 + DRV+RTV+FTC/TDF (Test, N = 29)**	
AUCtau (ng•h/mL)	3195.73	4426.70	138.52 (132.96, 144.31)
Cmax (ng/mL)	300.09	465.01	154.96 (144.79, 165.84)
Ctau (ng/mL)	56.87	86.26	151.68 (144.61, 159.10)
	ATV+RTV+FTC/TDF (Reference, N = 24)	**SOF/GS-5816 + ATV+RTV+FTC/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	4006.30	5197.90	129.74 (123.69, 136.10)
Cmax (ng/mL)	335.58	519.74	154.88 (143.15, 167.57)
Ctau (ng/mL)	73.79	102.41	138.79 (130.59, 147.50)
	LPV/RTV+FTC/TDF (Reference, N = 24)	**SOF/GS-5816 + LPV/RTV+FTC/TDF (Test, N = 24)**	
AUCtau (ng•h/mL)	3530.94	4314.04	122.18 (113.61, 131.40)
Cmax (ng/mL)	323.10	457.37	141.56 (127.39, 157.30)
Ctau (ng/mL)	66.00	84.73	128.38 (119.89, 137.47)
	RAL+FTC/TDF (Reference, N = 30)	**SOF/GS-5816 + RAL+FTC/TDF (Test, N = 30)**	
AUCtau (ng•h/mL)	2908.26	4066.86	139.84 (134.48, 145.41)
Cmax (ng/mL)	278.95	407.04	145.92 (138.58, 153.65)
Ctau (ng/mL)	56.45	95.79	169.70 (161.34, 178.50)

CI = confidence interval; GLSM = geometric least-squares mean

Table 9: Effect of SOF/GS-5816 on DRV Pharmacokinetics

DRV PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	DRV+RTV+FTC/TDF (Reference, N = 30)	SOF/GS-5816 + DRV+RTV+FTC/TDF (Test, N = 29)	
AUCtau (ng•h/mL)	92653.59	85657.74	92.45 (87.21, 98.00)
Cmax (ng/mL)	8907.42	8046.75	90.34 (86.23, 94.64)
Ctau (ng/mL)	1826.60	1580.31	86.52 (78.56, 95.28)

CI = confidence interval; GLSM = geometric least-squares mean

Table10: Effect of SOF/GS-5816 on RTV Pharmacokinetics

RTV PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	DRV+RTV+FTC/TDF (Reference, N = 30)	SOF/GS-5816 + DRV+RTV+FTC/TDF (Test, N = 29)	
AUCtau (ng•h/mL)	4894.75	5483.25	112.02 (105.08, 119.42)
Cmax (ng/mL)	662.61	707.25	106.74 (97.06, 117.38)
Ctau (ng/mL)	49.42	53.64	108.55 (102.24, 115.24)
	ATV+RTV+FTC/TDF (Reference, N = 24)	SOF/GS-5816 + ATV+RTV+FTC/TDF (Test, N = 24)	
AUCtau (ng•h/mL)	8953.07	8651.56	96.63 (89.29, 104.58)
Cmax (ng/mL)	1508.80	1347.16	89.29 (81.81, 97.45)
Ctau (ng/mL)	40.60	52.21	128.57 (114.83, 143.96)
	LPV/RTV +FTC/TDF (Reference, N = 24)	SOF/GS-5816 + LPV/RTV +FTC/TDF (Test, N = 24)	
AUCtau (ng•h/mL)	9493.29	9173.95	96.64 (88.98, 104.95)
Cmax (ng/mL)	1707.96	1608.45	94.17 (83.04, 106.80)
Ctau (ng/mL)a	40.48	43.24	106.81 (94.99, 120.10)

CI = confidence interval; GLSM = geometric least-squares mean

Table11: Effect of SOF/GS-5816 on ATV Pharmacokinetics

ATV PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	ATV+RTV+FTC/TDF (Reference, N = 24)	SOF/GS-5816 + ATV+RTV+FTC/TDF (Test, N = 24)	
AUCtau (ng•h/mL)	48050.52	57704.65	120.09 (109.84, 131.30)
Cmax (ng/mL)	5109.73	5591.97	109.44 (100.49, 119.18)
Ctau (ng/mL)	948.18	1313.89	138.57 (119.58, 160.58)

CI = confidence interval; GLSM = geometric least-squares mean

Table12: Effect of SOF/GS-5816 on LPV Pharmacokinetics

LPV PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	LPV/RTV +FTC/TDF (Reference, N = 24)	SOF/GS-5816 + LPV/RTV +FTC/TDF (Test, N = 24)	
AUCtau (ng•h/mL)	180,656.50	179,275.99	99.24 (93.06, 105.82)
Cmax (ng/mL)	15,174.81	14,689.66	96.80 (91.69, 102.20)
Ctau (ng/mL)	1243.95	1386.31a	111.44 (95.58, 129.95)

CI = confidence interval; GLSM = geometric least-squares mean

Table13: Effect of SOF/GS-5816 on RAL Pharmacokinetics

RAL PK Parameter	GLSM		%GLSM Ratio (90% CI) Test / Reference
	RAL+FTC/TDF (Reference, N = 30)	SOF/GS-5816 + RAL+FTC/TDF (Test, N = 30)	
AUCtau (ng•h/mL)	4594.62	4439.97	96.63 (72.89, 128.11)
Cmax (ng/mL)	1049.56	1080.53	102.95 (73.94, 143.34)
Ctau (ng/mL)	253.33	200.43	79.12 (42.20, 148.32)

CI = confidence interval; GLSM = geometric least-squares mean

Conclusion:

- The PK changes observed with SOF and SOF metabolites GS-566500 and GS-331007 in combination with the evaluated ARV regimens were not clinically significant and do not preclude coadministration.
- The observed increase in GS-5816 exposure when administered with an ATV-based regimen does not warrant dose modification.
- Modest changes in the trough concentrations of COBI, ATV, and RTV (as part of ATV+RTV+FTC/TDF) when administered with SOF/GS-5816 do not warrant dose modification.
- TFV exposure increases up to 55% when SOF/GS-5816 was coadministered with TDF containing regimens. The safety of higher TFV exposures when a TDF containing

regimen is coadministered with SOF/VEL for the duration of treatment is being evaluated in ASTRAL-5 (HIV/HCV coinfection trial).

4.1.4.7 GS-US-281-1058: A Phase 1, Open-Label, Drug Interaction Study Evaluating the Effect of GS-5816 on the Pharmacokinetics of a Representative Hormonal Contraceptive Medication, Norgestimate/Ethinyl Estradiol (by AbhayJoshi)

Study Rationale: Concomitant use of other drugs may affect the safety and/or efficacy of oral contraceptives (OC) due to drug interactions. This study evaluated the pharmacokinetics (PK) of ethinyl estradiol and norgestimate when coadministered with GS-5816.

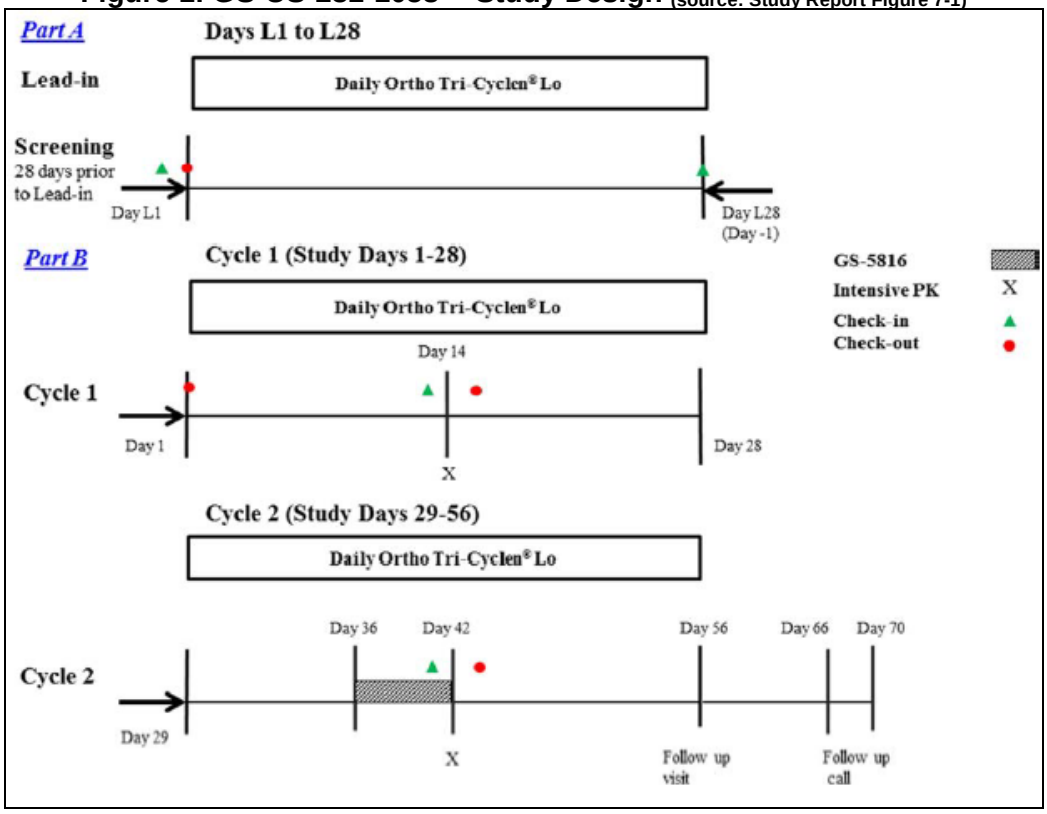
Primary Objective(s):

- To determine the effect of GS-5816 on the PK of a representative hormonal contraceptive medication, norgestimate/ethinyl estradiol (Ortho Tri-Cyclen Lo)
- To assess the effect of norgestimate/ethinyl estradiol on the PK of GS-5816

Study Design: Eligible subjects were either enrolled in a Lead-in period of 28 days (Part A, Cycle 1) during which they completed dosing with Ortho Tri-Cyclen Lo, or subjects with a documented history of taking an OC for at least 1 menstrual cycle, enrolled directly into (Part B, Cycle 1) of the study (Figure 1).

All subjects were directed to continue taking study drug from their Ortho Tri-Cyclen® Lo Dosing Pak once-daily with food in the AM at approximately the same time each day for two full 28-day menstrual cycles (until Day 56). Subjects received 100 mg (2 X 50-mg tablets) GS-5816 in the mornings of study days 36 to 42 at approximately the same time each day with 240 mL of water and within 30 minutes after initiation of a light-fat breakfast (~400 kcal and approximately 30% fat) Days 36 to 42 (Cycle 2). Intensive PK sampling was performed on Day 14 and Day 42 of the study.

Figure 1: GS-US-281-1058 -- Study Design (source: Study Report Figure 7-1)



The doses of ethinyl estradiol and norgestimate or inert tablets relative to menstrual cycle days are outlined in Table 1.

Table 1: Composition of Ortho Tri-Cyclen Lo (source: Study Report Table 7-2)

	Each Cycle Comprising 28 Days			
Ortho Tri-Cyclen Lo Hormones	Days 1-7	Days 8-14	Days 15-21	Days 22-28
Ethinyl Estradiol (mg/daily dose)	0.025	0.025	0.025	inert, "reminder pills"
Norgestimate (mg/daily dose)	0.180	0.215	0.250	

Formulation:
GS-5816 Tablets: 50 mg (Lot No# DL1203B1)
Ortho Tri-Cyclen Lo: norgestimate 0.180 mg/0.215 mg/0.250 mg -- ethinyl estradiol 0.025 mg (Lot No# 13HM805)

Pharmacokinetic Sampling: PK parameters for norgestimate, norelgestromin and norgestrel (metabolites of norgestimate), and ethinyl estradiol were estimated from the plasma concentrations on Study Days 14 and 42. PK parameters for GS-5816 were estimated from the plasma concentrations on Study Day 42 (Cycle 2).

Cycle 1: Study Day 14:
Serial blood samples were collected relative to OC administration in the morning at the following time points: predose (≤ 5 min), 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.

Cycle 2: Study Day 42:
Serial blood samples were collected relative to OC plus GS-5816 (Study Day 42) administration in the morning at the following time points: predose (≤ 5 min), 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.

Analytical methods: Concentrations of GS-5816, norgestrel, norgestimate, 17-desacetyl norgestimate, and ethinyl estradiol in plasma samples were determined using validated high-performance liquid chromatography-tandem mass spectroscopy (LC/MS/MS). All samples were analyzed within the timeframe supported by frozen stability storage data. The assays for GS-5816, norgestrel, norgestimate, 17-desacetyl norgestimate, and ethinyl estradiol were all performed and validated by (b) (4). Assay validation parameters are summarized in Table 2.

Table 2: Bioanalytical assay validation parameters (source: Study Report Table 7-7)

Parameter	GS-5816	Norgestrel	Norgestimate	17-Desacetyl Norgestimate	Ethinyl Estradiol
Linear range (ng/mL)	1 to 1000	0.02 to 20	0.05 to 50	0.02 to 10	0.0025 to 0.5
Lower limit of quantitation (ng/mL)	1	0.02	0.05	0.02	0.0025
Interday precision range (%CV)	1.6 to 3.8	1.3 to 5.6	1.8 to 3.8	2.3 to 8.0	3.8 to 7.9
Interday accuracy range (%RE)	-2.0 to 4.5	-2.3 to 4.3	-10.8 to 5.8	-5.2 to 3.1	-4.1 to 1.2
Stability in frozen matrix (days)	60 days at –20°C and –70°C	129 days at -20°C and -70°C	129 days at -20°C and -70°C	153 days at -20°C and -70°C	182 days at -20°C and -70°C

Results: In total, 15 subjects were continued to Part B of the study. All 15 subjects were included in each PK and PD analysis set, except for the GS-5816 PK analysis set, which included 13 subjects due to early termination of two subjects before GS-5816 dosing.

PK of norgestimate
The sponsor reports that the observed plasma concentrations of norgestimate were below quantification limit (< 50 pg/mL) for all subjects at most time points; therefore, PK parameters of norgestimate were not evaluable.

PK of Norelgestromin
Mean plasma norelgestromin concentrations (Figure 2) and PK parameter estimates (Table 3) were comparable following administration of OC alone or in combination with GS-5816. The confidence intervals (CI) of all parameters are contained within the conventional “no effect” bounds of 80% and 125% (Table 4).

Figure 2: Mean (SD) Plasma concentration-time profile of Norelgestromin following administration of Norgestimate/Ethinyl Estradiol (OC) alone or in combination with GS-5816 (source: Study Report Figure 10-1)

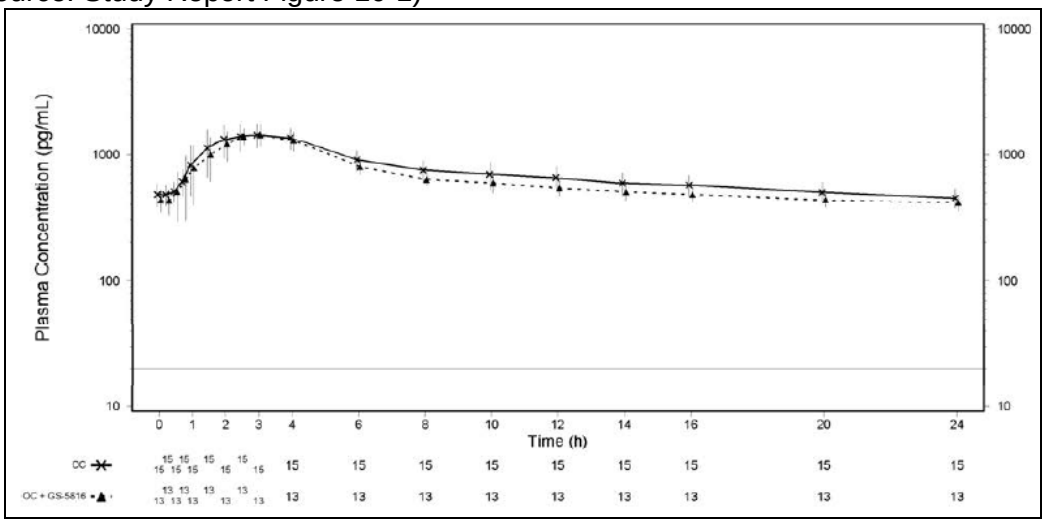


Table 3: Norelgestromin plasma PK parameters following administration of Norgestimate/Ethinyl Estradiol (OC) alone or with GS-5816 (source: Study Report Table 10-1)

Norelgestromin PK Parameter	Mean (% CV)	
	OC+GS-5816 (N=13)	OC (N=15)
AUC_{tau} (hr*pg/mL)	15736.4 (11.2)	17689.6 (16.7)
C_{max} (pg/mL)	1595.4 (13.7)	1654.7 (16.8)
C_{tau} (pg/mL)	416.0 (14.3)	453.6 (18.5)
T_{max} (hr)[a]	3.00 (2.50, 3.00)	3.00 (2.50, 3.00)
T_{last} (hr)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
$t_{1/2}$ (hr)[a]	29.14 (24.66, 41.17)	23.89 (17.62, 32.23)

a Median (Q1, Q3)

Table 4: Statistical comparison of Norelgestromin PK parameters following administration of Norgestimate/Ethinyl Estradiol (OC) alone or with GS-5816 (source: Study Report Table 10-2)

Norelgestromin PK Parameter	GLSM		% GLSM Ratio (90% CI)
	OC+GS-5816 (N=13)	OC (N=15)	OC+GS-5816 versus OC (N=13)
AUC_{tau} (hr*pg/mL)	15648.12	17465.40	89.59 (81.73, 98.22)
C_{max} (pg/mL)	1580.69	1634.55	96.71 (87.63, 106.72)
C_{tau} (pg/mL)	411.92	446.35	92.29 (82.68, 103.01)

PK of Norgestrel

Mean plasma norgestrel concentrations (Figure 3) and PK parameter estimates (Table 5) were comparable following administration of OC alone or in combination with GS-5816. The confidence intervals (CI) of all parameters are contained within the sponsor's pre-specified bounds of 70% and 143% (Table 6).

Figure 3: Mean (SD) plasma concentration-time profile of Norgestrel following administration of Norgestimate/Ethinyl Estradiol (OC) alone or in combination with GS-5816 (source: Study Report Figure 10-2)

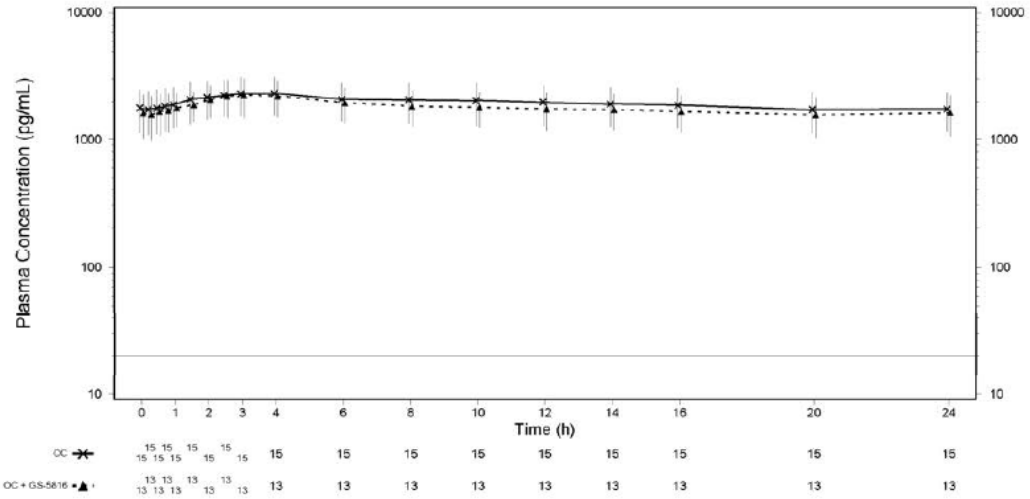


Table 2: Norgestrel plasma PK parameters following administration of Norgestimate/Ethinyl Estradiol (OC) alone or with GS-5816 (source: Study Report Table 10-3)

Norgestrel PK Parameter	Mean (% CV)	
	OC+GS-5816 (N=13)	OC (N=15)
AUC_{tau} (hr*pg/mL)	43021.8 (32.4)	46974.8 (34.4)
C_{max} (pg/mL)	2332.3 (31.5)	2408.7 (30.6)
C_{tau} (pg/mL)	1641.5 (35.7)	1755.9 (34.4)
T_{max} (hr)[a]	3.00 (2.50, 3.00)	3.00 (3.00, 4.00)
T_{last} (hr)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
$t_{1/2}$ (hr)[a]	78.11 (49.95, 122.56)	65.98 (48.10, 96.47)

a Median (Q1, Q3)

Table 3: Statistical comparison of Norgestrel PK parameters following administration of Norgestimate/Ethinyl Estradiol (OC) alone or with GS-5816 (source: Study Report Table 10-4)

Norgestrel PK Parameter	GLSM		% GLSM Ratio (90% CI)
	OC+GS-5816 (N=13)	OC (N=15)	OC+GS-5816 versus OC (N=13)
AUC_{tau} (hr*pg/mL)	40645.20	44440.57	91.46 (72.62, 115.19)
C_{max} (pg/mL)	2219.60	2304.12	96.33 (78.20, 118.66)
C_{tau} (pg/mL)	1533.18	1659.62	92.38 (72.50, 117.72)

PK of Ethinyl Estradiol

Mean peak plasma ethinyl estradiol concentrations were slightly higher following OC+GS-5816 administration compared with OC administered alone (Figure 4). However, mean ethinyl estradiol concentrations (C_{tau}) were slightly lower at the end of the dosing interval following OC+GS-5816 administration compared with OC administered alone (Figure 4, Table 7). The upper bound of 90% CI of C_{max} estimate was 165.5%, which was outside of the sponsor's pre-specified bounds of 70% and 143% (Table 8). The lower bound of 90% CI of C_{tau} estimate was 65.27%, which was also outside of the sponsor's pre-specified bounds of 70% and 143% (Table 8).

Figure 4: Mean (SD) plasma concentration-time profile of Ethinyl Estradiol following administration of Norgestimate/Ethinyl Estradiol (OC) alone or in combination with GS-5816 (source: Study Report Figure 10-3)

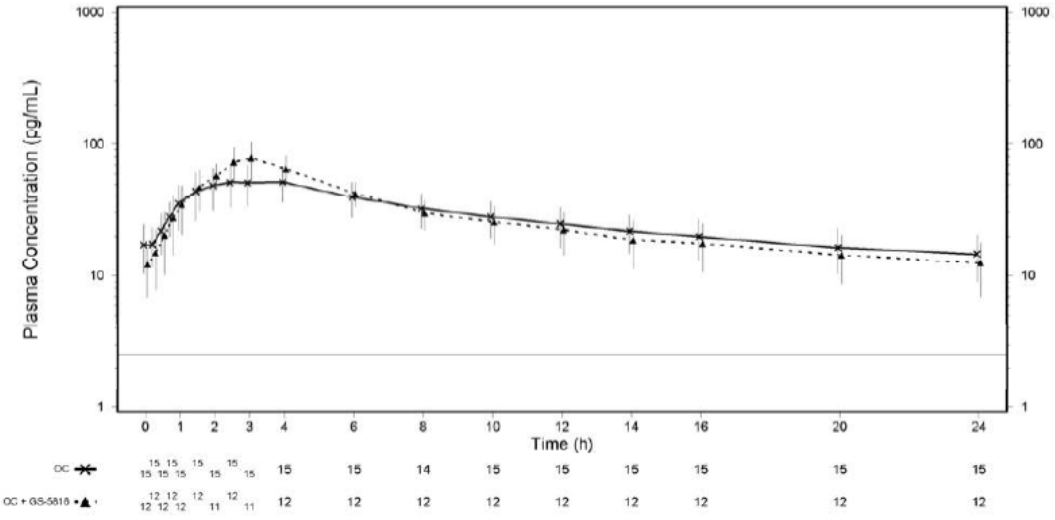


Table 4: Ethinyl Estradiol plasma PK parameters following administration of Norgestimate/Ethinyl Estradiol (OC) alone or with GS-5816 (source: Study Report Table 10-5)

Ethinyl Estradiol PK Parameter	Mean (% CV)	
	OC+GS-5816 (N=12)	OC (N=15)
AUC_{tau} (hr*pg/mL)	686.4 (27.3)	665.8 (30.7)
C_{max} (pg/mL)	79.9 (28.4)	57.5 (27.3)
C_{tau} (pg/mL)	12.4 (43.9)	14.8 (39.3)
T_{max} (hr)[a]	3.00 (2.74, 3.00)	3.00 (2.50, 3.00)
T_{last} (hr)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
$t_{1/2}$ (hr)[a]	15.21 (12.79, 18.44)	16.83 (14.74, 18.41)

a Median (Q1, Q3)

Table 5: Statistical comparison of Ethinyl Estradiol PK parameters following administration of Norgestimate/Ethinyl Estradiol (OC) alone or with GS-5816 (source: Study Report Table 10-6)

Ethinyl Estradiol PK Parameter	GLSM		% GLSM Ratio (90% CI)
	OC+GS-5816 (N=12)	OC (N=15)	OC+GS-5816 versus OC (N=12)
AUC_{tau} (hr*pg/mL)	666.39	640.04	104.12 (87.16, 124.37)
C_{max} (pg/mL)	77.35	55.65	139.00 (116.75, 165.50)
C_{tau} (pg/mL)	11.55	13.89	83.15 (65.27, 105.95)

PK of GS-5816

The sponsor concludes that, when coadministered with OC, the GS-5816 plasma PK parameter estimates (Table 9) were consistent with values observed in other Phase 1 studies.

Table 6: GS-5816 plasma PK parameters following coadministration of Norgestimate/Ethinyl Estradiol (OC) with GS-5816 (source: Study Report Table 10-7)

GS-5816 PK Parameter	Mean (% CV)
	OC+GS-5816 (N=13)
AUC_{tau} (hr*ng/mL)	4684.9 (35.1)
C_{max} (ng/mL)	625.6 (22.0)
C_{tau} (ng/mL)	68.3 (47.6)
T_{max} (hr)[a]	4.00 (3.00, 4.00)
T_{last} (hr)[a]	24.00 (24.00, 24.00)
$t_{1/2}$ (hr)[a]	14.02 (11.95, 16.13)
CL/F (mL/hr)	24067.7 (40.0)

a Median (Q1, Q3)

Pharmacodynamic evaluation:
Reported Luteinizing hormone (LH), follicle-stimulating hormone (FSH), and progesterone concentrations were similar for both treatment cycles (Table 10). LH and FSH median values were at the low end of expected values for the ovulatory phase. Progesterone levels were lower than the expected range for the luteal phase. The sponsor states that these results are consistent with a decrease in serum LH and FSH caused by oral hormonal contraceptives and absence of ovulation.

Table 7: Summary of LH, FSH, and Progesterone concentrations following administration of Norgestimate/Ethinyl Estradiol (OC) alone or with GS-5816 (source: Study Report Table 10-8)

	Median (Q1, Q3)	
PD Analyte	**OC (N=15)**	**OC+GS-5816 (N=13)**
LH (mIU/mL)	8.0 (2.7, 12.9)	9.3 (5.4, 14.4)
FSH (mIU/mL)	3.6 (2.0, 6.0)	2.6 (2.2, 5.1)
Progesterone (ng/mL)	0.24 (0.15, 0.40)	0.27 (0.18, 0.80)

Conclusions:

- The PK of a representative hormonal contraceptive medication; norgestimate/ethinyl estradiol (Ortho Tri-Cyclen Lo), was not affected to a clinically significant extent when coadministered with GS-5816.
- The PK of GS-5816 was not affected when coadministered with a representative hormonal contraceptive medication; norgestimate/ethinyl estradiol (Ortho Tri-Cyclen Lo)
- Based on a consultation with the OCP repro-uro team, these results with norgestimate/ethinyl estratiol cannot be extrapolated to other oral contraceptives.

4.1.5 *In vitro* Studies

1. Absorption

Summary: The in vitro permeability studies on VEL were carried out in Caco-2 cell monolayers. However, permeability values of VEL could not be reliably obtained due to low recovery of the compound and poor reproducibility. Therefore, the report was not submitted.

2. Distribution

- AD-281-2001: Protein binding determination of GS-5816
- AD-281-2009: Human plasma protein binding determination of GS-5816

Summary: GS-5816 is greater than 99.5% bound to human plasma proteins and protein binding is independent of drug concentration over the range of 0.1 µM to 2 µM.

3. Metabolism and elimination

- AD-281-2006: Metabolic Stability of GS-5816 in Hepatic Subcellular Fractions and in Cryopreserved Human Hepatocytes
- AD-281-2007: Cytochrome P450 Metabolic Reaction Phenotyping of GS-5816

Summary: Velpatasvir has a low hepatic clearance and is a substrate of CYP2B6, CYP2C8, and CYP3A4 with slow turnover.

4. Drug interaction potential

- AD-281-2008: CYP inhibition potential of GS-5816
- AD-281-2009: CYP induction potential of GS-5816
- AD-281-2025: Induction potential of GS-5816 in cultured human hepatocytes
- AD-281-2016: Human UGT1A1 Inhibition Potential of GS-5816
- AD-281-2041: Effect of P-glycoprotein and BCRP Expression on GS-5816 Accumulation
- AD-281-2011: Potential of GS-5816 as a substrate of human OATP1B1 and OATP1B3
- AD-281-2010: GS-5816 inhibition of human OATP1B1, OATP1B3, P-gp and BCRP
- AD-281-2040: Potential of GS-5816 as an Inhibitor of human OATP1A2 or OATP2B1
- AD-281-2026: Potential of GS-5816 as an Inhibitor of OCT1, OCT2, MATE1, OAT1, and OAT3 or Substrate for OCT1
- AD-281-2012: GS-5816 inhibition potential for human MRP2, BSEP, and NTCP
- AD-334-2023: In vitro inhibition of human P-gp by high concentrations of SOF
- AD-334-2024: In vitro inhibition studies of P-gp, OCT1, OCT2, MATE1, OAT3, BSEP and MRP2 transporters by high concentrations of GS-331007
- AD-334-2025: Metabolism of Irinotecan following coincubation with Either Sofosbuvir or GS-331007 in primary human hepatocytes
- AD-334-2026: In vitro assessment of human hepatic microsomal cytochrome P450 mechanism-based inhibition potential of GS-7977

Summary: Most of the in vitro studies for SOF and GS-331007 have been reviewed in NDA 204671 and NDA 205834. The applicant submitted additional in vitro studies to evaluate the inhibition potential of SOF and GS-331007 at higher concentrations. This review also evaluates the potential of CYP-based, UGT-based and transporter-based drug interactions for VEL.

The following table provides the pharmacokinetic parameters for SOF, GS-331007 and VEL following oral administration of the SOF/VEL FDC to humans, used for assessment of drug-drug interactions potential.

Steady State Pharmacokinetic Parameters in HCV-Infected Subjects for SOF, Metabolite GS-331007, and VEL

	SOF	GS-331007	VEL
Dose (mg)	400	-	100
Total Cmax (μM)[a]	1.07	3.34	0.293
Unbound Cmax (μM)[b]	0.409	3.10	0.000879 (0.00293[e])
Intestinal (μM)[c]	3020	-	453
$C_{hep,\ inlet}$ (μM)[d]	51.5	-	7.84
Unbound $C_{hep,\ inlet}$ (μM)[d]	19.7	-	0.0235 (0.0784[e])

a Values are the mean C_{max} based on population PK modeling using data from HCV-infected subjects.
b Unbound C_{max} calculated based on protein binding values measured in vitro of 61.8% for SOF (measured at 1.89 μM), 7.2% GS-331007 (measured at 3.85 μM), and 99.7% for VEL (measured at 2 μM).
c Intestinal concentrations are calculated based on an intestinal volume of 250 mL.
d Liver inlet concentration = $C_{max} + (k_a \times dose \times F_aF_g/Q_h)$; where k_a and F_aF_g have not been determined and are assumed to be 0.1 min-1 and 1, respectively. Qh for human is 1500 mL/min.
e Concentration based on an arbitrary 1% free concentration used to assess drug-drug interaction potential.

Conversions- SOF: 1 nM = 0.529 ng/mL; GS-331007: 1 nM = 0.260 ng/mL; VEL: 1 nM = 0.883 ng/ml

SOF showed no inhibition of P-gp at up to the highest concentration tested (300 μM). GS-331007 showed no inhibition of P-gp, OCT1, MATE1 and MRP2 mediated transport at test concentrations up to 300 μM. At 300 μM, GS-331007 inhibited OCT2-mediated transport of TEA, OAT3-mediated transport of E3S, and BSEP-mediated transport of taurocholate by 13%, 34% and 17%, respectively (IC50 values > 300 μM), which was not considered clinically significant.

SOF and GS-331007

SOF is metabolized by CES1 to form an intermediate metabolite GS-566500. Further metabolism leads to form of GS-331007 and the active metabolite. Irinotecan is also hydrolyzed by CES1 to a putatively active metabolite, SN-38. Therefore, the potential drug-drug interactions between sofosbuvir and irinotecan were examined in primaryl human hepatocytes (Study AD-334-2025). While GS-331007 had no effect on SN-38 formulation (IC50 > 300 μM), sofosbuvir weakly inhibited the formation of SN-38 with an IC50 value of 29.2 μM. This concentration is greater than 30-fold the Cmax achieved in the clinic (~1 μM). No change in levels of sofosbuvir's active metabolite, GS-461203, was observed upon co-administration of SOF or GS-331007 with 10 μM irinotecan. These results suggest clinically relevant drug-drug interactions between sofosbuvir and irinotecan are unlikely.

GS-7977 showed no potency for mechanism-based inhibition against CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 or CYP3A.

VEL

P-gp and BCRP transport of GS-5816 was determined using non-transfected (MDCKII-WT) and transfected (MDCKII-MDR1 and MDCKII-BCRP) cells in the presence and absence of known

Pgp and BCRP inhibitors. The results showed that GS-5816 was a substrate for both P-gp and BCRP mediated transport.

OATP1B1 and OATP1B3 transport of GS-5816 was evaluated using transfected Chinese hamster ovary (CHO) cells in the presence and absence of the OATP inhibitor rifampicin. The results showed that GS-5816 is not a substrate of OATP1B1 or OATP1B3 in transfected CHO cells. However, a clinical study with a single dose of the perpetrator drug rifampin indicates that VEL is weakly affected by OATP1B1 and/or OATP1B3.

VEL has Cmax of approximately 0.3 µM. based on the value of Cmax/IC50 (<0.01), VEL does not inhibit the activities of any of the tested human enzymes, including CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4 (IC50 > 25 µM).

Velpatasvir had an inhibitory effect on the activity of human UGT1A1, with an IC50 value of 1.56 µM. Although based on the total Cmax, Cmax/IC50 is >0.1, the unbound Cmax /IC50 is less than 0.002. In addition, results of a clinical drug-drug interaction study between SOF/VEL and dolutegravir, a UGT1A1 substrate, demonstrated no inhibition by VEL at clinical concentrations.

In cultured human hepatocytes, Velpatasvir caused little or no induction of CYP mRNA or enzyme activities. Small increases in CYP2B6 and 3A4 activity and mRNA levels observed at the highest concentration tested of 10 µM were less than 20% of those caused by the positive controls. There was no concentration-dependent increase in CYP2C9 mRNA (fold over vehicle control ranged from 1.37 to 1.57), P-gp mRNA (fold change above vehicle control ranged from 1.37 to 1.47), or UGT1A1 mRNA (fold change above vehicle control ranged from 1.50 to 1.57). These results indicate that VEL has a low likelihood to act as a clinically relevant inducer. Additional study was conducted to evaluate the potential of GS-5816 to activate the aryl hydrocarbon receptor (AhR) and the pregnane X receptor (PXR). At concentrations up to 50 uM, GS-5816 does not activate AhR and may be a weak activator of PXR. Induction of CYP3A (regulated by PXR) is possible at pharmacological concentrations of GS-5816 (0.5 uM or less), while induction of CYP1A2 (regulated by AhR) is less likely. No evidence for induction has been observed during clinical drug-drug interaction studies. For example, SOF/VEL did not meaningfully decrease levels of coadministered antiretroviral drugs including nonnucleoside inhibitors (efavirenz and rilpivirin), integrase inhibitors (raltegravir, dolutegravir, and elvitegravir), or boosted protease inhibitors (atazanavir, lopinavir or darunavir). Similarly, VEL had minimal effect on coadministered cyclosporine.

The potential for VEL to inhibit drug transporters has been assessed in vitro using cell lines transfected with individual transporters or using membrane vesicle preparations. The results are summarized in the following table.

Inhibition Potential of Transporters by Velpatasvir

Transporter	Maximum Inhibition at Highest Concentration Tested (Concentration)	IC_{50} (µM)	Report
P-gp	66% (40 µM)	20.6 ± 5.7	AD-281-2010
BCRP	100% (40 µM)	0.30 ± 0.04	AD-281-2010
MRP2	No Inhibition (40 µM)	> 40	AD-281-2012
BSEP	90% (40 µM)	0.64	AD-281-2012
NTCP	No Inhibition (40 µM)	> 40	AD-281-2012
OATP1B1	80% (40 µM)	1.5 ± 0.5	AD-281-2010
OATP1B3	92% (40 µM)	0.26 ± 0.03	AD-281-2010
OATP1A2	No Inhibition (10 µM)	> 10	AD-281-2040
OATP2B1	30% (10 µM)	> 10	AD-281-2040
OCT1	22% (4 µM)	> 4	AD-281-2026
OCT2	45% (4 µM)	> 4	AD-281-2026
OAT1	No Inhibition (4 µM)	> 4	AD-281-2026
OAT3	No Inhibition (4 µM)	> 4	AD-281-2026
MATE1	19% (4 µM)	> 4	AD-281-2026

BCRP = breast cancer resistance protein; BSEP = bile salt export pump; IC_{50} = concentration resulting in 50% inhibition; P-gp = P-glycoprotein; MATE = multidrug and toxin extrusion protein; MRP = multidrug resistance associated protein; NTCP = Na+-taurocholate cotransporting polypeptide; OAT = organic anion transporter; OATP = organic anion-transporting polypeptide; OCT = organic cation transporter

VEL showed dose-dependent inhibition of P-gp, BCRP, BSEP, OATP1B1, and OATP1B3, with IC50 values of 20.6, 0.30, 0.64, 1.5, and 0.26 µM, respectively. Marginal inhibition of OCT1, OCT2, and MATE1 by VEL was observed, with 22%, 45%, and 19% inhibition, respectively, at the highest concentration tested (4 µM). Based on an estimated unbound hepatic inlet concentration of 0.0784 µM (assuming plasma protein binding of 1%), and IC50s for OATP1B1 and OATP1B3, R is approximately 1.05 for OATP1B1 and 1.3 for OATP1B3. GS-5816 showed some potential to inhibit the hepatic uptake transporters OATP1B1 and OATP1B3 during first pass. Based on $[I]_2/IC_{50}$, VEL has the potential to inhibit the intestinal P-gp, BCRP and OATP2B1($[I]_2/IC_{50}$ >10).

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

Introduction

This study evaluated the protein binding of GS-5816 in Sprague-Dawley rat, beagle dog, cynomolgus monkey, rhesus monkey, and human plasma via equilibrium dialysis. Additionally, the relative protein binding of GS-5816 to cell culture medium (CCM) and human plasma was also determined by direct competitive dialysis.

Materials and Methods

The binding of GS-5816 in plasma from Sprague-Dawley rat, beagle dog, cynomolgus monkey, rhesus monkey, and human was performed at [redacted] (b) (4). The competitive

dialysis studies between cell culture medium (CCM) and human plasma was performed at Gilead Sciences, Inc. The quantification method utilized for GS-5816 detection was different at these locations.

Commercially available chemicals were obtained from [redacted] (b) (4). Dialysis membrane ([redacted] (b) (4)) had a molecular weight cutoff of 10 kDa.

Plasma protein binding assay:
Sprague- Dawley rat, beagle dog, cynomolgus monkey, rhesus monkey, and human plasma was spiked with GS-5816 at final concentrations of 2 µM. Equilibrium dialysis was conducted at 37°C by placing spiked plasma (1 ml) and compound-free phosphate buffer into opposite sides of the assembled dialysis cells. The dialysis was performed in triplicate for 3 hours. Percent unbound drug was calculated using the equation below.

$$\%\,Unbound = \frac{C_f}{C_t} \times 100$$

C_f = post-dialysis buffer concentration
C_t = post-dialysis cell culture medium or plasma concentration

Competitive protein binding assay:
Competitive equilibrium dialysis was performed at 37°C with opposed dialysis cells containing 100% human plasma and the CCM (Gibco Dulbecco's Modified Eagle Medium with 10% (v/v) fetal bovine serum). Both matrices were spiked with GS-5816 to a final concentration of 2 µM. The relative binding was calculated using the following equation.

$$Ratio = \frac{C_{Plasma}}{C_{CCM}}$$

Results
Detected free fraction of GS-5816 was very low for all species (Table 1) and the relative protein binding of GS-5816 in CCM, was high (Table 2).

Table 1: Protein binding of GS-5816 in plasma from different species (source: Study Report Table 1)

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

a: Initial concentration in protein-containing dialysis cell
b: Mean ± Standard Deviation (n = 3)

Table 2: Relative protein binding of GS-5816 in CCM and human plasma (source: Study Report Table 2)

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

a: Initial concentration in each dialysis cell
b: Corrected final concentration in plasma / corrected final concentration in CCM; Mean ± Standard Deviation, (n = 2)

Conclusion
The protein binding of GS-5816 in plasma was high (>99.5%) in all species tested. In the competitive dialysis study, the concentration of GS-5816 in human plasma was 52-fold higher than in CCM. These results indicate that for GS-5816, deviations in the plasma protein binding extent could affect the pharmacokinetic properties and consequently, the efficacy.

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

Introduction
The purpose of this study was to assess the extent of human plasma protein binding of GS-5816 at concentrations of 0.1, 0.25, 0.5, 1 and 2 µM.

Materials and Methods
This study was performed at Gilead Sciences, Inc. Commercially available chemicals were obtained from (b) (4). Dialysis membrane ((b) (4)) had a molecular weight cutoff of 10 kDa.

Equilibrium dialysis was performed at 37°C using human plasma spiked with GS-5816 at final concentrations of 0.1 µM, 0.25 µM, 0.5 µM, 1 µM, and 2 µM. Compound free phosphate buffer was placed into opposite sides to the plasma in the assembled dialysis cells. The dialysis was performed in duplicate for 3 hours. Percent unbound drug was calculated using the equation below.

$$\%\,Unbound = \frac{C_f}{C_t} \times 100$$

C_f = post-dialysis buffer concentration
C_t = post-dialysis cell culture medium or plasma concentration

Results
The estimated protein bound fraction was >99.5% over the concentration range from 0.1 to 2 µM (Table 1).

Table1: Protein binding of GS-5816 in human plasma at different GS-5816 concentrations (source: Study Report Table 1)

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

a: Initial concentration in protein-containing dialysis cell

b: Mean ± Standard Deviation (n = 2)

Conclusion

The human plasma protein binding of GS 5816 was high (>99.5%) and was independent of drug concentration over the range of 0.1 µM to 2 µM. Thus, minute alterations in the extent of plasma protein binding may have an impact on efficacy or pharmacokinetics of GS-5816.

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

Introduction

This in vitro study assessed the potential for hepatic clearance of GS-5816 using pooled hepatic microsomal fractions from Sprague-Dawley rat, beagle dog, cynomolgus monkey, and human. The rate of metabolism was also assessed using cryopreserved human hepatocytes.

Materials and Methods

Pooled hepatic microsomal fractions, cryopreserved hepatocytes, hepatocyte thawing (HT) medium, and KHB medium was provided by (b) (4). NADPH regenerating system was from (b) (4). All other chemicals were purchased from (b) (4).

Internal Standard/Quench (IS/Q) solution was (b) (4) This IS/Q solution was used to stop reactions in microsome and hepatocyte incubations.

Metabolic Stability in Hepatic Microsomal Fraction

Verapamil was used as the metabolic stability standards. Metabolic stability was assessed in the reaction mixture containing 3 µM test compound (GS-5816 or verapamil), 0.5 mg microsomal protein/ml, 1.25 mM NADP, 3.3 mM glucose-6-phosphate, 0.4 U/ml glucose-6-phosphate dehydrogenase and 3.3 mM $MgCl_2$ in 50 mM potassium phosphate buffer (pH 7.4). At 0, 2, 5, 10, 15, 30, 45, and 60 min, 25 µl aliquots of the reaction mixture were transferred to plates containing 250 µl of IS/Q quenching solution. After quenching, the plates were centrifuged and aliquots of the supernatant were analyzed by Liquid Chromatography - Mass Spectrometry.

Metabolic Stability in Cryopreserved Hepatocytes

To the cell suspension of thawed cryopreserved hepatocytes, KHB medium was added to obtain a target density of 2 × 106 cells/ml. For incubations, aliquots of hepatocyte suspension was mixed with GS-5816 or metabolic stability controls (7-Hydroxycoumarin and testosterone) to achieve final concentration of 2 µM. From incubation, aliquots were removed after 0, 1, 3, and 6 hours and added to 100 µl IS/Q quenching solution. After quenching, the plates were centrifuged and aliquots of the supernatant were analyzed by Liquid Chromatography - Mass Spectrometry.

Rate of GS-5816 disappearance was calculated using the following equation.

$$C_t = C_0 \times e^{-K \times t}$$

C_t = % of parent remaining at time = t
C_0 = % of parent remaining at time = 0
K = First order elimination rate constant

The hepatic clearance was predicted for GS-5816 using the following equations.

$$CL_{int} = K \times V \times \frac{Y}{P}$$

$$CL_h = \frac{(CL_{int} \times Q_h)}{(CL_{int} + Q_h)}$$

CL_h = Predicted hepatic clearance (L/hr/kg body weight)
CL_{int} = Intrinsic hepatic clearance (L/hr/kg body weight)
V = Incubation volume (L)
Y = Microsome protein yield (mg protein/kg body weight) or hepatocyte yield
(millions of hepatocytes/kg body weight)
P = Mass of protein in the incubation (mg) or number of hepatocytes (× 106)
Q_h = Hepatic blood flow (L/kg body weight)

Values used for calculation of the predicted hepatic clearance are presented in the table below.

Table 1: Values used for the predicted hepatic clearance calculations (source: Study Report Table 1)

Species	Hepatocytes			Hepatic Microsomes			Q_h (L/kg)
	V (L)	Y (× 106/kg)	P (× 106/kg)	V (L)	Y (mg)	P (mg/kg)	
Rat				0.001	0.5	45	4.2
Monkey				0.001	0.5	20	1.6
Dog				0.001	0.5	20	1.8
Human	0.00025	5000	0.25	0.001	0.5	20	1.3

Results

The metabolic stability of GS-5816 in cryopreserved human hepatocytes and hepatic microsomal fractions was assessed by estimating the half-lives and percent hepatic extraction (Table 2 and Table 3). The predicted hepatic clearance, based on the cryopreserved hepatocytes, was low (<0.07 L/h/kg). Additionally, with hepatic microsomes, the predicted clearance estimates were low in all species.

Table 2: *In Vitro* rate of metabolism of GS-5816 in cryopreserved human hepatocytes (source: Study Report Table 2)

Species	t½ (h)	Predicted Hepatic Cl (L/hr/kg)	Predicted Hepatic Extraction (%)
Human	> 39.5	< 0.07	< 5.1

Table 3: *In Vitro* rate of metabolism of GS-5816 in hepatic microsomes (source: Study Report Table 3)

Species	t½ (min)	Predicted Hepatic Cl (L/hr/kg)	Predicted Hepatic Extraction (%)
Rat	192	0.743	17.7
Dog	163	0.369	20.5
Monkey	> 395	< 0.17	< 10.6
Human	> 395	< 0.17	< 12.7

Conclusion

These study results of metabolic stability in cryopreserved human hepatocytes and from other species' microsomes, suggests low hepatic metabolic clearance of GS-5816.

AD-281-2007: Cytochrome P450 Metabolic Reaction Phenotyping of GS-5816 (by Abhay Joshi)

Introduction

This in vitro study was designed to identify whether GS-5816 is a substrate for particular CYPenzymes. GS-5816 was incubated with individual cDNA expressed human CYP450 enzyme preparations. Compounds known to be metabolized by each CYP450 enzyme were used as a positive control.

Materials and Methods

This study was conducted by [redacted](b) (4). Bacterially expressed human CYP enzyme preparations co-expressed with human NADPH cytochrome P450 reductase were supplied from [redacted](b) (4). All other chemicals were from [redacted](b) (4) or equivalent vendors.

GS-5816 (5 µM) or control compound was incubated with the individual Bactosome preparations. CYP concentrations were CYP1A2, 100 pmol/ml; CYP2B6, 100 pmol/ml; CYP2C8, 50 pmol/ml; CYP2C9, 25 pmol/ml; CYP2C19, 100 pmol/ml;CYP2D6, 50 pmol/ml; and CYP3A4, 25 pmol/ml. NADPH was used to initiate the reaction. Each compound was incubated individually for 0, 5, 15, 30, and 45 min with each enzyme. After stopping the reaction, the plates were centrifuged and aliquots of the supernatant were analyzed by Liquid Chromatography - Mass Spectrometry

The peak area ratios were plotted against incubation time and the half-life for loss of parent compound was determined using the following equation:

$$C_t = C_0 \times e^{-\frac{\ln 2}{t_{1/2}} \times t}$$

C_t = Concentration of compound at time t
C_0 = Concentration of compound at time 0
$T_{½}$ = In vitro half-life
t = Time

The rate of metabolism (min^{-1}) was subsequently calculated using the following equation:

$$Rate = \frac{\ln 2}{T_{1/2}} \times \frac{[S]}{[P450]}$$

[S] = Substrate concentration (5000 pmol/mL)
[P450] = CYP protein concentration (pmol/mL)

Results

Rates of metabolism of GS-5816 and the positive controls by individual CYP enzymes are reported in Table 1. GS-5816 was not a substrate for recombinant CYP1A2, CYP2C9, CYP2C19, or CYP2D6. Metabolism of GS-5816 with CYP2B6, CYP2C8, and CYP3A4 was detectable; however, observed rate of metabolism was low.

Table 8: Rates of metabolism of GS-5816 and control substrates by major human CYP450 enzymes (source: Study Report Table 1)

Compound	Metabolism Rate (min-1)						
	CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP3A4
GS-5816 (% Positive Control)	< 0.12 (< 0.8%)	0.13 (6.6%)	1.26 (5.5%)	< 0.47 (< 2.2%)	< 0.12 (< 12%)	< 0.23 (< 1.0%)	2.09 (18%)
Ethoxycoumarin	14.2	-	-	-	-	-	-
Efavirenz	-	1.97[a]	-	-	-	-	-
Amodiaquine	-	-	23.1	-	-	-	-
Diclofenac	-	-	-	21.5	-	-	-
Diazepam	-	-	-	-	0.96[b]	-	-
Dextromethorphan	-	-	-	-	-	22.8	-
Testosterone	-	-	-	-	-	-	11.5

a Efavirenz is a selective substrate for CYP2B6 but is metabolized slowly

b Diazepam is a selective substrate for CYP2C19 but is metabolized slowly

Reviewer's note:

- *Reported metabolism rate estimate for the positive control used: Efavirenz, was low compared to the rate estimate reported under similar experimental conditions, by the same analytic institute (Based on information from the Study site's catalog* (b) (4) *). However, it is recommended to use the probe substrate (positive control) that has the proven reproducibility history under the specific experimental conditions.*

- *Highest metabolism rate estimates (compared to control) were reported for CYP3A4, CYP2B6 and CYP2C8. These findings were consistent with subsequent clinical study results. When coadministered with the CYP3A4 inductive agent (efavirenz), GS-5816 exposures were slightly reduced (mean Cmax ratio {90% CI} = 0.8 {0.7-0.9}). When coadministered with the moderate CYP2C9 and CYP2C8 inductive agent (rifampin), GS-5816 exposures were significantly reduced (mean Cmax ratio {90% CI} = 0.29 {0.2-0.4}).*

Conclusion

These in vitro study results indicate that GS-5816 is a substrate of CYP3A4, CYP2B6, and CYP2C8.

AD-81-2008: In Vitro Assessment of Human Liver Cytochrome P450 Inhibition Potential of GS-5816

Introduction

In this study, the potential for GS-5816 to inhibit human cytochrome P450 (CYP) isoforms was assessed using isoform-specific probe substrates in human liver microsomal fractions.

Materials and Methods

Probe substrates were incubated with human liver microsomes and NADPH at 37ºC in the presence of GS-5816 (up to 25 uM) or control inhibitors (Table 1). Drug concentrations were

assessed by LC-MS/MS and were used to calculate IC_{50} values. Assays were conducted by (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	7-ethoxyresorufin	0.5	5	α-napthoflavone	0-3
CYP2B6	bupropion	110	5	ticlopidine	0-10
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-5816 IC_{50} values were high for all CYP isoforms evaluated (>25 uM, Table 2). In contrast, control inhibitors had low IC_{50} values, indicating potent inhibition.

Table 2: Effects of GS-5816 and Positive Control Inhibitors on the Activities of Major Human Cytochromes P450 (source: Study Report Table 1)

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

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

Conclusion

At concentrations up to 25 μM, GS-5816 had no inhibitory effect on the activities of human hepatic microsomal CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6 or CYP3A enzymes (IC50 > 25 μM). Thus, GS-5816 is unlikely to cause drug-drug interactions through inhibition of these enzymes.

AD-281-2009: Induction of metabolizing enzymes by GS-5816

Introduction

In this study, the potential of GS-5816 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 CYP1A2-DRE (expressing AhR and a luciferase reporter linked to enhancer regions of CYP1A2) and DPX2 (expressing PXR and a luciferase reporter linked to enhancer regions of CYP3A4).

Materials and Methods

DPX2 and CYP1A2-DRE cells were plated in 96-well plates and allowed to recover for 24 h. Cells were incubated in 150 uL/well containing 0.15-50 uM GS-5816 for 24 h, Postive controls including 0.1-20 uM b-naphthoflavone (AhR activator, CYP1A2-DREcells) or 0.1-20 uM rifampicin (PXR activators, DPX2 cells) and the DMSO negative control were included on each plate. Medium was replaced with 25 µL of phosphate-buffered saline and 25 µL CellTiter-Fluor assay buffer (Promega Corporation, Madison, WI). The plates were incubated for a further 1 hour and fluorescence determined in a Perkin-Elmer Victor 2 fluorometer. Following the toxicity assessment, 50 µL of ONE-Glo™ luciferase substrate (Promega Corporation) was added. The plates were incubated at room temperature for 5 minutes and then luminescence determined in a BMG luminometer. Three replicates were evaluated.

Results

Table 1 shows the results for the activation of PXR and AhR by GS-5816 and positive control compounds. Treatments with the PXR inducer, rifampicin, led to up to 12.7-fold activation of reporter gene expression. In contrast, at a concentration of 15 µM, the extent of activation of PXR by GS-5816 reached only 16.3% of the maximum achieved by the positive control. Treatment with the AhR inducer, β-Naphthoflavone, resulted in up to 70.0-fold activation compared to vehicle control; While GS-5816 with concentrations up to 50 µM resulted in less than 1% of activation elicited by β-naphthoflavone.

Table 1: Human PXR Activation by GS-5816 and Positive Controls (source: Study Report Tables 2 and 3)

Fold induction over 0.1% DMSO ctrl	PXR (CYP3A4)		AhR (CYP1A2)	
Conc. (uM)	GS-5816	rifampicin	GS-5816	α-naphthoflavone
0.1	-	1.68	-	1.61
0.15	1.30	-	0.97	-
0.5	1.67	4.45	0.81	2.80
1	-	6.23	-	4.15
1.5	2.48	-	0.76	-
5	2.53	11.6	0.73	21.5
10	-	12.7	-	41.6
15	2.91	-	0.61	-
20	-	12.6	-	70.0
50	2.70	-	0.77	-

Conclusion
At concentrations up to 50 uM, GS-5816 does not activate AhR and may be a weak activator of PXR. Induction of CYP3A (regulated by PXR) is possible at pharmacological concentrations of GS-5816 (0.5 uM or less), while induction of CYP1A2 (regulated by AhR) is less likely.

AD-281-2025: Evaluation of induction potential of Velpatasvir (VEL) in cultured human hepatocytes

Introduction
In this study, the induction potential of cytochrome P450 (CYP) isoforms, UDP glucuronosyl transferase (UGT) 1A1, and P-glycoprotein (P-gp) by test article VEL in cultured human hepatocytes was evaluated using primary cultured human hepatocytes from three donors.

Materials and Methods
The cryopreserved human hepatocytes from 3 donors were plated in collagen I-coated 24-well plates and incubated with VEL at 1, 3, or 10 uM or positive control inducers (CYP1A2: 50 uM omeprazole, CYP2B6 and P-gp: 1 mM phenobarbital, CYP2C9 and CYP3A4: 10 uM rifampicin, UGT1A1: 20 uM β-naphthoflavone) for three days. Induction of CYP1A2, CYP2B6, and CYP3A activity were measured using catalytic activity assays (probe substrates: 100 uM phenacetin, 250 uM bupropion, and 200 uM 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 at (b) (4).

Results
The activity results demonstrated no concentration-dependent increases in CYP1A2, CYP2B6, and CYP3A activity over the vehicle control for all three hepatocyte lots in response to VEL at concentrations of 1 to 10 µM. Fold induction values ranged from 0.59 to 1.3, (CYP1A2, Table 6 of Study Report), 1.1 to 2.0 (CYP2B6, Table 8 of Study Report) and 0.40 to 3.2 (CYP3A, Table 10 of Study Report). In general, the CYP1A2, CYP2B6, and CYP3A4 mRNA results were consistent with the activity results, with fold induction values ranging from 0.32 to 1.1 (CYP1A2, Table 7 of Study Report), 0.95 to 2.6 (CYP2B6, Table 9 of Study Report), and 2.7 to 30 (CYP3A4, Table 11 of Study Report) in a concentration-independent manner. No increases in CYP2C9, P-gp, and UGT1A1 mRNA expression levels resulted in the hepatocyte cultures in response to VEL. Fold induction values ranged from 0.89 to 2.2 (CYP2C9, Table 12 of Study Report), 1.2 to 1.8 (P-gp, Table 13 of Study Report), and 1.2 to 1.8 (UGT1A1, Table 14 of Study Report). Table 1 and Table 2 below summarize the results from the 3 donors. At concentrations 1 to 10 µM, the increase in CYP3A4 mRNA expression is above the cut-off of 4-fold for induction (Fahmi et al. DMD 2010), while the increase of CYP1A2, CYP2B6, CYP2C9, P-gp and UGT1A1 mRNA expressions are below the 4-fold cut-off. Therefore, based on this study, VEL may induce CYP3A4, but the induction potential is much less than the active control, rifampin (<20%). The results are consistent with the results from AD-281-2009, the PXR and AhR activation study.

Table 1. Effect of Velpatasvir Treatment on CYP Activity in Cultured Human Hepatocytes

Treatment	Fold Increase of CYP Activity over Vehicle Control (Percent Increase Relative to Positive Control)		
	CYP1A2	CYP2B6	CYP3A
VEL (1 μM)	1.10 (3.1%)	1.73 (20%)	1.58 (6.2%)
VEL (3 μM)	0.92 (2.6%)	1.73 (20%)	1.69 (6.7%)
VEL (10 μM)	0.83 (2.4%)	1.67 (20%)	1.67 (6.6%)
Omeprazole (50 μM)	35.3	NA	NA
Phenobarbital (1000 μM)	NA	8.53	NA
Rifampin (10 μM)	NA	NA	25.3

CYP = cytochrome P450; NA= not applicable
Probe substrates were phenacetin, bupropion, and testosterone for CYP1A2, 2B6, and 3A, respectively.
Data represent the mean from 3 donors

Table 2. Effect of Velpatasvir Treatment on mRNA Levels in Cultured Human Hepatocytes

Treatment	mRNA Fold Increase over Vehicle Control					
	CYP1A2	CYP2B6	CYP3A4	CYP2C9	UGT1A1	P-gp
VEL (1 μM)	0.86 ± 0.22	1.90 ± 0.61	7.70 ± 5.76	1.57 ± 0.55	1.57 ± 0.21	1.37 ± 0.12
VEL (3 μM)	0.76 ± 0.22	1.43 ± 0.32	13.5 ± 14.3	1.37 ± 0.25	1.53 ± 0.15	1.47 ± 0.25
VEL (10 μM)	0.77 ± 0.39	1.58 ± 0.68	11.9 ± 11.5	1.46 ± 0.52	1.50 ± 0.30	1.43 ± 0.32
Omeprazole (50 μM)	50.0 ± 50.3	NA	NA	NA	NA	NA
Phenobarbital (1000 μM)	NA	17.0 ± 5.0	NA	NA	NA	2.23 ± 0.15
Rifampin (10 μM)	NA	NA	114 ± 70	3.17 ± 0.32	NA	NA
β-naphthoflavone (20 μM)	NA	NA	NA	NA	2.60 ± 1.15	NA

CYP = cytochrome P450; NA = not applicable; P-gp = P-glycoprotein; UGT = uridine diphosphate glucuronosyl transferase
Data are the mean ± SD from 3 donors

Conclusion

VEL may induce CYP3A4, but the induction is not dose-dependent and with the potency much less than rifampin. In addition, in vivo studies show that VEL did not decrease the exposure of CYP3A substrates. Therefore, the potential of CYP3A induction by VEL is not expected to be clinically significant.

AD-281-2016: In vitro assessment of human UGT1A1 inhibition potential of GS-5816

Introduction

In this study, inhibition of UGT1A1 catalytic activity by GS-5816 was determined using microsomal fractions from baculovirus-expressed human UGT1A1 insect cells (Supersomes™).

Materials and Methods
The UGT1A1 substrate estradiol (10 uM) was incubated with Supersomes™ (0.25 mg/mL protein), UDP-glucuronic acid (5 mM), and alamethicin (25 ug/mL) in the presence or absence of GS-5816 (concentration range: 0.4-100 uM) or the positive control inhibitor silybin (concentration range: 0-100 uM) for 30 min at 37ºC. 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 by (b) (4).

Results
The GS-5816 IC_{50} value for UGT1A1 was 1.56 µM, compared to an IC_{50} of 1.99 uM for the UGT1A1 inhibitor silybin (Table 1).

Table 1. Effects of GS-5816 and Positive Control Inhibitor on the Activity of Human UGT1A1 (source: Study Report Table 1)

Enzyme	Activity	Calculated IC_{50} (µM)	
		Control Inhibitor[a]	GS-5816
UGT1A1	Estradiol Glucuronidation	1.99	1.56

[a] Control Inhibitors: UGT1A1, silybin (0-100 µM)

Conclusion
The ratio of the total C_{max} to IC_{50} is greater than 0.1 (0.2). However, the unbound Cmax /IC50 is less than 0.02. Therefore, UGT1A1 inhibition by GS-5816 is less likely.

AD-281-2041: Effect of P-glycoprotein and BCRP Expression on GS-5816 Accumulation

Introduction
In this study, P-gp and BCRP transport of GS-5816 was determined using non-transfected (MDCKII-WT) and transfected (MDCKII-MDR1 and MDCKII-BCRP) cells in the presence and absence of known Pgp and BCRP inhibitors.

Materials and Methods
[^{14}C]GS-5816 at 50 nM or prazosin (a positive control) at 10 µM was dosed in the culture media in presence or absence of various inhibitors and incubated with wild type (WT), P-gp- or BCRP-overexpressing Madin-Darby canine kidney (MDCKII) cells at 37°C for 60 minutes. Compound concentration in cell lysate was determined by liquid scintillation counter or by LC/MS/MS. Prazosin was used as a positive control and tested under the same assay conditions.

Results
The amount of GS-5816 and prazosin detected in WT, Pgp and BCRP-transfected cells are summarized in Figure 1 and Figure 2.

The average accumulation of GS-5816 in MDCKII-MDR1 and MDCKII-BCRP cells was ~10-fold and 2-fold lower than in MDCKII-WT cells, respectively. P-gp inhibitors verapamil (100 µM) and CsA (5 µM) increased the accumulation of GS-5816 to a level comparable to those in MDCKII-WT cells. In MDCKII-BCRP cells, coincubation of GS-5816 with BCRP inhibitors novobiocin, Ko143 and CsA increased the cellular accumulation of GS-5816 to a level close to the level observed in WT cells.

For positive control substrate prazosin, the accumulation in MDCKII-MDR1 and -BCRP cells was ~6-fold and ~3-fold lower than in MDCKII-WT cells. Verapamil and CsA increased the level of prazosin accumulation in MDCKII-MDR1 cells close to the level seen in WT cells. All inhibitors of BCRP significantly increased the levels of prazosin accumulation in MDCKII-BCRP cells, the most pronounced effects were seen in presence of novobiocin and CsA. While inhibitors also had slight effects on prazosin uptake into MDCKII-WT cells, MDCKII-BCRP cells were substantially more sensitive and an increase in the ratio of uptake into the two cell types (MDCKII-BCRP/MDCKII-WT) was observed in the presence of all inhibitors tested.

Figure 1. Accumulation of GS-5816 (Assayed in Triplicates) and Prazosin (Assayed in Duplicates) in MDCKII-WT and MDCKII-MDR1 Cells (Data from Two Independent Assays Done in Triplicate)

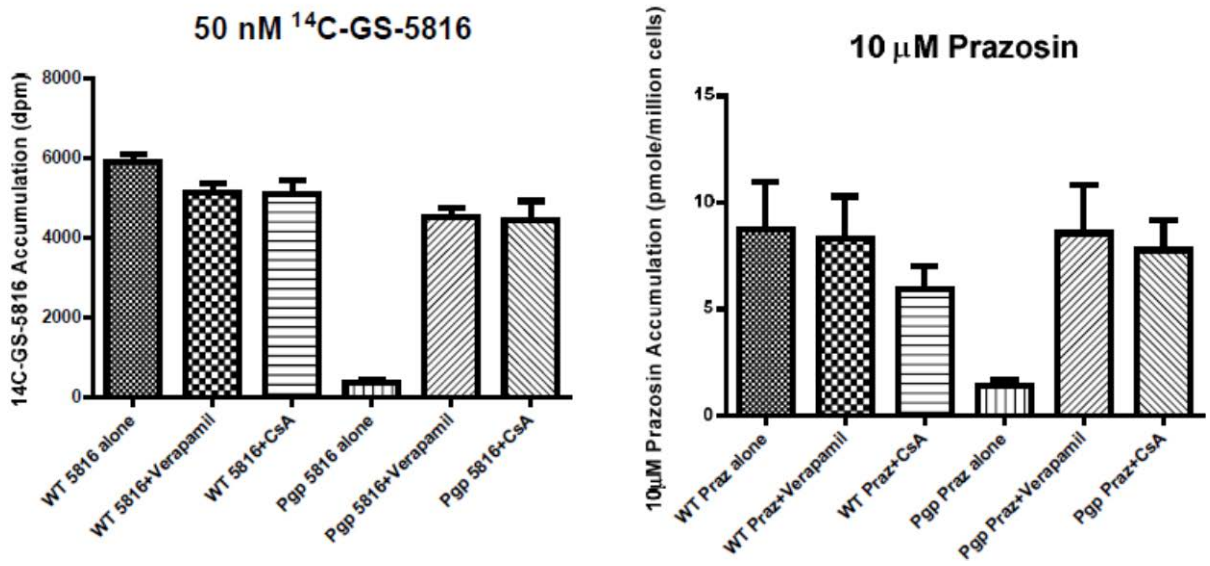
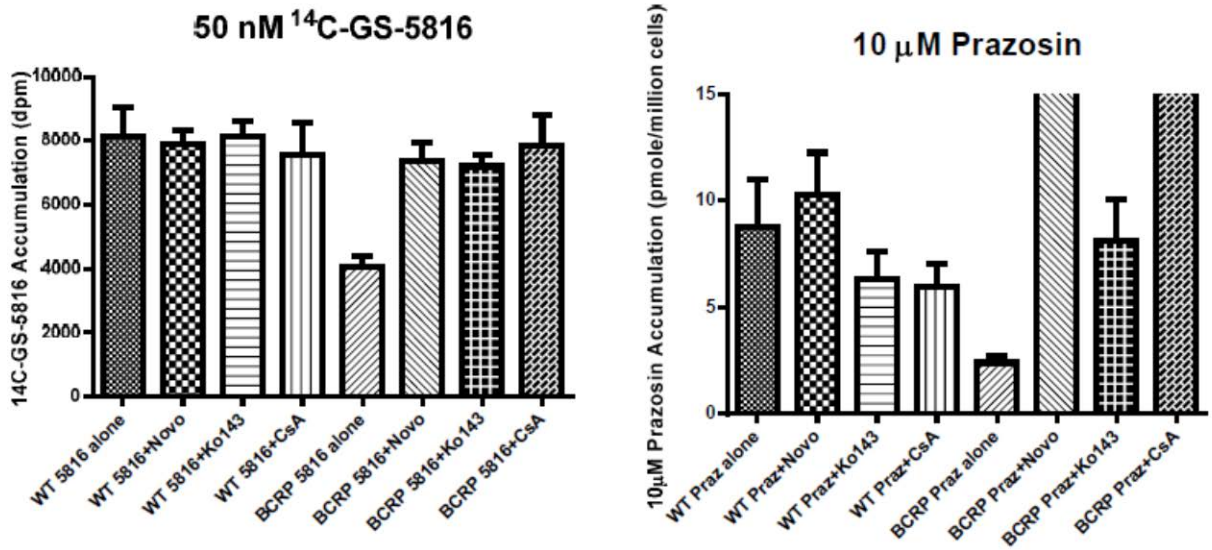


Figure 2. Accumulation of GS-5816 (Assayed in Triplicates) and Prazosin (Assayed in Duplicates) in MDCKII-WT and MDCKII-BCRP Cells (Data from Two Independent Assays Done in Triplicate)



Conclusion
GS-5816 was a substrate for both P-gp and BCRP mediated transport.

AD-281-2011: Potential of GS-5816 as a substrate of human OATP1B1 and OATP1B3

Introduction
In this study, OATP1B1 and OATP1B3 transport of GS-5816 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-5816 (final concentration: 0.1 µM) in the presence or absence of rifampicin (final concentration: 40 uM) at 37ºC 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 OATP1B1/WT and OATP1B3/WT ratios for GS-5816 were 0.8 and 1.7, respectively. Positive control atorvastatin showed OATP1B1/WT ratio of 16 and OATP1B3/WT ratio of 17. Passive permeability control antipyrin had OATP1B1/WT ratio of 1.0 and OATP1B3/WT ratio of 1.1. In the presence of 40µM rifampicin, GS-5816 rate of uptake in WT, OATP1B1, and OATP1B3 transfected cells were similar to GS-5816 dosed alone. For positive control atorvastatin, the uptake rate decreased 8 fold in OATP1B1 cells and 14 fold in OATP1B3 cells in the presence of rifampicin. For the negative control, antipyrin, the addition of rifampicin did

not significantly affect the uptake rate. The results indicate GS-5816 is not a substrate of OATP1B1 or OATP1B3.

Uptake activities of GS-5816 and control compounds are summarized in Table 1.

Table 1: Uptake rate of GS-5816 and control compounds in transfected and wild-type CHO cells (source: Study Report Tables 2 and 3)

Uptake Rate (pmol/min/1x10^6cells)	0.1 µM GS-5816		0.1 µM atorvastatin		10 µM antipyrin	
Rifampicin	-	+	-	+	-	+
CHO-WT	3.1	4.2	0.4	0.6	16	16
CHO-OATP1B1	2.5	2.4	6.9	0.8	16	17
CHO-OATP1B3	5.4	3.2	7.4	0.5	17	17
OATP1B1/WT ratio	0.8		16		1.0	
OATP1B3/WT ratio	1.7		17		1.1	

Conclusion
GS-5816 is not a substrate of OATP1B1 or OATP1B3 in transfected CHO cells.

AD-281-2010: GS-5816 inhibition of human OATP1B1, OATP1B3, P-gp and BCRP

Introduction
In this study, inhibition of uptake transporters OATP1B1 and OATP1B3 or efflux transporters P-gp and BCRP by GS-5885 was determined using cell lines transfected with the individual transporters and fluorescent model substrates.
Materials and Methods
GS-5816 (0.05 to 40 µM) or a positive control (rifampicin) was incubated for 1 h with OATP1B1- or OATP1B3-overexpressing Chinese hamster ovary (CHO) cells in black 96-well plates with clear bottoms. Following incubation and washing, the cells were lysed and each well was analyzed for Fluo 3 fluorescence at an excitation of 485 nm and emission of 530 nm. For the P-gp assay, VEL or a positive control (verapamil) was incubated for 1 h with MDCKII cells. Following incubation and washing, the cells were lysed and each well was analyzed for calcein fluorescence at an excitation of 494 nm and an emission of 517 nm. For BCRP assay, VEL or a positive control (Fumitremorgin C (FTC)) was incubated for 18 h with MDCKII-ABCG2. Following incubation and washing, the cells were lysed and each well was analyzed for PhA fluorescence at an excitation of 415 nm and an emission of 675 nm.

Results
GS-5816 showed dose-dependent inhibition of OATP1B1 and OATP1B3 with IC_{50} of 1.5 ± 0.5 µM and 0.26 ± 0.03 µM, respectively. The positive control rifampicin had an IC_{50} value of 3.7 ± 0.3µM for OATP1B1 and 3.0 µM ± 1.0 for OATP1B3. Based on an estimated unbound hepatic inlet concentration of 0.305 µM (assuming plasma protein binding of 1%), GS-5816 showed some potential to inhibit the hepatic uptake transporters OATP1B1 and OATP1B3 during first pass.

GS-5816 showed concentration-dependent inhibition of Pgp with IC_{50} of 20.6 ± 5.7 µM. The positive control verapamil had IC_{50} value of 5.1 ± 2.8 µM. GS-5816 showed concentration-dependent inhibition of BCRP with IC_{50} of 0.30 ± 0.04 µM**.** The positive control FTC had IC_{50}

value of 0.23 ± 0.08 µM. Based on an estimated maximal intestinal concentration of 453 µM, VEL has the potential to inhibit intestinally expressed P-gp, and BCRP.

Table 1. Inhibition of OATP1B1/ 1B3-Mediated Transport of Fluo3, BCRP-Mediated Transport of Pheophorbide A, and Pgp-Mediated Transport of Calcein AM by GS-5816 and control compounds (source: Study Report Table 1)

	Uptake Transporter IC_{50} (µM)	
Transporters	OATP1B1	OATP1B3
GS-5816	1.5 ± 0.5	0.26 ± 0.03
Rifampicin	3.7 ± 0.3	3.0 ± 1.0
	Efflux Transporters IC_{50} (µM)	
Transporters	P-gp	BCRP
GS-5816	20.6 ± 5.7	0.30 ± 0.04
Verapamil	5.1 ± 2.8	NA
Fumitremorgin C (FTC)	NA	0.23 ± 0.08

Conclusion

- Based on an estimated unbound hepatic inlet concentration of 0.0784 µM (assuming plasma protein binding of 1%), and IC50s for OATP1B1 (IC50 = 1.5 µM) and OATP1B3 (IC50 = 0.26 µM), R is approximately 1.05 for OATP1B1 and 1.3 for OATP1B3. GS-5816 showed some potential to inhibit the hepatic uptake transporters OATP1B1 and OATP1B3 during first pass.
- Based on an estimated maximal intestinal concentration of 453 µM, VEL has the potential to inhibit intestinally expressed P-gp, and BCRP.

AD-281-2040: Potential of GS-5816 as an Inhibitor of human OATP1A2 or OATP2B1

Introduction

In this study, inhibition of uptake transport via OATP1A2 or OATP2B1 by GS-5816 was determined using cell lines transfected with the individual transporters and radiolabeled model substrate E3S.

Materials and Methods

OATP1A2–overexpressing human embryonic kidney 293 (HEK293) cells or OATP2B1-overexpressing Madin-Darby canine kidney (MDCK) cells were incubated with [^{3}H]estrone-3-sulfate (E3S) (1 µM for OATP1A2 and 0.2 µM for OATP2B1) in 96-well cell culture plates in the absence or presence of GS-5816 (at 7 concentrations from 0.014 to 10 µM). [^{3}H]E3S-derived radioactivity in cell lysates was determined with liquid scintillation counter. Sulfobromophthalein at 500 µM served as a positive control for OATP1A2 inhibition. Fluvastatin at 10 µM served as a positive control for OATP2B1 inhibition.

Results

GS-5816 showed no inhibition of OATP1A2 transport of E3S at concentrations up to 10 µM. GS-5816 inhibited OATP2B1 mediated E3S transport by 30% at 10 µM. Positive control sulfobromophthalein (SBP, 500 µM) inhibited OATP2A1 transport of E3S by 81% and fluvastatin

(10μM) inhibited OATP2B1 transport of E3S by 90%. Based on an estimated maximal intestinal concentration of 453 μM, VEL has the potential to inhibit intestinally expressed OATP2B1.

Table 1. Summary of the Results of the OATP1A2 and OATP2B1 Uptake Transporter Inhibition Assays (source: Study Report Table 1)

Test article	Transporter	IC_{50} (μM)	Observed effect (% inhibition at 10 μM)
GS-5816	OATP1A2	NA	no inhibition
	OATP2B1	>10	30

NA: not applicable

Conclusion
GS-5816 showed no inhibition of OATP1A2 transport of E3S at concentrations up to 10 μM. GS-5816 has the potential to inhibit intestinally expressed OATP2B1.

AD-281-2026: Potential of GS-5816 as an Inhibitor of OCT1, OCT2, MATE1, OAT1, and OAT3 or Substrate for OCT1

Introduction
Inhibition of the human organic anion uptake transporters OAT1 (SLC22A6) and OAT3 (SLC22A8), organic cation uptake transporters OCT1 (SLC22A1) and OCT2 (SLC22A2), and multidrug and toxin extrusion transporter MATE1 (SLC47A1) was studied in transfected cell lines. Transporter specific accumulation into OCT1 transporter-expressing cells was investigated to determine if GS-5816 is a substrate for this transporter.

Materials and Methods
For OAT1, OCT1, OCT2 and MATE1 transporter inhibition, GS-5816 (0.02 to 12 μM) or a positive control (benzbromarone for OAT1, verapamil for OCT1 and OCT2, quinidine for MATE1) was incubated with Chinese hamster ovary (CHO) cells in the presence of probe substrates (*p*-aminohippuric acid for OAT1, triethylamine for OCT1, OCT2 and MATE1. For OAT3 inhibition, increasing concentrations of GS-5816 (0.02 to 12 μM) was incubated with FlpIn293 cells in the presence of a probe substrate (estrone-3-sulfate). Transporter specific accumulation of the probe substrate in the cells was measured. Accumulation of GS-5816 into OCT1-overexpressing CHO cells was investigated at 2 concentrations (1 and 10 μM) and at 2 incubation time points (1 and 10 min) in an OCT1 substrate assay. The studies were performed by [redacted] (b) (4)

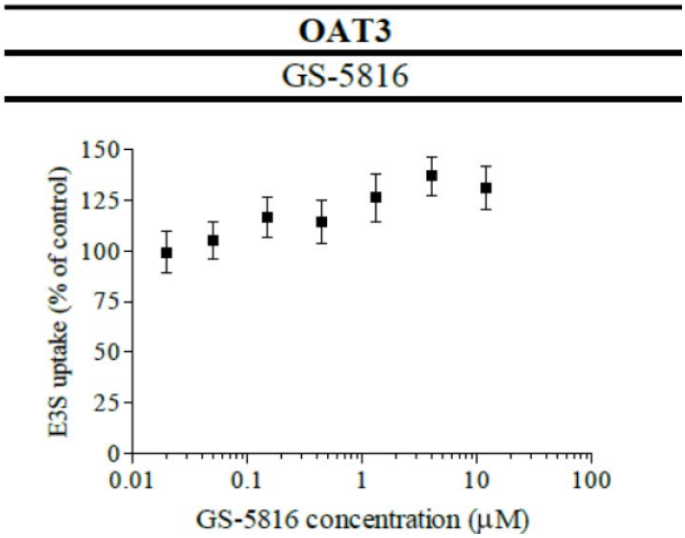
Results
Inhibition data are summarized in Table 1. The positive control inhibitors for each transporter showed > 90% inhibition in each assay. The highest concentration tested for GS-5816 was 12 μM, however, due to solubility concerns at this concentration the values measured at 4 μM are reported.

GS-5816 at 4 μM showed weak inhibition of OCT1, OCT2 and MATE1-mediated transport of TEA at 22%, 45%, and 19% of control, respectively. GS-5816 showed no transporter specific inhibition of OAT1 or OAT3. Stimulation of OAT3 was observed at higher concentrations (Figure 1); the clinical relevance of this observation is not clear.

Table 1. Inhibition of Human OCT1, OCT2, OAT1, OAT3 and MATE1 Transporters by GS-5816 (source: Study Report Table 3)

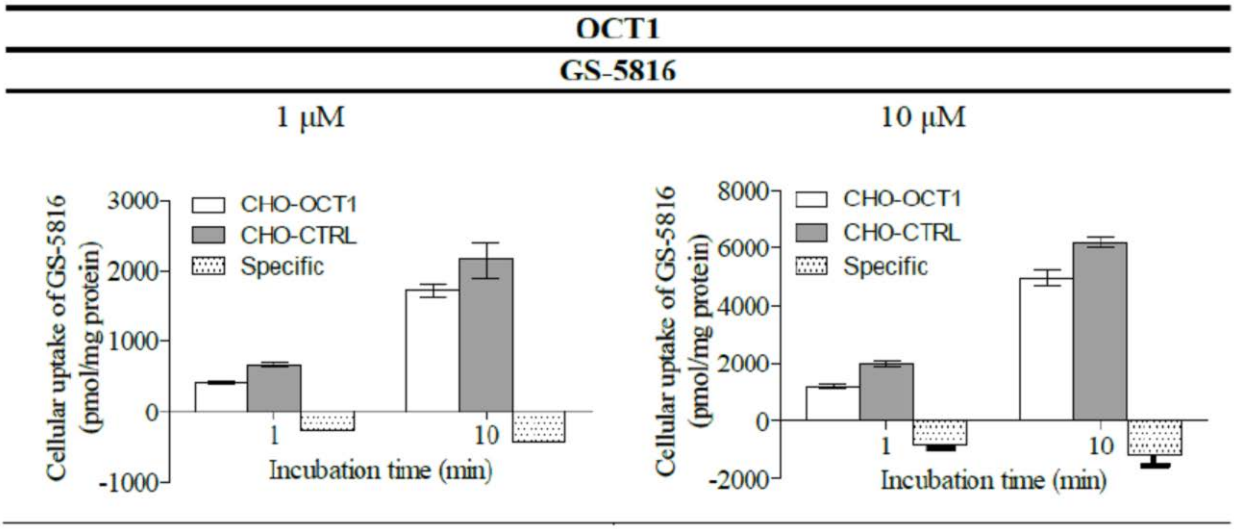
Uptake transporter inhibition		
Transporter	**Maximum inhibition at 4 µM (% of control)**	**IC_{50} (µM)**
OCT1	22%	> 4
OCT2	45%	> 4
OAT1	NA	> 4
OAT3	NA (37% stimulation)	> 4
MATE1	19%	> 4

Figure 1: GS-5816 Inhibition of OAT3-Mediated E3S Uptake Transport (source: Study Report Figure 6)



No transporter specific accumulation of GS-5816 into OCT1 transporter-expressing cells was observed under the current assay conditions (Figure 2).

Figure 2. Accumulation of GS-5816 in OCT1 Transporter Expressing and Control Cells in the Uptake Transporter Substrate Assay (source: Study Report Figure 6)



Conclusion
GS-5816 at 4 µM inhibited OCT1, OCT2 and MATE1 transporter activity by 22%, 45% and 19%, respectively. No inhibition of OAT1 or OAT3 was observed for GS-5816 at up to 4 µM. Stimulation of OAT3 (37%) was observed for GS-5816 at 4 µM. GS-5816 was not a substrate of OCT1.

AD-281-2012: GS-5816 inhibition potential for human MRP2, BSEP, and NTCP

Introduction
In this study, inhibition of transport via MRP2, BSEP, NTCP by GS-5885 was determined using cell membrane vesicles and cell lines transfected with the individual transporters.

Materials and Methods
GS-5816 was incubated with membrane vesicle preparations (total protein: 50 µg/well) and probe substrates, taurocholate (2 µM) for BSEP or E217βG (50 µM) for MRP2, in the absence or presence of ATP. The amount of probe substrates inside the filtered vesicles was determined by liquid scintillation after washing. Positive control used for MRP2 was benzbromarone (100 µM) and positive control used for BSEP was cyclosporine A (20 µM). To measure NTCP transporter inhibition, increasing concentrations of GS-5816 was incubated with Chinese hamster ovary (CHO) cells in the presence of probe substrates taurocholate (2 µM) for NTCP. Reference inhibitor taurochenodeoxycholate (TCDC) was used as a positive control

Results
GS-5816 inhibited human BSEP transporter with IC50 value of 0.64 µM. Based on an estimated unbound Cmax of <3 nM, GS-5816 is less likely to inhibit the hepatic efflux transporter BSEP in vivo. No inhibition was determined for MRP2 and NTCP up to the highest concentration of GS-5816 (40 µM). The MRP2-mediated E217βG transport increased slightly (by up to 30%) by GS-5816 at lower concentrations (up to 4 µM). The pharmacologic relevance of this finding is not clear. All positive control inhibitors inhibited the transport of probe substrates by ≥ 96 % in all tests.

Table 1: Effect of GS-5816 on Activity of Human BSEP, MRP2 and NTCP (source: Study Report Table 2)

Transporter	Probe substrate	IC_{50} (µM)	Maximum inhibition (%)
BSEP	Taurocholate	0.64	90
MRP2	$E_2$17βG	> 40	130 % activation at 1.48 µM
NTCP	Taurocholate	> 40	ND

E217βG: estradiol-17-beta-glucuronide
ND Not Determined

Conclusion
GS-5816 showed dose-dependent inhibition of human BSEP transporter with IC50 of 0.64 µM. Based on an estimated unbound Cmax of <3 nM, GS-5816 is less likely to inhibit the hepatic efflux transporter BSEP in vivo. No inhibition was determined for MRP2 and NTCP up to 40 µM (IC50 >40 µM). Up to 30% of activation of MRP2 was observed for GS-5816 at concentrations up to 4 µM. The pharmacologic relevance of this finding is not clear.

AD-334-2023: In vitro inhibition of human P-gp by high concentrations of SOF

Introduction
In this study, the inhibition of the ATP-Binding Cassette (ABC) efflux transporter P-glycoprotein (P-gp) by sofosbuvir (SOF) at concentrations up to 300 µM was determined *in vitro* using a cell line transfected with the individual transporter and a fluorescent model substrate.

Materials and Methods
SOF (tested at 6 concentrations ranging from 1.23 to 300 µM) or a positive control (verapamil) was incubated for 1 h with MDCKII cells in a buffer containing 10 µM Calcein AM in 96-well black cell culture plates with clear bottoms at a density of 5 x 104 cells/well. Following incubation and washing, the cells were lysed and each well was analyzed for calcein fluorescence at an excitation of 494 nm and an emission of 517 nm.

Results
SOF showed no inhibition of P-gp at up to the highest concentration tested (300 µM). The positive control verapamil had an IC50 value of 6.2 ± 2.5 µM.

Table 1. Inhibition of P-gp Mediated Transport of Calcein AM by Sofosbuvir and Verapamil (source: Study Report Table 1)

	Efflux Transporter IC_{50} (µM)
Test Compounds	P-gp
Sofosbuvir	>300
Verapamil	6.2 ± 2.3

Conclusion
SOF showed no inhibition of P-gp at up to the highest concentration tested (300 µM).

AD-334-2024: In vitro inhibition studies of P-gp, OCT1, OCT2, MATE1, OAT3, BSEP and MRP2 transporters by high concentrations of GS-331007

Introduction

In this study, the inhibition of P-gp, OCT1, OCT2, MATE1, OAT3, BSEP and MRP2 transporters by GS-331007 at concentrations up to 300 µM was determined *in vitro* using model substrates and transfected cell lines or membrane vesicles.

Materials and Methods

For P-gp assay, GS-331007 or a positive control (verapamil) was incubated for 1 h with MDCKII cells in a buffer containing 10 µM Calcein AM in 96-well black cell culture plates with clear bottoms at a density of 5 x 104 cells/well. Following incubation and washing, the cells were lysed and each well was analyzed for calcein fluorescence at an excitation of 494 nm and an emission of 517 nm. To measure OCT1, OCT2 and MATE1 transporter inhibition, increasing concentrations of GS-331007 was incubated with Chinese hamster ovary (CHO) cells in the presence of probe substrates triethylamine for OCT1, OCT2, and MATE1. For OAT3 inhibition, increasing concentrations of GS-331007 was incubated with FlpIn293 cells in the presence of a probe substrate (estrone-3-sulfate). Transporter specific accumulation of the probe substrate in the cells was measured. For BSEP and MRP2 assay, GS-331007 was incubated with membrane vesicle preparations (total protein: 50 µg/well) and probe substrates, taurocholate (2 µM) for BSEP or E217βG (50 µM) for MRP2, in the absence or presence of ATP. The amount of probe substrates inside the filtered vesicles was determined by liquid scintillation after washing.

Results

GS-331007 showed no inhibition of P-gp, OCT1, MATE1 and MRP2-mediated transport at test concentrations up to 300 µM. At 300 µM, GS-331007 inhibited OCT2-mediated transport of TEA, OAT3-mediated transport of E3S, and BSEP-mediated transport of taurocholate by 13%, 34% and 17%, respectively (IC50 values > 300 µM) (Figures 1-3). Based on the Cmax/IC50 values of much less than 0.1, the potential of GS-331007 inhibit OCT2 and OAT3 in vivo is low. The positive control for P-gp, verapamil, showed an average of 84% inhibition of P-gp transport at 40 µM. The positive controls for OCT1, OCT2, MATE1, OAT3, BSEP and MRP2 showed almost complete inhibition of transport of the specific transporters.

Figure 1. The Effect of GS-331007 on OCT2-Mediated TEA transport (source: Study Report Figure 4)

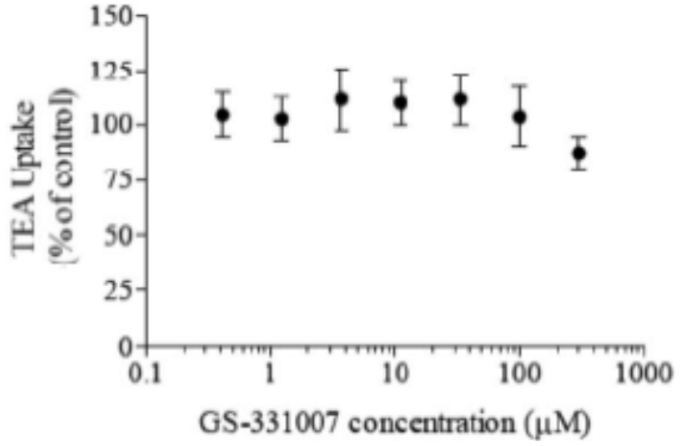


Figure 2. The Effect of GS-331007 on MATE3-Mediated TEA Transport (source: Study Report Figure 6)

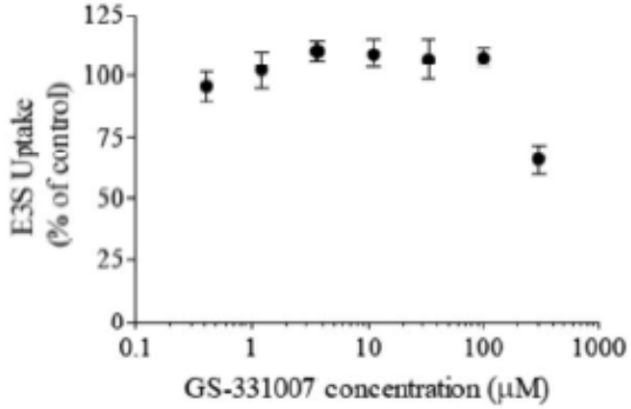
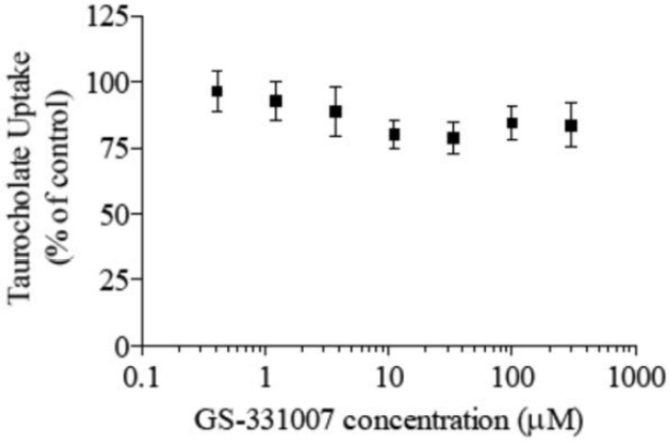


Figure 3. The Effect of GS-331007 on BSEP-Mediated Taurocholate Transport (source: Study Report Figure 7)



Conclusion

GS-331007 showed no inhibition of Pgp-, OCT1-, MATE1- and MRP2-mediated transport at test concentrations up to 300 μM. At 300 μM, GS-331007 inhibited OCT2-mediated transport of TEA, OAT3-mediated transport of E3S, and BSEP-mediated transport of taurocholate by 13%, 34% and 17%, respectively (IC50 values > 300 μM), which was not considered clinically significant.

AD-334-2025: Metabolism of Irinotecan following coincubation with Either Sofosbuvir or GS-331007 in primary human hepatocytes

Introduction

SOF is metabolized by CES1 to form an intermediate metabolite GS-566500. Further metabolism leads to form of GS-331007 and the active metabolite. Irinotecan is also hydrolyzed by CES1 to a putatively active metabolite, SN-38. Therefore, the potential drug-drug interactions between sofosbuvir and irinotecan were examined.

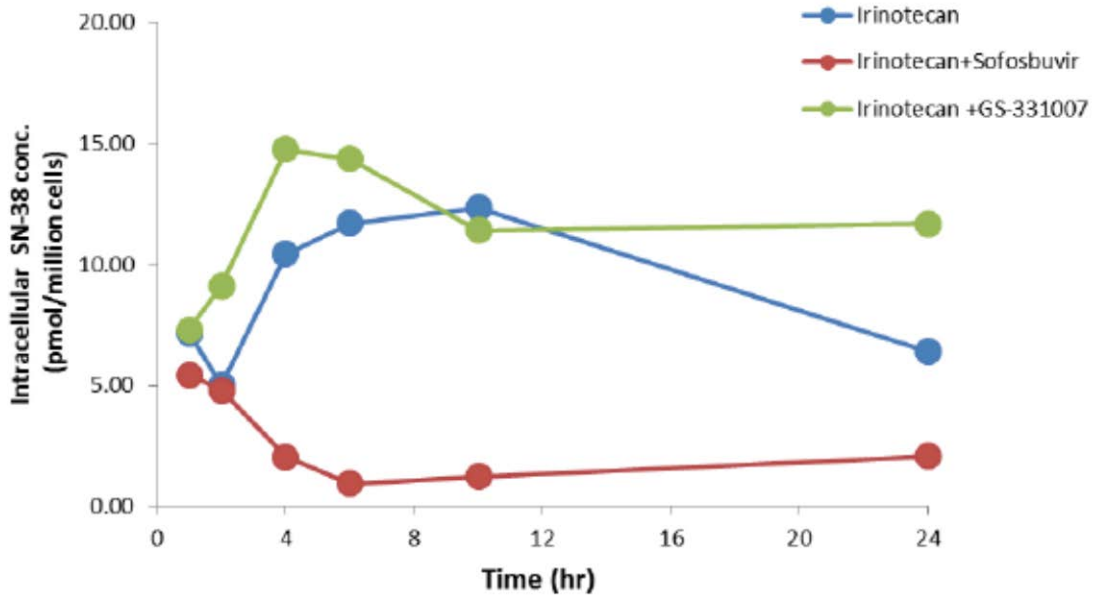
Materials and Methods

Primary human hepatocytes from 2 different donors were incubated with either 10 µM irinotecan alone or in combination with either 100 µM sofosbuvir or 300 µM GS-331007 for 24 hours to access the effect of sofosbuvir or GS-331007 on the metabolism of irinotecan in primary human hepatocytes. The intracellular metabolite SN-38 was extracted and analyzed by LC/MS/MS. The effect of irinotecan on the metabolism of sofosbuvir or GS-331007 was also examined in the primary human hepatocytes incubated with 100 µM sofosbuvir or 300 µM GS-331007 in the absence or the presence of 10 µM irinotecan. Levels of the triphosphate metabolite, GS-461203, was extracted and analyzed by LC/MS/MS.

Results

The SN-38 concentrations were lower with co-administration of sofosbuvir at 100 µM but were not affected by 300 µM GS-331007 (Figure 1). The intracellular levels of irinotecan and SN-38 ranged from 201 – 353 and 3.84-12.8 pmol/million cells, respectively; when incubated alone, indicating that irinotecan is inefficiently metabolized in primary human hepatocytes.

Figure 1. Intracellular SN-38 Formed During a 24 h Continuous Incubation with 10 µM Irinotecan Alone or in the Presence of 100 µM Sofosbuvir or 300 µM GS-331007 in Primary Human Hepatocytes (Mean of Duplicate Wells) (source: Study Report Figure 3)

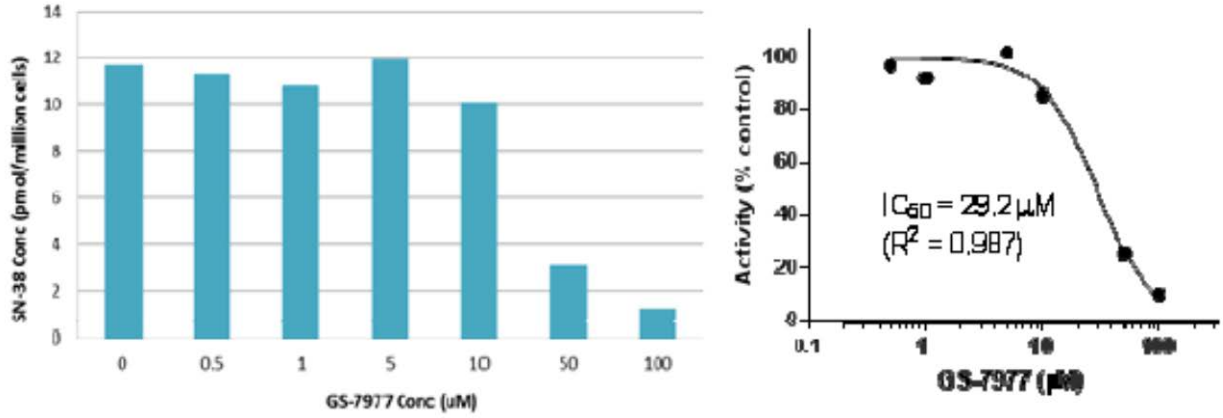


The effect of irinotecan on the metabolism of sofosbuvir or GS-331007 was also examined in the primary human hepatocytes incubated with 100 µM sofosbuvir or 300 µM GS-331007 in the absence or the presence of 10 µM irinotecan. Levels of the triphosphate metabolite, GS-461203, were unchanged with co-administration of irinotecan. Sofosbuvir was efficiently metabolized forming between 660 and 908 pmol/million after the 24 h incubation in the presence or absence of irinotecan. Levels of GS-461203 were also monitored during the incubation of 300 µM GS-331007. Consistent with its poor phosphorylation, incubation with GS-331007 only resulted in the formation of low levels of GS-461203 (approximately 13 pmol/million at 24 h).

In order to understand the dose-dependence of the effect of sofosbuvir on irinotecan, formation of SN-38 was assessed at a single time point of 4 h. A concentration-dependent decrease in

SN-38 formation was observed in hepatocytes in the presence of sofosbuvir at concentrations equal to or greater than 50 µM and a nonlinear regression analysis yielded an IC50 of 29.2 µM with a goodness of the fit (R2 value) of 0.987 (Figure 2). Formation of SN-38 was not affected upon co-administration with GS-331007 at concentrations up to 300 µM.

Figure 2. Intracellular SN-38 Formed During a 4 h Incubation with 10 µM Irinotecan Either Alone or in the Presence of Various Concentrations of Sofosbuvir in Primary Human Hepatocytes (Mean of Duplicate Wells) (source: Study Report Figure 6)



Conclusion

While GS-331007 had no effect on SN-38 formation (IC50 > 300 µM), sofosbuvir weakly inhibited the formation of SN-38 with an IC50 value of 29.2 µM. This concentration is greater than 30-fold the Cmax achieved in the clinic (~1 µM). No change in levels of sofosbuvir's active metabolite, GS-461203, was observed upon co-administration with 10 µM irinotecan. These results suggest clinically relevant drug-drug interactions between sofosbuvir and irinotecan are unlikely.

AD-334-2026: In vitro assessment of human hepatic microsomal cytochrome P450 mechanism-based inhibition potential of GS-7977 (Sofosbuvir)

Introduction

In a previous study (AD-334-2020) GS-7977 was shown to display undetectable or very weak inhibition of seven human CYP enzymes (IC50 ≥ 53.1 µM) and was also shown to display no potential for mechanism-based inhibition of human hepatic microsomal CYP3A. In this study, the potential for GS-7977 to be a mechanism-based inhibitor (to show preincubation time-dependent and NADPH cofactor-dependent changes in inhibitory potency) of the other six major human hepatic microsomal cytochrome P450 drug-metabolizing enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6) was assessed in vitro, using pooled human liver microsomal fraction.

Materials and Methods

GS-7977 (up to 50 µM) was incubated with pooled human liver microsomal fraction and NADPH in the presence of individual probe substrates for each CYP enzyme (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 or CYP2D6). All assays were designed so that conditions were linear with respect to time and protein concentration. Substrates were present at concentrations equal to or lower than their respective Km values. Positive control inhibitors were tested in parallel.

Results
GS-7977 showed no significant change in inhibitory potency against CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6. GS-7977 has also been shown previously to display no potential for mechanism-based inhibition of human CYP3A.The %Change values for the respective control inhibitors and GS-7977 are summarized in Table 1. Data for CYP3A from AD-334-2020 are provided for comparison. The positive control mechanism-based inhibitors incubated at 25 µM all achieved the expected NADPH cofactor-dependent and preincubation time-dependent changes in CYP inhibitory potency (%Change > 40%).

Table 1. %Change Values for Time- and Cofactor-Dependent Inhibition of Major Human Hepatic Microsomal CYP Enzymes by GS-7977 (source: Study Report Table 1)

CYP Enzyme	Probe Activity	Calculated %Change	
		Control Inhibitor[a]	GS-7977
CYP1A2	Ethoxyresorufin O-deethylase	67.1 ± 6.64 53.1 ± 1.52	-8.8 ± 3.41
CYP2B6	Bupropion 4-hydroxylase	76.5 ± 0.42	-0.9 ± 6.96
CYP2C8	Paclitaxel 6α-hydroxylase	48.4 ± 5.60	4.9 ± 0.72
CYP2C9	Diclofenac 4'-hydroxylase	79.2 ± 0.44	4.0 ± 1.46
CYP2C19	S-Mephenytoin 4'-hydroxylase	61.3 ± 1.03	2.2 ± 1.86
CYP2D6	Dextromethorphan O-demethylase	81.7 ± 0.62	3.4 ± 2.07
CYP3A[b]	Midazolam 1'-hydroxylase	61.8 ± 0.79 82.2 ± 0.18	8.4 ± 1.75

a. CYP1A2, furafylline and resveratrol; CYP2B6, ticlopidine; CYP2C8, gemfibrozil glucuronide; CYP2C9, tienilic acid; CYP2C19, ticlopidine; CYP2D6, paroxetine; CYP3A, mibefradril and mifepristone

b. Data from AD-334-2020

Conclusion
GS-7977 is unlikely to act as a mechanism-based inhibitor of human hepatic microsomal CYP450 enzymes.

4.1.6 Pharmacometric Review (Fang)

1 Summary of Findings

1.1 Key Review Questions

The purpose of this review is to address the following key questions.

1.1.1 What covariates significantly influence velpatasvir pharmacokinetics and are any dose adjustments necessary based on these changes?

Covariates identified to significantly influence velpatasvir oral clearance (CL/F) in the Applicant's population PK analysis for velpatasvir (GS-5816), were sex, disease status, and hepatic impairment. Females subjects had 30% lower CL/F than male subjects, Subjects with moderate or severe hepatic impairment (Child Pugh [CPT] B or C) had 35% higher CL/F than those with normal hepatic function or mild hepatic impairment (CPT-A). Healthy subjects had 38% lower CL/F than HCV infected subjects.

Covariates identified to significantly influence volume of distribution (Vc/F) were sex, disease status, and hepatic impairment. Female subjects had 26% lower Vc/F than male subjects.

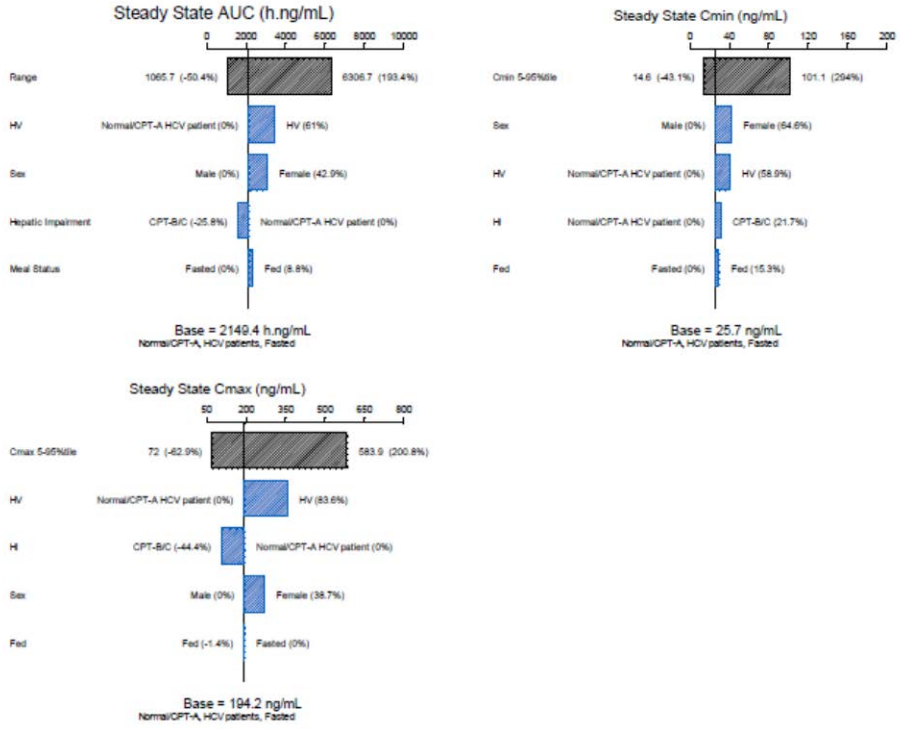
Food increased the bioavailability of velpatasvir by 9%. Food also decreased first-order absorption rate constant and increased lag time. Both of these effects result in delayed absorption. Other evaluated covariates, such as age, body weight, BMI, race (While vs. non-White), were not found to significantly effect velpatasvir pharmacokinetics or exposure. The effect of covariates on velpatasvir PK and exposure is summarized below in Table 1 and Figure 1

Table 1: Effect of Covariates on Key PK Velpatasvir Parameters

PK Parameters and Baseline Covariates	Estimate	Change from Typical (%)	Inter-Individual Variability
Typical CL (L/hr, Male, fasted, HCV infected subjects with normal hepatic function or CPT-A)	46.5	-	50.8%
Female	32.6	-30	
Hepatic impairment (CTP-B/C)	62.7	34.9	
HV	28.9	-37.9	
Typical Vc (L)	392	-	68.9%
Female	288	-26.4	
Hepatic impairment (CTP-B/C)	950	143	
HV	193	-50.7	
Typical Q (L/hr)	10.8	-	
Typical Vp (L)	219	-	50.8%
Typical ka (1/hr, fasted)	0.78	-	54.2%
Food	0.51	-34.7	
Typical lag time (hr, fasted)	0.295	-	
Food	0.466	57.8	
F1 (fasted)	1	-	
Food	1.09	8.76	
Residual variability as coefficient of variation (%)	56.7	-	

Source: Table A on page 13 of applicant's population PK report for velpatasvir

Figure 1: Effect of Covariates on Velpatasvir Steady-State Exposure (AUC, C_{min} and C_{max})



Source: Figure 12 on page 46 of applicant's population PK report for GS-5816

Of note, healthy volunteers were estimated to have 61% higher steady state AUC than HIV infected subjects, and female subjects had 43% higher steady state AUC than male subjects. Subjects with moderate and severe hepatic impairment (CPT-B/C) had 26% lower AUC than subjects with normal hepatic function. However, the difference in AUC caused by these factors is not clinically significant and no VEL dose adjustments are necessary based on these factors.

1.1.2 What covariates significantly influence sofosbuvir pharmacokinetics and are any dose adjustments necessary based on these changes?

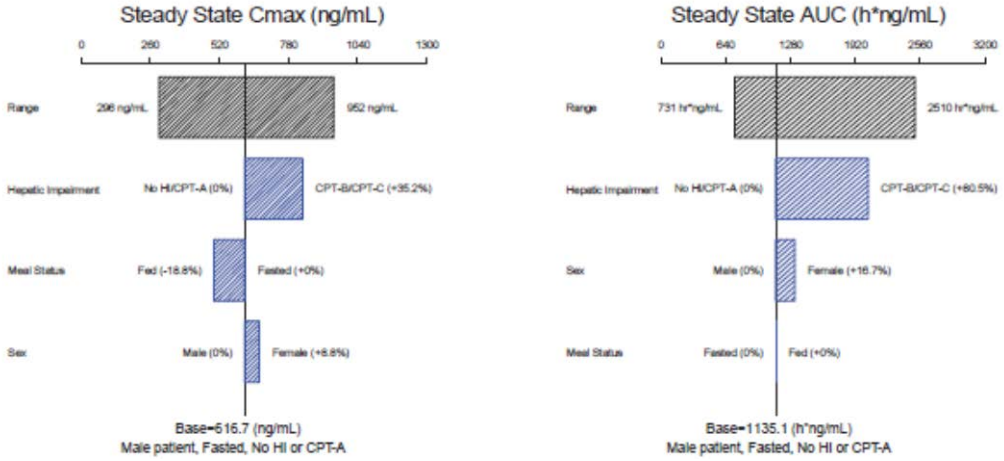
Covariates identified to have significant influence on sofosbuvir oral clearance (CL/F) in the Applicant's population PK analysis for sofosbuvir (GS-7977) after administration of SOF and velpatasvir (VEL) or SOF/VEL fixed-dose combination, were disease status and sex. Subjects with moderate and severe hepatic impairment (CPT-B/C) had a 44.6% lower CL/F than subjects with normal hepatic function. Female subjects were estimated to have 14% lower CL/F than male subjects. Food slowed drug absorption and decreased ka by 29.8%. The effect of covariates on sofosbuvir PK and exposure is summarized below in Table 2 and Figure 2.

Table 2: Effect of Covariates on Key SOF PK Parameters

PK Parameters and Baseline Covariates		Baseline Covariate Value	Estimate	Change from Typical (%)	Inter-individual Variability (%)
Typical CL (male, No HI/CPT-A, L/hr)			**352.4**	—	**48.18**
Hepatic Impairment	***CPT-B/CPT-C***		*195.2*	*-44.61*	—
Sex	*Female*		*302.0*	*-14.29*	
Typical Vc (L)			**197.2**	—	**94.94**
Typical k_a (fasted, hr^{-1})			**1.247**	—	**4.606**
Food	*Fed*		*0.875*	*-29.81*	—
Typical lag time (hr)			**0.0925**	—	—
Residual variability as coefficient of variation (%)		*HV*	**119.9**	—	—
		Patient	**108.8**		

Source: Table A on page 15 of applicant's population PK report for sofosbuvir

Figure 2: Effect of Covariates on VEL Steady-State Exposure (AUC, and C_{max})



Source: Figure B on page 16 of applicant's population PK report for sofosbuvir

As shown in Figure 2, female subjects were estimated to have 17% higher steady state AUC than male subjects. Subjects with moderate or severe hepatic impairment (CPT-B/C) were estimated to have 80% higher AUC than subjects with normal hepatic function. The difference in AUC caused by these factors did not translate into differences in efficacy (SVR12 rate). Therefore, the differences are not considered clinically significant, and no SOF dose adjustments are necessary based on these factors.

1.1.3 Is there any evidence of exposure-response relationship for efficacy?

There was no VEL dose-response relationship for efficacy in treatment-naïve subjects. As indicated in Phase 2 study GS-US-342-0102, the SVR12 rates after 12- week treatment with SOF 400 mg + VEL 100 mg (N =77) and SOF 400 mg + VEL 25 mg (N=76) were almost identical (Table 3). Both treatment arms showed high SVR12 rates of 96%. Despite a 4-fold VEL dose range, the 100 mg dose did not demonstrate greater efficacy than the 25 mg dose in the combination treatment.

Table 3: SVR12 Rate in Treatment-Naïve Subjects (GT 1-6) after 12-Week Combinational Treatment with SOF 400 mg + VEL 100 or 25 mg (Phase 2 Study GS-US-342-0102)

ARM*	Duration	Total	SVR12-Yes	SVR12-No	VEL AUCτ (h.ng/mL)	VEL Cmax (ng/mL)
SOF 400 mg + VEL 100 mg	12 weeks	77	74 (96.1%)	3 (3.90%)	2833.6 (N=9)	353.4 (N=9)
SOF 400 mg + VEL 25 mg	12 weeks	76	73 (96.05%)	3 (3.95%)	487.1 (N=15)	78.9 (N=15)

*Genotype 1 2 3 4 5 6 combined.

However, a dose-response relationship for efficacy was evident in treatment-experienced subjects with HCV genotype 3 infection. In study GS-US-342-0109, the SVR12 rate after 12-week treatment with SOF 400 mg + VEL 100 mg was 96.3% (77/80) while the SVR12 rate was only 81% (64/79) after treatment with SOF 400 mg + VEL 25 mg (Table 4). All subjects who failed treatment were infected with HCV genotype 3 (Table 5), suggesting that a higher dose is needed in treatment-experienced genotype 3 subjects.

Table 4: SVR12 Rate in Treatment-Experienced Subject after 12-Week Combinational Treatment with SOF 400 mg + VEL 100 or 25 mg (Phase 2 Study GS-US-342-0109)

ARM*	Duration	Total	SVR12-Yes	SVR12-No	VEL AUCτ (h.ng/mL)	VEL Cmax (ng/mL)
SOF 400 mg + VEL 100 mg	12 weeks	80	77 (96.3%)	3 (3.7%)	2053.4 (N=11)	285.0 (N=11)
SOF 400 mg + VEL 25 mg	12 weeks	79	64 (81.0%)	15 (19.0%)	478.8 (N=5)	71.6 (N=5)

*Genotype 1 and 3 combined.

Table 5: SVR12 Rate in Treatment-Experienced Subject after 12-Week Combinational Treatment with SOF 400 mg + VEL 100 or 25 mg Stratified by Genotype (Phase 2 Study: GS-US-342-0109)

ARM*	Genotype	Total	SVR12-Yes	SVR12-No	VEL AUCτ (h.ng/mL)	VEL Cmax (ng/mL)
SOF 400 mg + VEL 100 mg 12 weeks	genotype 1	27	27(100%)	0(0%)	2053.4 (N=11)	285.0 (N=11)
	genotype 3	53	50(94.3%)	3(5.7%)		
SOF 400 mg + VEL 25 mg 12 weeks	genotype 1	27	27(100%)	0(0%)	478.8 (N=5)	71.6 (N=5)
	genotype 3	52	37(71.1%)	15(28.9%)		

An information request was sent to the Applicant requesting evaluation of the exposure-response relationship between VEL exposure and SVR12 in patients infected with HCV genotype 3. However, the Applicant replied that such an analysis is not feasible as sparse samples from the 25 mg VEL treatment arms were not analyzed. As the applicant is seeking

approval of VEL 100 mg as part of the fixed-dose combination (rather than VEL 25 mg), the lack of PK information for VEL 25 mg is not considered as an issue for approval of the fixed-dose combination of SOF 400 mg/VEL 100 mg.

1.2 Recommendations

The Division of Pharmacometrics in the Office of Clinical Pharmacology has reviewed data and analysis submitted in this application and recommends approval of sofosbuvir /velpatasvir (400 mg/100 mg) fixed dose combination once daily for 12 weeks for the treatment of chronic HCV infection genotypes 1, 2, 3, 4, 5, and 6. The reviewer agrees with the Applicant's conclusions from the population PK analyses for sofosbuvir and velpatasvir.

2. PERTINENT REGULATORY BACKGROUND

Gilead Sciences Inc. submitted this application to seek marketing approval for sofosbuvir/velpatasvir (400 mg/100 mg) as an oral fixed-dose combination (FDC) tablet for the treatment of chronic hepatitis C virus (HCV) infection in adults. Sofosbuvir is a nucleotide analog nonstructural protein 5B (NS5B) polymerase inhibitor previously approved by FDA for use in combination with other agents for the treatment chronic HCV infection in adults. Velpatasvir (VEL, GS-5816) is a novel HCV nonstructural protein 5A (NS5A) inhibitor that is being developed in combination with SOF (b) (4) for the treatment of HCV infection.

In this application, Gilead submitted four Phase 3 clinical studies (GS-US-342-1138 (ASTRAL-1), GS-US-342-1139 (ASTRAL-2), GS-US-342-1140 (ASTRAL-3), and GS-US-342-1137 (ASTRAL-4) to evaluate the efficacy and safety of SOF/VEL in a range of HCV-infected subject populations, including treatment-naïve and treatment-experienced, and those with and without cirrhosis. In addition, the sponsor submitted three population PK reports to characterize the PK profile of sofosbuvir, velpatasvir, as well as GS-331007 (main sofosbuvir metabolite) and evaluate covariates that may significantly influence PK and exposure of those compounds.

The population PK of sofosbuvir has been extensively reviewed by the Agency in previous submissions. In this review, the pharmacometrics reviewer focused on reviewing the data and population PK analysis for velpatasvir, sofosbuvir, as well as GS331007. The dose-response analysis of velpatasvir for efficacy was also explored with data from two Phase 2 studies where the PK and efficacy after multiple doses of velpatasvir were evaluated.

3 RESULTS OF SPONSOR'S ANALYSIS

3.1 Population PK Analysis: Velpatasvir

The applicant performed population pharmacokinetic analysis of GS-5816 (velpatasvir) using the data collected from 11 clinical studies.

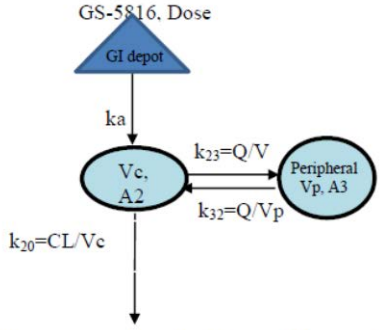
Objectives: the objective of the analysis was to develop a model to characterize velpatasvir PK and the effects of demographic, pathophysiologic, and HCV disease-related covariates on velpatasvir PK parameters. Model predicted individual velpatasvir exposure was also provided for additional exposure-response analysis for safety and efficacy of velpatasvir.

Data: Data for population PK analysis were pooled from 11 clinical studies (GS-US-342-0102, GS-US-342-0104, GS-US-342-0109, GS-US-337-0122, GS-US-342-1137, GS-US-342-1138, GS-US-342-1139, GS-US-342-1140 GS-US-342-1167, GS-US-342-1326, and GS-US-342-1346). A total of 18,459 data points from 2022 subjects were included in the model development dataset.

Population PK Model Development

Base Model: The selected base model that best described the velpatasvir data was a two-compartment model with first-order absorption, first-order elimination from the central compartment, and a lag time. The PK model was parameterized with clearance (CL), central volume (V_c), inter-compartment clearance (Q), peripheral volume (V_p) absorption rate constant (Ka) and a lag time (T_{lag}). The impact of food on Ka and T_{lag} were also included in the base model. The scheme of the model is depicted in Figure 3.

Figure 3: Two-compartment PK Model with First Order Absorption



Ka = first-order absorption rate
K_{20} = rate constant for the linear elimination
K_{23} = rate constant from central to peripheral compartment
K_{32} = rate constant from peripheral to central compartment
CL = linear elimination clearance
Q = distribution clearance
V_c = central volume
V_p = peripheral volume

The system was described by the following first-order differential equations:

$$\frac{dA_2}{dt} = ka \cdot A_1 - (k_{20} + k_{23}) \cdot A_2 + k_{32} \cdot A_3$$

$$\frac{dA_3}{dt} = k_{23} \cdot A_2 - k_{32} \cdot A_3$$

Final Model

Full population PK Model was constructed via forward inclusion of covariates of interest followed by a reduction step removing covariates using a stepwise backward elimination method. The criterion for retention was a change in likelihood ratio > 10.98 for 1 parameter ($p < 0.001$). The final model includes the following parameter-covariate relationships:

$$CL_i = exp(\theta_1 + \theta_{11} \cdot SEXF + \theta_{12} \cdot (CPT - B/C) + \theta_{13} \cdot HV)$$

$$Vc_i = exp(\theta_2 + \theta_{14} \cdot SEXF + \theta_9 \cdot (CPT - B/C) + \theta_{10} \cdot HV)$$

$$K_a = \exp(\theta_5 + \theta_7 \cdot Food)$$

$$Tlag = \exp(\theta_6 + \theta_8 \cdot Food)$$

$$F_1 = \exp(\theta_{15} \cdot Food)$$

The parameter estimates of the final model were summarized in the Table 6. Goodness-of-fit plots and visual predictive check (VPC) plots are shown in Figure 4 and Figure 5, respectively.

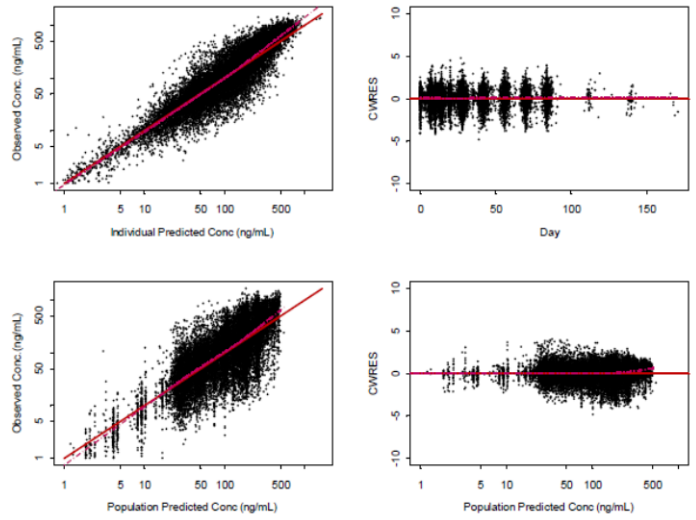
Table 6: Parameter Estimates for Velpatasvir Final Model and Bootstrap Results

Parameter	Parameter Description	Final model estimate [95% CI]	Bootstrap estimate Median [2.5 - 97.5%tile]
$\exp(\theta_1)$	Apparent oral clearance CL (L/hr)	46.5 [44.2, 48.3]	44.9 [42.9, 49]
$\exp(\theta_2)$	Apparent central volume, Vc (L)	392 [359, 417]	379 [343, 420]
$\exp(\theta_3)$	Inter-compartment clearance (L/hr)	10.8 [9.64, 11.8]	10.5 [9.24, 11.9]
$\exp(\theta_4)$	Peripheral volume (L)	219 [194, 240]	213 [190, 246]
$\exp(\theta_5)$	Absorption rate constant Ka (1/hr)	0.78 [0.704, 0.842]	0.772 [0.689, 0.857]
$\exp(\theta_6)$	Absorption lag time (hr),	0.295 [0.294, 0.296]	0.294 [0.292, 0.295]
θ_7	Food on ka	-0.426 [-0.529, -0.351]	-0.404 [-0.523, -0.29]
θ_8	Food on Tlag	0.456 [0.438, 0.47]	0.456 [0.434, 0.474]
θ_9	HI (CPT-B/C) on Vc/F	0.886 [0.746, 0.989]	0.882 [0.744, 1.02]
θ_{10}	HV on Vc/F	-0.707 [-0.799, -0.639]	-0.71 [-0.807, -0.612]
θ_{11}	SEXF on CL	-0.357 [-0.408, -0.319]	-0.356 [-0.41, -0.306]
θ_{12}	HI (CPT-B/C) on CL/F	0.299 [0.214, 0.361]	0.294 [0.209, 0.381]
θ_{13}	HV on CL/F	-0.476 [-0.532, -0.435]	-0.476 [-0.535, -0.421]
θ_{14}	SEXF on Vc/F	-0.306 [-0.394, -0.241]	-0.308 [-0.397, -0.22]
$\exp(\theta_{15})$	F1 with Food	1.09 [1.04, 1.13]	1.01 [1, 1.12]
$\omega^2_{CL/F}$	IIV of CL/F	0.258 [0.234, 0.276]	0.259 [0.236, 0.285]
$\omega^2_{CL/F,Vc/F}$	Interaction of CL/F and Vc/F	0.319 [0.285, 0.344]	0.32 [0.289, 0.357]
$\omega^2_{Vc/F}$	IIV of Vc/F	0.475 [0.41, 0.523]	0.473 [0.409, 0.545]
$\omega^2_{Vp/F}$	IIV of Vp/F	0.258 [0.0889, 0.382]	0.255 [0.0956, 0.447]
ω^2_{ka}	IIV of Ka	0.294 [0.207, 0.358]	0.3 [0.222, 0.454]
σ^2	Residual	0.321 [0.306, 0.332]	0.321 [0.308, 0.336]

Note: Bootstrap results were summarized from 990 out of 1000 successful runs.

Source: Table 9 on page 37 of applicant's population PK report for velpatasvir

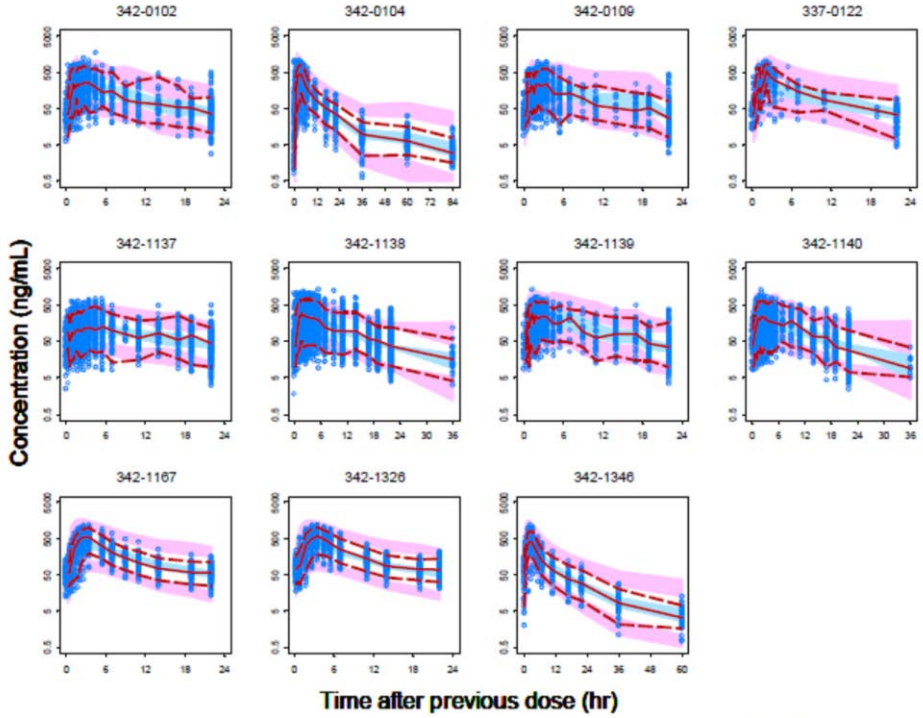
Figure 4: Goodness-of-Fit Plots for the Final Model of Velpatasvir



Top panel: Observed versus individual predicted concentrations (left) and CWRES versus time (right) for the final PopPK model. Bottom panel: Observed versus population predicted concentrations (left), and CWRES versus population predicted concentrations (right) for the final PopPK model.

Source: Figure 5 on page 38 of applicant's population PK report for velpatasvir

Figure 5: pcVPC of Velpatasvir Plasma Concentration-Time Profiles Stratified by Study



Circles are observed GS-5816 plasma concentrations, solid red lines represent the median observed value, and dashed lines represent 5%ile and 95%iles of the observed values. Blue shaded areas represent the spread of the median predicted values (5th to 95th %ile), and red shaded areas represent the spread (5%ile and 95%ile) of the 5th and 95th predicted percentile concentrations.

Source: Figure 11 on page 43 of applicant's population PK report for velpatasvir

Reviewer's Comment: The applicant's PK parameter estimates for velpatasvir appear reasonable. The observed velpatasvir concentrations were generally captured by the final population PK model. The predictive performance of the model as indicated in the pcVPC is acceptable.

However, the goodness-of-fit plots may not have been adequately described, especially for subjects with sparse samples, either due to data quality or the overall model/covariate structure. Independent analysis from the reviewer to verify the applicant's final model resulted in the same conclusion.

In addition, the applicant's analysis did not include data from velpatasvir 25 mg dose. All records related to velpatasvir 25 mg were either not available or excluded from model building dataset. As a result, only velpatasvir 100 mg PK data were in the final population PK dataset. This model should only be considered as fitting concentrations from the 100 mg dose of velpatasvir.

3.2 Population PK Analysis: Sofosbuvir

The applicant performed population pharmacokinetic analysis of sofosbuvir using the data collected from 11 clinical studies. The studies included treatments with VEL (100 mg or 25 mg) in combination with SOF 400 mg, or SOF/VEL (400 mg/100 mg) FDC treatment.

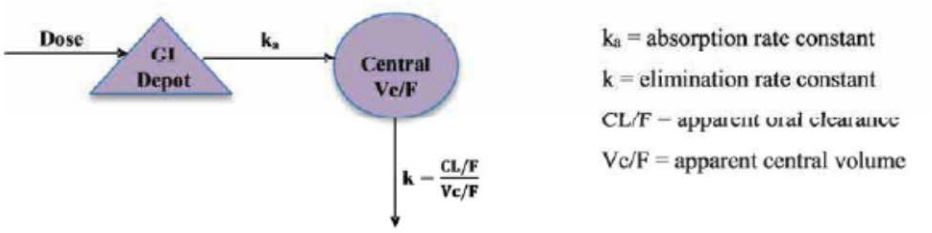
Objectives: The objective of the analysis was to develop a model to characterize sofosbuvir 400 mg pharmacokinetics in the presence of velpatasvir, and the effects of demographic, pathophysiologic, and HCV disease-related covariates on sofosbuvir PK parameters. Model predicted individual sofosbuvir exposure was also provided for additional exposure-response analysis for safety and efficacy.

Data: Data for population PK analysis were pooled from 11 clinical studies (GS-US-342-0102, GS-US-342-0104, GS-US-342-0109, GS-US-337-0122, GS-US-342-1137, GS-US-342-1138, GS-US-342-1139, GS-US-342-1140 GS-US-342-1167, GS-US-342-1326, and GS-US-342-1346). A total of 8507 data points from 1519 subjects were included in the model development dataset.

Population PK Model Development

Base Model: The selected base model that best described the sofosbuvir data was a one-compartment model with first-order absorption. The PK model was parameterized with clearance (CL), volume of distribution (V),) absorption rate constant (Ka) and a lag time (T_{lag}). The impact of food on Ka and T_{lag} were also included in the base model. The scheme of the model is depicted in Figure 6.

Figure 6: One-compartment PK Model with First Order Absorption



Final Model

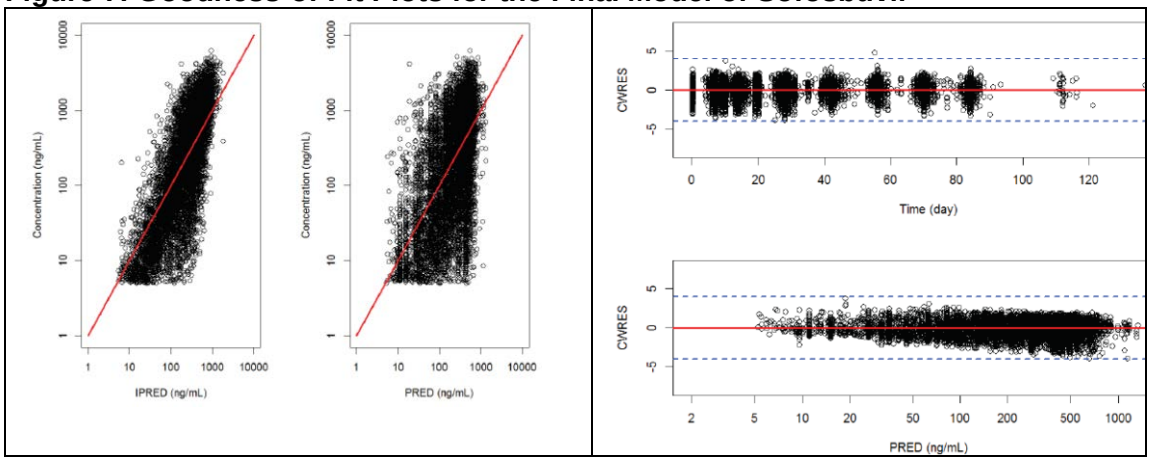
The full population PK Model was constructed via forward inclusion of covariates of interest followed by a reduction step removing covariates using a stepwise backward elimination method. The criterion for retention was a change in likelihood ratio > 10.98 for 1 parameter ($p< 0.001$). Assessed covariates included baseline demographic covariates (age, body weight, BMI, sex, ethnicity, and race), pathophysiological covariates (CLCR, EGFR, HI, CIRR, IL28B, HCV genotype, disease status, and concomitant medications), RBV usage, and food.
The parameter estimates of the final model were summarized below in the Table 7. The model goodness-of-fit plots and VPC plots are shown in Figure 7 and Figure 8.

Table 7: Population PK Parameters for Sofosbuvir (Final PopPK Model)

Parameter	Parameter Description	Final PopPK Model Estimated (2.5th, 97.5th Percentiles)	Bootstrap Final Model Median (2.5th, 97.5th Percentiles)
$exp(\theta_1)$	Apparent oral clearance, CL/F (L/hr)	352.4 (335.7, 369.9)	351.4 (334.9, 369.8)
θ_5	Influence of Sex on CL/F	-0.1542 (-0.2234, -0.0851)	-0.1554 (-0.2256, -0.0894)
θ_7	Influence of HI on CL/F	-0.5907 (-0.688, -0.4934)	-0.5864 (-0.6932, -0.4958)
$exp(\theta_2)$	Apparent central volume, Vc/F (L)	197.2 (182.1, 213.5)	197.4 (180.2, 216.3)
$exp(\theta_3)$	Absorption rate constant, Ka (1/hr, fasted)	1.247 (1.184, 1.313)	1.251 (1.190, 1.331)
θ_6	Influence of Food on Ka	-0.3540 (-0.4214, -0.2865)	-0.3565 (-0.4292, -0.2885)
$exp(\theta_4)$	Lag time (hr)	0.0925 (0.0874, 0.0979)	0.0927 (0.0783, 0.0974)
Inter-individual variability (%)	CL/F	48.18 (43.02, 52.83)	48.10 (42.70, 52.73)
	Vc/F	94.94 (84.92, 104.0)	94.99 (85.40, 104.8)
	Ka	4.605 (0, 11.39)	5.037 (0.0495, 13.09)
$\omega^2_{CL/F,Vc/F}$	Covariance between CL/F and Vc/F	0.1383 (0.0714, 0.2051)	0.1415 (0.0714, 0.2146)
σ^2	Residual error (%, HV)	119.9 (115.7, 123.9)	119.7 (115.2, 123.4)
	Residual error (%, Patient)	108.8 (105.6, 111.8)	108.7 (105.7, 111.8)

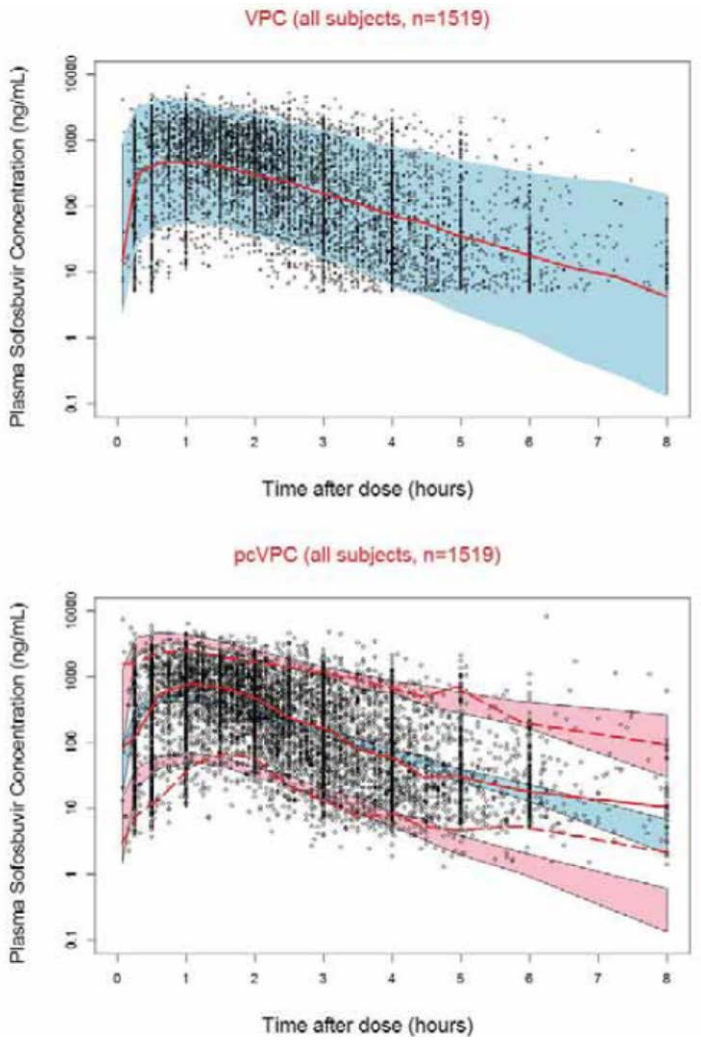
Source: Table 7 on page 36 of applicant's population PK report for sofosbuvir

Figure 7: Goodness-of-Fit Plots for the Final Model of Sofosbuvir



Source: Adapted from Figure 5 and Figure 6 on page 37-38 of applicant's population PK report for sofosbuvir

Figure 8: **VPC and pcVPC of Sofosbuvir Plasma Concentration-Time Profiles For Once Daily of SOF/VEL (400 mg/ 100 mg) FDC**



VPC: Points are the observed plasma SOF concentrations. The red lines are the median of the predicted concentrations by the final PopPK model (1000 trials). The blue shaded areas are the spread (5th to 95th percentile) of the predicted concentrations. pcVPC: The solid red line represents the median observed plasma concentration, and the semitransparent blue field represents a simulation-based 95% confidence interval for the median. The observed 5% and 95% percentiles are presented with dashed red lines, and the 95% confidence intervals for the corresponding model predicted percentiles are shown as semitransparent pink fields. The observed plasma concentrations (prediction corrected in the pcVPC) are represented by black circles.

Source: Figure 11 on page 43 of applicant's population PK report for sofosbuvir

Reviewer's Comment: The model characterized the PK profile of a single dose of sofosbuvir (400 mg) in the presence of velpatasvir (100 mg).The population PK parameter estimates appear reasonable. The inter-individual variability was modest (48%) for clearance (CL/F) but large (95%) for the central volume compartment (Vc/F) suggesting that the central volume may not be reliably estimated from available data. Identified covariates that significantly influence sofosbuvir exposure include hepatic impairment and sex. Moderate and severe hepatic impairment (CPT-B/C) was estimated to increase sofosbuvir steady AUC by about 80%, and female subjects were estimated to increase steady state AUC by 18%. This magnitude of change in AUC is not considered as clinically significant, and no dose adjustments are necessary based on hepatic function or sex.

However, while the final model generally captured the observed data, the fitting of the data as shown in the goodness-of-fit plots is poor, either due to data quality or the model/covariate structure.

3.3 Population PK Analysis: GS-331007

The applicant performed population pharmacokinetic analysis of GS-331007 (sofosbuvir metabolite) using the data collected from 11 clinical studies. The studies included treatments with VEL (100 mg or 25 mg) in combination with SOF 400 mg, or SOF/VEL (400 mg/100 mg) FDC treatment.

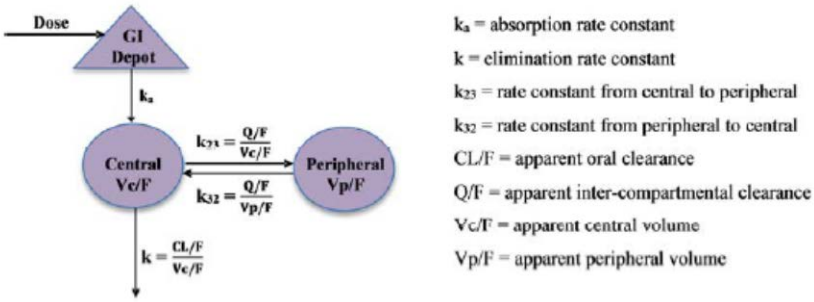
Objectives: The objective of the analysis was to develop a model to characterize GS-331007 pharmacokinetics following the combination treatment with sofosbuvir (400 mg) and velpatasvir (100 mg). The effects of demographic, pathophysiologic, and HCV disease-related covariates on GS-331007 PK parameters were also explored. Model predicted individual sofosbuvir exposure was generated for additional exposure-response analysis for safety and efficacy.

Data: Data for population PK analysis were pooled from 11 clinical studies (GS-US-342-0102, GS-US-342-0104, GS-US-342-0109, GS-US-337-0122, GS-US-342-1137, GS-US-342-1138, GS-US-342-1139, GS-US-342-1140 GS-US-342-1167, GS-US-342-1326, and GS-US-342-1346). A total of 18515 samples from 2025 subjects were included in the model development dataset.

Population PK Model Development

Base Model: The selected base model that best described the GS-331007 data was a two-compartment model with first-order absorption, first order elimination from the central compartment and a lag time, as illustrated in Figure 9. The PK model was parameterized in apparent clearance (CL/F), volume of distribution (Vc/F), apparent inter-compartment clearance (Q/F), apparent peripheral volume (Vp/F), and absorption rate constant K_a.

Figure 9: Two-compartment Model Describing Plasma GS-331007 Concentration Time Course Data Following an Oral Dose



Final Model

The full population PK Model was constructed via forward inclusion of covariates of interest followed by a reduction step removing covariates using a stepwise backward elimination method. The criterion for retention was a change in likelihood ratio > 10.98 for 1 parameter ($p< 0.001$). Assessed covariates included baseline demographic covariates (age, body weight, sex,

ethnicity, and race), pathophysiological covariates (CLCR, EGFR,CIRR, IL28B, HCV genotype, disease status, and concomitant medications (anti-coagulants, SSRIs, statins, calcium channel blockers, H2RAs, and diuretics), RBV usage, and food. The final model only contained covariates that met the pre-defined statistical criteria.

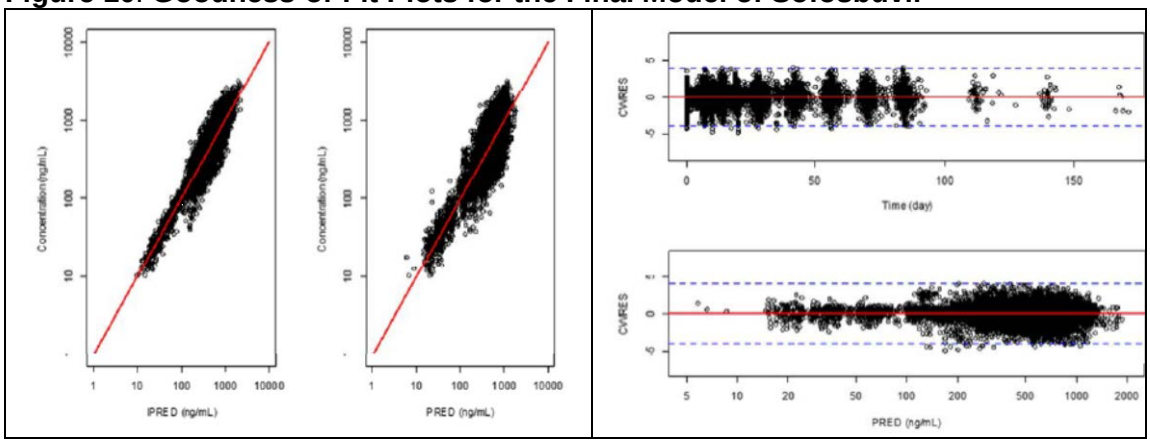
The parameter estimates of the final model were summarized below in the Table 8. The model goodness-of-fit plots and VPC plots are shown in Figure 10 and Figure 11.

Table 8: **Population PK Parameters for GS-331007 (Final PopPK Model)**

Parameter	Parameter Description	Final PopPK Model Estimated	Bootstrap Final Model Median (2.5th, 97.5th Percentiles)
$exp(\theta_1)$	Apparent oral clearance, CL/F (L/hr)	30.26	30.28 (29.56, 31)
θ_7	Influence of CLCR on CL	0.3864	0.3662 (0.3238, 0.4169)
θ_8	Influence of HV on CL	0.05562	0.08475 (0.04059, 0.1244)
θ_9	Influence of TE patient on CL	0.05571	0.05671 (0.02759, 0.08311)
θ_{10}	Influence of sex on CL	-0.1683	-0.1704 (-0.1957, -0.1459)
θ_{13}	Influence of RBV on CL	0.2058	0.2069 (0.1635, 0.2448)
θ_{15}	Influence of CMSTAT on CL	-0.1204	-0.1203 (-0.1911, -0.05339)
θ_{16}	Influence of ETH on CL	0.07785	0.0725 (0.03411, 0.1149)
θ_{17}	Influence of race on CL	0.09321	0.09445 (0.06405, 0.1276)
$exp(\theta_2)$	Apparent central volume, Vc/F (L)	320	319.8 (299.2, 342)
θ_6	Influence of weight on Vc	0.4716	0.4594 (0.2705, 0.6364)
θ_{11}	Influence of sex on Vc	-0.1978	-0.1988 (-0.275, -0.1234)
θ_{14}	Influence of RBV on Vc	0.377	0.3764 (0.2784, 0.4882)
$exp(\theta_3)$	Apparent inter-compartmental clearance, Q/F (L/hr)	40.89	40.82 (39.1, 42.84)
$exp(\theta_4)$	Apparent peripheral volume, Vp/F (L)	693.6	695.4 (668.2, 720)
$exp(\theta_5)$	Absorption rate constant, Ka (1/hr)	0.3729	0.3734 (0.3505, 0.3982)
θ_{12}	Influence of food on Ka	-0.4766	-0.4765 (-0.5529, -0.4029)
Inter-individual variability (%)	CL/F	23.9	23.88 (22.85, 25)
	Vc/F	44.25	44.16 (39.62, 48.29)
	Q/F	31.53	31.3 (26.01, 36.87)
	Vp/F	16.77	16.49 (9.423, 22.53)
	Ka	25.32	25.65 (18.74, 32)
$\omega^2_{CL/F,Vc/F}$	Covariance between CL/F and Vc/F	0.04544	0.04581 (0.03585, 0.05571)
σ^2	Residual error (%)	27.32	27.3 (26.72, 27.86)

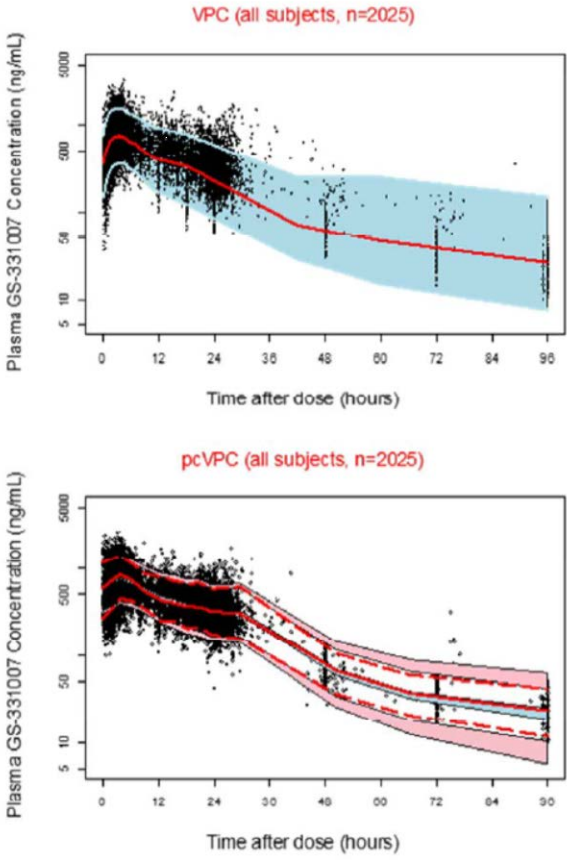
Source: Table 7 on page 36 of applicant's population PK report for GS-331007

Figure 10: **Goodness-of-Fit Plots for the Final Model of Sofosbuvir**



Source: Adapted from Figure 5 and Figure 6 on page 40 of applicant's population PK report for GS-331007

Figure 11: **VPC and pcVPC of GS-331007 Plasma Concentration-Time Profiles For Once Daily of SOF/VEL (400 mg/ 100 mg) FDC**



Source: Figure 11 on page 45 of applicant's population PK report for GS-331007

Effect of Covariates

The Effect of covariates on GS-331007 PK parameters are summarized in Table 9. The sensitive plot comparing the effect of covariates on GS-331007 exposure is shown in Figure 12 Disease status, ETH, race, and stain useage were identified as statistically significant covariates. A 10% decrease in CRCL resulted in a 4.0% drop in CL/F to 29.05 L/hr. Females were 15.5% lower in CL and 17.9% lower in Vc compared to males. Patients with RBV use was 22.8% higher in CL and 45.5% higher in Vc. However, this magnitude of change is not considered as clinically significant.

Table 9: **Effect of Covariates on GS-331007 PK Parameters**

PK Parameters and Baseline Covariates	Baseline Covariate Value	Estimate	Change from Typical (%)	Inter-individual Variability (%)
Typical CL (male, TN patient, L/hr)		**30.26**	—	**23.90**
Disease status	*HV*	31.99	5.72	—
	TE patient	31.99	5.73	—
CLCR (mL/min)	*5%tile*	24.90	-17.72	—
	95%tile	35.31	16.68	—
Sex	*Female*	25.57	-15.49	—
RBV	*Yes*	37.17	22.85	—
Statin	*Yes*	26.83	-11.34	—
Ethnicity	*hispanic/latino*	32.71	8.10	—
Race	*Non-White*	33.22	9.77	—
Typical Vc (L)		**320.0**	—	**44.25**
Body weight (kg)	*5%tile*	270.5	-15.48	—
	95%tile	376.6	17.69	—
Sex	*Female*	262.6	-17.94	—
RBV	*Yes*	466.6	45.80	—
Typical k_a (fasted, hr^{-1})		**0.373**	—	**25.32**
Food	*Fed*	*0.232*	*-37.91*	—

Figure 12: **Sensitive Plot Comparing the Effect of Covariates on GS-331007 Steady State Exposure**

Reviewer's Comment: The applicant's population PK analysis for GS-331007 was acceptable. The model adequately characterized the PK profile of GS-331007 after sofosbuvir (400 mg) /velpatasvir (100 mg). The inter-individual variability expressed as CV% was modest for clearance (24%) and the central volume compartment (44%)) suggesting that key PK parameter were reasonably estimated from available data. The population PK of GS-331007 in this application is not very much different from previous analysis (see Clinical Pharmacology Review 2014 for Ledipasvir/Sofosbuvir, NDA205834). No new findings or conclusions were derived from the analysis.

4. Reviewer's Analysis

4.1 Introduction

The population PK analysis for VEL is new in this application while population PK models for SOF and GS-331007 were developed and assessed in previous submissions. Therefore, it is of primary interest to know the adequacy of the model for VEL in describing the observed data and the effects of covariates of interest (such as age, race, and body weight) on VEL PK.

As such, the pharmacometrics reviewer performed independent analysis to verify the sponsor's analysis. The primary objective was to evaluate whether the results from VEL population PK analysis will support the applicant's labeling claims.

4.2 Objectives

Analysis objectives are to:

1. Evaluate the adequacy of the applicant's final model in describing the observed VEL concentrations for the proposed dosing regimen.
2. Evaluate the effect of covariates of interest, such as, age, weight, race, and other factors, on VEL exposure (e.g., steady state AUC).

4.3 Methods

Data sets used are summarized in Table 10.

Table 10: Analysis Data Sets

Study Number	Name	Link to EDR
Report Number 15-0001	vel.xpt	\\cdsesub1\evsprod\NDA208341\0001\m5\datasets\pop-pk-vel\analysis\legacy\datasets

4.3.1 Software

NONMEM (Version 7.2) installed on a 48-core Linux cluster was used for the population PK analysis. An R package "popPK" developed by FDA was used for population PK graphing and reporting; SAS for windows 9.4 was used for all other graphing and statistical analyses.

4.3.2 Models

The applicant's population PK dataset and final model (run109) were used for testing the adequacy of the submitted final model and estimating PK parameters. The dataset name and its location are summarized in Table 10.

4.4 Results

4.4.1 Population PK Analysis

The reviewer conducted population PK analysis with the applicant's models. The results of the applicant's population PK analysis were replicated by the reviewer. The PK parameter estimates from the reviewer's model were similar to those of the applicant's analysis.

4.4.2 Goodness-of-fit plots for the final model

The final model was evaluated by assessing the goodness-of-fit plots as shown in Figure 13, which shows the population prediction and individual prediction versus observed VEL concentrations for all subjects. The individual predictions versus observed concentrations stratified by study are shown in Figure 14. As shown, the fittings are good across all studies, suggesting that the observations were generally captured, though there is evidence of bias in the estimation at low (model over predicts) and high (model under predicts) concentrations.

Figure 13: Predicted versus Observed Plasma VEL concentrations (the black lines are line of identity and the dashed red lines are smooth lines)

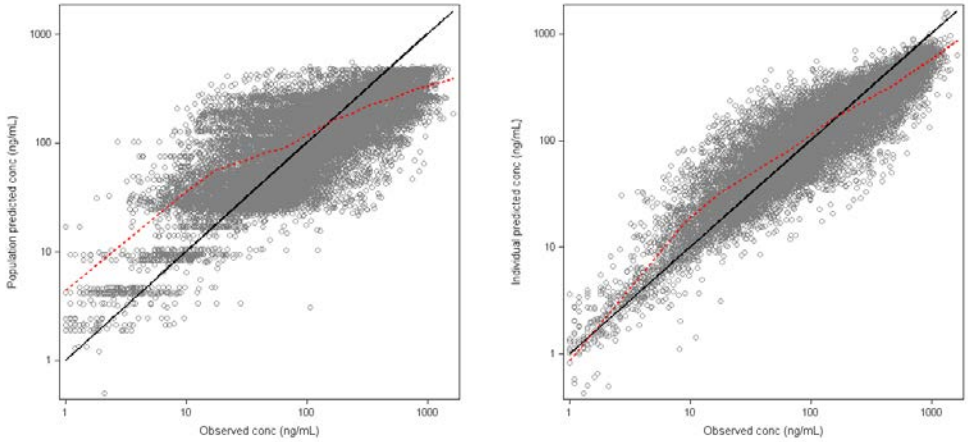
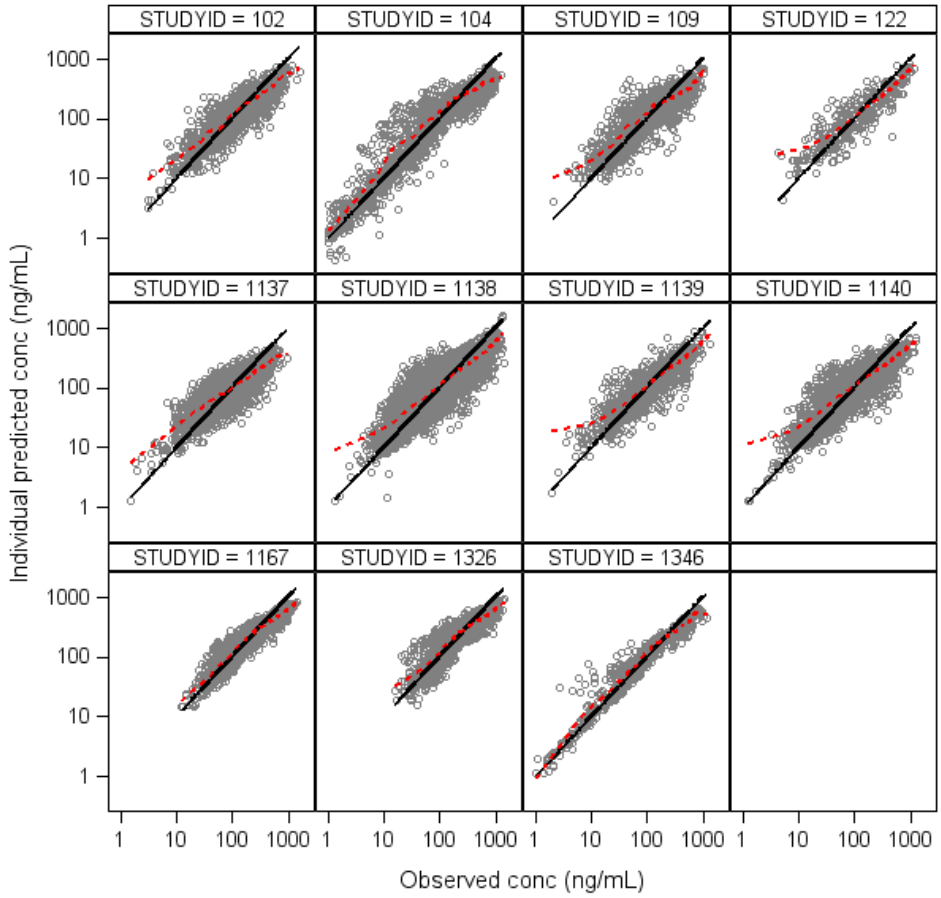


Figure 14: Individual Predicted Concentrations versus Observed Plasma Concentrations by Study



4.4.3 Parameter Estimates

The reviewer's population PK parameter estimates results were similar to those from the applicant.

Summary of post-hoc estimates of VEL individual clearance and AUC_{0-24} after 100 mg QD in combination with SOF 400 mg dose or in FDC versus covariates of interest is summarized in Figure 15 and Table 11. There was no clinically relevant difference in exposure with respect to race (Caucasians, African Americans, and Asians), sex (male versus female), or age groups (<65 or ≥65 years of age).

Figure 15: VEL PK parameter-covariate relationships

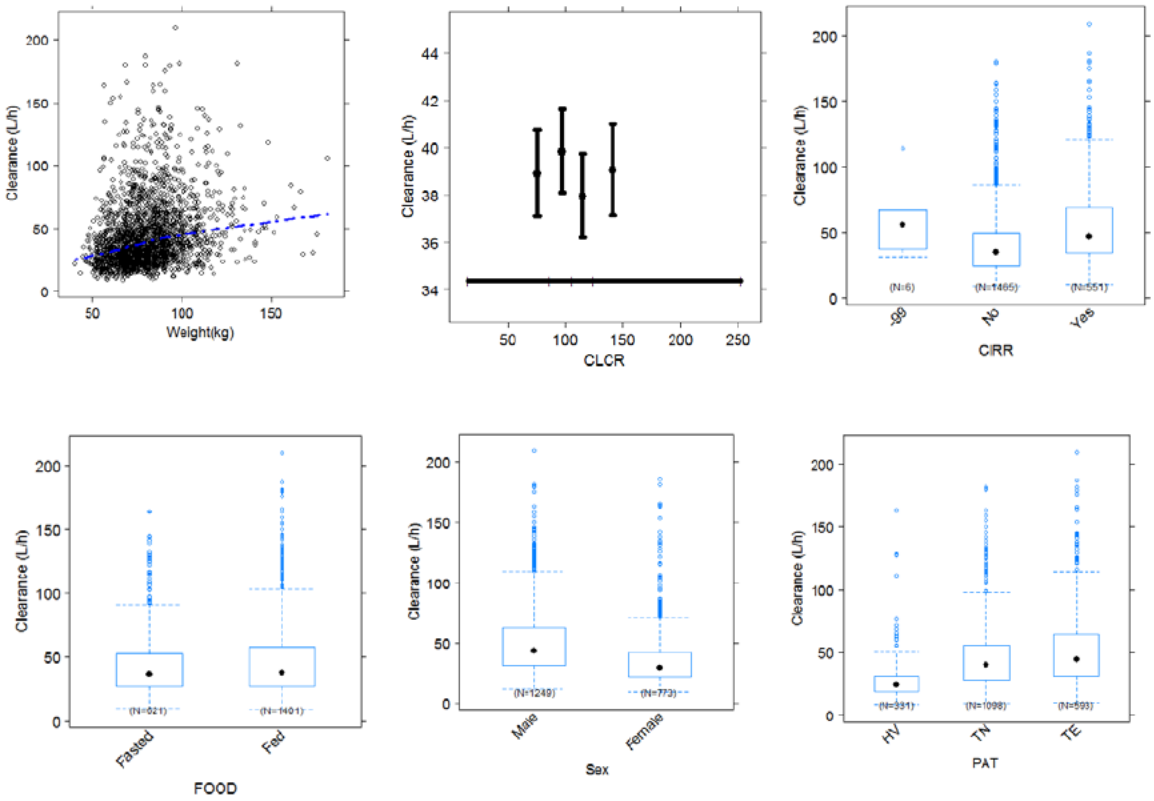


Table 11: Summary of Individual Empirical Bayes' Estimates for VEL

Parameters		N	Mean	SD	5th percentile	95th percentile
CL/F (L/h)	Caucasian	1712	45.4	27.6	17.0	99.6
	Black	159	44.6	28.8	17.4	119.6
	Asian	100	41.2	24.0	15.3	79.2
	Others	51	49.3	25.1	21.0	83.9
	Male	1249	51.1	28.3	20.4	111.5

	Female	773	35.8	23.2	14.6	76.5
	< 65 years	1840	44.9	27.4	16.9	99.7
	≥ 65 years	182	48.3	28.2	18.3	98.4
	Non-Cirrhotic	1465	40.9	24.0	16.3	87.9
	Cirrhotic	551	56.8	32.5	21.5	125.0
	Healthy Volunteers	331	27.9	16.3	14.1	56.4
	HCV-infected	1691	48.6	28.0	19.2	105.7
$AUC_{ss(0-24)}$ (ng.h/mL)	Caucasian	1712	3118	1659	520	12183
	Black	159	3184	1614	692	9858
	Asian	100	3364	1742	725	9793
	Others	51	2662	1350	702	8135
	Male	1249	2661	1313	520	8521
	Female	773	3872	1862	583	12184
	< 65 years	1840	3150	1670	520	12184
	≥ 65 years	182	2863	1461	600	7900
	Non-Cirrhotic	1465	3372	1688	603	12184
	Cirrhotic	551	2474	1363	520	11050
	Healthy Volunteers	1691	4556	1478	520	11502
	HCV-infected	331	2844	1761	612	12184

Listing of Analyses Codes and Output Files

File Name	Description	Location in \\cdsnas\pharmacometrics\
eff_pk.sas	Post NONMEM analysis of the final model as well as ER analysis	~\SOF_VEL_NDA208341_FL\ER Analyses\eff_pk

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/s/

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