CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

209195Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

CLINICAL PHARMACOLOGY REVIEW ADDENDUM

NDA	209195 (SDN 1)
SUBMISSION DATE	12/08/2016
DRUG	VOSEVI® (Sofosbuvir/velpatasvir/voxilaprevir (SOF/VEL/VOX))
SPONSOR	Gilead Sciences
SUBMISSION TYPE	NME; Priority
OCP DIVISION	DCP4
OND DIVISION	DAVP
REVIEWER	Qin Sun, Ph.D.
TEAM LEADER	Shirley Seo, Ph.D.
INDICATION	Treatment of adult patients with chronic HCV infection
FORMULATION	Fixed dose combination tablets; SOF/VEL/VOX 400/100/100 mg
DOSAGE AND ADMINISTRATION	One tablet orally once daily with food

The Clinical Pharmacology Review for NDA 209195 was completed on May 8, 2017 while active labeling negotiations were still underway with the sponsor. This addendum documents the final language for the co-administration of VOSEVI with dabigatran and statins, including pitavastatin, atorvastatin, fluvastatin, lovastatin, and simvastatin in Section 7.

The original and final versions of the labeling for those updates are summarized below (original recommendation from FDA is in black and final agreed upon wording is in red.):

Concomitant Drug Class: Drug Name	Effect on Concentration	Clinical Effect/Recommendation
Anticoagulants:		
dabigatran etexilate	↑ dabigatran	(b) (4) [redacted] Clinical monitoring of dabigatran is recommended when coadministered with VOSEVI. Refer to dabigatran etexilate prescribing information for dose modification recommendations in the setting of moderate renal impairment.

Upon further internal discussion and consideration, it was decided that a reference to the current dabigatran USPI would be the most appropriate way to direct the reader to the most up-to-date dosing recommendations for dabigatran.

HMG-CoA Reductase Inhibitors:		
Pitavastatin	↑ pitavastatin	(b) (4) [redacted]
atorvastatin fluvastatin lovastatin simvastatin	↑ atorvastatin ↑ fluvastatin ↑ lovastatin ↑ simvastatin	Coadministration with VOSEVI may increase the concentration of pitavastatin and is not recommended, due to an increased risk of myopathy, including rhabdomyolysis. Coadministration with VOSEVI may increase the concentrations of atorvastatin, fluvastatin, lovastatin, and simvastatin. Increased statin concentrations may increase the risk of myopathy, including rhabdomyolysis. Use the lowest approved statin dose. If higher doses are needed, use the lowest necessary statin dose based on a risk/benefit assessment.

Upon further internal discussion and consideration, wording for the clinical recommendation for the concomitant use of certain statins (atorvastatin, fluvastatin, lovastatin, and simvastatin) was revised to emphasize the use of clinical judgment in guiding statin doses.

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/s/

QIN SUN
07/07/2017

SHIRLEY K SEO
07/07/2017

Office of Clinical Pharmacology Review

NDA Number	209195
Link to EDR	\\CDSESUB1\evsprod\NDA209195\0000
Submission Date	12/08/2016
Submission Type	NME; Priority
Brand Name	Vosevi®
Generic Name	Sofosbuvir/velpatasvir/voxilaprevir (SOF/VEL/VOX)
Dosage Form and Strength	Fixed dose combination tablets; SOF/VEL/VOX 400/100/100 mg
Route of Administration	Oral
Proposed Indication	Treatment of adult patients with chronic HCV infection
Applicant	Gilead Sciences
Associated IND(s) and NDA(s)	INDs 125751 (SOF/VEL/VOX), 119926 (VOX only) and NDAs 208341 (Epclusa®), 204671 (Sovaldi®), 205834 (Harvoni®)
OCP Review Team	Qin Sun, PhD; Shirley Seo, PhD; Jenny Zheng, PhD; Jeffrey Froude, PhD; Fang Li, PhD; Jeffry Florian, PhD
OCP Final Signatory	John Lazor, PharmD Division IV Director Office of Clinical Pharmacology

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1 EXECUTIVE SUMMARY

Gilead Sciences is seeking approval of sofosbuvir (SOF, GS-7977), velpatasvir (VEL, GS-5816), and voxilaprevir (VOX, GS-9857) formulated as an oral fixed-dose combination (FDC) tablet (SOF/VEL/VOX 400/100/100 mg) for the treatment of chronic hepatitis C virus (HCV) infection in adult patients, without cirrhosis or with compensated cirrhosis [Child-Pugh-Turcotte (CPT) A] for 12 weeks. It is indicated for genotype 1, 2, 3, 4, 5, or 6 in nonstructural protein 5A (NS5A) inhibitor-experienced patients, and for genotype 1a or 3 in nucleotide analog NS5B polymerase inhibitor-experienced patients who are NS5A inhibitor-naïve (no additional benefit of SOF/VEL/VOX has been established over SOF/VEL in genotypes 1b, 2, 4, 5 and 6) (final indication wording is still under negotiation at the time of this review).

Despite high SVR rates following DAA treatment, there is a growing population of patients who fail DAA-based therapies. Treatment options are limited for patients who fail DAA-only treatment, particularly regimens that include NS5A inhibitor. Retreatment of NS5A inhibitor-experienced patients with currently marketed DAAs, including Harvoni® and Epclusa® for 24 weeks leads to SVR rates as low as 70% for certain genotypes.

The efficacy and safety for SOF/VEL/VOX FDC tablets once daily with food were evaluated in four Phase 3 studies (POLARIS-1 (NS5A inhibitor experienced), POLARIS-2 (DAA naïve), POLARIS-3 (DAA naïve), and POLARIS-4 (non-NS5A DAA inhibitor experienced)). SOF/VEL/VOX demonstrated high SVR rates of > 95% in both DAA-experienced and DAA-naïve patients across different genotypes, without cirrhosis or with compensated cirrhosis. There were no significant safety concerns identified in any of the Phase 3 studies.

1.1 Recommendations

The Office of Clinical Pharmacology has reviewed the clinical pharmacology information contained in NDA 209195. This NDA is considered approvable from a clinical pharmacology perspective. The key review issues with specific recommendations and comments are summarized below:

Review Issues	Recommendations and Comments
Pivotal or Supportive evidence of effectiveness	The primary evidence of effectiveness is provided by two Phase 3 studies: Study GS-US-367-1171 (POLARIS-1) and Study GS-US-367-1170 (POLARIS-4). The supportive evidence for efficacy comes from two Phase 3 studies (Study GS-US-367-1172 (POLARIS-2) and Study GS-US-367-1173 (POLARIS-3)), four Phase 2 studies (Study GS-US-337-1468 (LEPTON, Cohorts 4 and 5), Study GS-US-367-1168, Study GS-US-367-1169, and Study GS-US-367-1871), and a

	Phase 1b dose-ranging study for VOX monotherapy (Study GS-US-338-1121).
General dosing instructions	The proposed dose for SOF/VEL/VOX FDC tablets at 400/100/100 mg once daily with food is effective and appears to be safe based on four Phase 2 and four Phase 3 studies, and is acceptable from a clinical pharmacology perspective.
Dosing in patient subgroups (intrinsic and extrinsic factors)	No dose adjustments are recommended based on intrinsic and extrinsic factors. SOF/VEL/VOX FDC tablets are **NOT** recommended to be adminstered in patients with the following intrinsic factors: • Severe renal impairment • End stage renal disease (ESRD) • Moderate hepatic impairment • Severe hepatic impairment Coadministration with certain drugs due to drug interactions (DDIs) is not recommended and coadministration with rifampin is contraindicated. (Refer to **Section 2.2.2**)
Labeling	Generally acceptable. The review team has specific content and formatting recommendations. (Refer to **Section 2.4**)
Bridge between the to-be-marketed and clinical trial formulations	Not applicable. To-be-marketed formulation was used in all pivotal Phase 3 studies.

1.2 Post-Marketing Requirements and Commitments

None.

2 Summary of Clinical Pharmacology Assessment

2.1 Pharmacology and Clinical Pharmacokinetics

SOF/VEL/VOX was developed as a FDC tablet (400/100/100 mg) to be given once daily with food for the treatment of HCV infection in adults, without cirrhosis or with compensated cirrhosis (CPT A) for 12 weeks. It is indicated for genotype 1, 2, 3, 4, 5, or 6 in NS5A inhibitor-experienced patients, and for genotype 1a or 3 in NS5B inhibitor-experienced patients who are NS5A inhibitor-naïve (final indication wording is still under negotiation at the time of this review).

Mechanism of Action: VOX is a pangenotypic HCV NS3/4A protease inhibitor (PI) with potent antiviral activity and an improved resistance profile compared with other HCV NS3/4A PIs. VEL is an HCV NS5A inhibitor. SOF is an HCV nucleotide analog NS5B polymerase inhibitor.

ADME: The absorption, distribution, metabolism, and excretion (ADME) profiles of the components of SOF/VEL/VOX are summarized in **Table 2.1-1**:

Table 2.1-1 ADME profiles of the components of SOF/VEL/VOX

	SOF	VEL	VOX
Absorption			
T_{max} (h)	2	4	4
Combination *vs.* individual (fasted)[a]	↔	↔	↓ 63%
Light-fat meal *vs.* fasted[a]	↑ 118%	↑ 166%	↑ 112%
Moderate-fat meal *vs.* fasted[a]	↑ 144%	↑ 129%	↑ 185%
High-fat meal *vs.* fasted[a]	↑ 64%	↑ 40%	↑ 435%
Distribution			
% Bound to human plasma proteins	61 to 65	> 99	> 99
Blood-to-plasma ratio	0.7	0.5−0.7	0.5−0.8
Metabolism			
Metabolism	SOF: Cathepsin A, CES1, HINT1	CYP2B6, CYP2C8, CYP3A4	CYP1A2, CYP2C8, CYP3A4
Elimination			
Major route of elimination	SOF: metabolism GS-331007[b]: glomerular filtration and active tubular secretion	Biliary excretion	Biliary excretion
$t_{1/2}$ (h)[c]	SOF: 0.5 GS-331007: 29	17	33
% Of dose excreted in urine[d]	80%[e]	0.4%	none
% Of dose excreted in feces[d]	14%	94% (77% parent)	94% (40% parent)

CES1 = carboxylesterase 1; HINT1 = histidine triad nucleotide-binding protein 1

[a] Values refer to mean systemic exposure. Light-fat meal = ~ 400 kcal, 10% fat; moderate-fat meal = ~600 kcal, 25-30% fat; high fat meal = ~1000 kcal, 45-55% fat.

[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 or [^{14}C] VOX in mass balance studies.

[e] Predominantly as GS-331007.

2.2 Dosing and Therapeutic Individualization

2.2.1 General Dosing

The Applicant's proposed dosage regimen of SOF/VEL/VOX FDC tablet at 400/100/100 mg taken orally once daily with food for 12 weeks is supported by the PK, efficacy and safety data from the clinical trials submitted in this NDA, and the recommended oral dose regimen is acceptable (see **Section 3.3.2** for additional discussion).

2.2.2 Therapeutic Individualization

Intrinsic factors:

• *Hepatic impairment*
The review team concurs with the Applicant's recommendation that no dosage adjustment is required for patients with mild hepatic impairment, and SOF/VEL/VOX is not recommended in patients with moderate or severe hepatic impairment due to higher exposures of VOX in these patients.

- *Renal impairment*

The review team concurs with the Applicant's recommendation that no dosage adjustment is required for patients with mild or moderate renal impairment, and no dosage recommendation can be given for patients with severe renal impairment or with end stage renal disease (ESRD) due to higher exposures (up to 20-fold) of GS-331007 in these patients.

Extrinsic factors:

The review team reviewed the relevant drug interaction studies, and the proposed management strategy for clinically important DDIs is generally acceptable. The agreed upon recommendations (at the time of this review) for clinical management of specific drugs are summarized in detail in **Section 3.3.4** (**Table 3.3.4-5**) and the general recommendations for potentially clinically important DDIs are listed below:

- P-gp inducers and/or moderate to potent CYP inducers (e.g., St. John's wort, carbamazepine) may decrease concentrations of SOF, VEL, and/or VOX, and are **NOT** recommended for co-administration.
- Inhibitors of OATP may increase VOX exposure substantially, and are **NOT** recommended for co-administration.
- SOF/VEL/VOX may increase the exposure of BCRP substrates (e.g., methotrexate, mitoxantrone, imatinib, irinotecan, lapatinib, rosuvastatin, sulfasalazine, topotecan) substantially, and thus are **NOT** recommended for co-administration with BCRP substrates.

2.3 Outstanding Issues

None.

2.4 Summary of Labeling Recommendations

The Office of Clinical Pharmacology recommends the following labeling concepts be included in the final package insert (**Table 2.4-1**).

Table 2.4-1 Summary of Labeling Issue Identification and Recommendations

Section/heading	Acceptable to OCP?			Comment
	A	AWE	NA	
Highlights/Contraindications	☐	☐	☒	Add "Coadministration with rifampin is contraindicated."
Section 4/Contraindications	☐	☐	☒	Add "Vosevi is contraindicated with rifampin."
Section 7.1/ Potential for other drugs to affect Vosevi	☐	☒	☐	• Update CYP enzymes for VOX metabolism
Section 7.2/ Potential for Vosevi to affect other drugs	☐	☐	☒	• Add "Coadministration of Vosevi with BCRP substrates (e.g., methotrexate, mitoxantrone, imatinib, irinotecan, lapatinib, rosuvastatin, sulfasalazine, topotecan) is not recommended"
Section 7.3/ Established and potentially significant drug interactions	☐	☐	☒	• Revise recommendation for PPIs: omeprazole 20 mg can be administered with Vosevi, and use with other proton-pump inhibitors has not been studied. • Revise recommendation of digoxin for concentration increases with unclear magnitude. • Revise recommendation for dabigatran etexilate and add dose adjustment for patients with moderate renal impairment • Add contraindication for rifampin coadministration • Add "Tipranavir/ritonavir coadministration is not recommended" • Add additional statins: for pitavastatin, the coadministration is not recommended due to an increased risk of severe myopathy at > 4 mg in premarketing clinical studies. For other unstudied statins, including atorvastatin, fluvastatin, lovastatin, simvastatin, the lowest necessary dose should be used when coadministered with Vosevi.
Section 7.4/ Drugs without clinically significant interactions with Vosevi	☐	☒	☐	Revise the DDI summary to match the drugs used for each DDI study
12.3/Pharmacokinetic properties	☐	☒	☐	Update VOX metabolism enzymes (b) (4) to the summary of "% of dose excreted in feces"
12.3/Specific populations/Race and Sex	☐	☒	☐	Combine summary for race and sex
12.3/DDI	☐	☒	☐	• Update VOX metabolism enzymes • Table 5, update dose regimen of SOF/VEL/VOX +VOX from "single dose" to "once daily", when coadministered with dabigatran etexilate, pravastatin, rosuvastatin • Revise DDI summary to match the drugs used for each DDI study for drugs with no effect with Vosevi or its component(s)

A = Acceptable; AWE=Acceptable with minor edits; NA=not acceptable/substantive disagreement (must provide comment)

3 Comprehensive Clinical Pharmacology Review

3.1 Overview of the Product and Regulatory Background

SOF/VEL/VOX FDC at 400/100/100 mg is indicated for the treatment of chronic HCV infection in adults, without cirrhosis or with compensated cirrhosis (CPT A) for 12 weeks. It is indicated for genotype 1, 2, 3, 4, 5, or 6 in NS5A inhibitor-experienced patients, and for genotype 1a or 3 in NS5B inhibitor-experienced patients who are NS5A inhibitor-naïve (final indication wording is still under negotiation at the time of this review). SOF, VEL, and VOX are HCV NS5B, NS5A, and NS3/4A inhibitors, respectively. VEL has been approved as part of a FDC with SOF (Epclusa®, NDA 208341) for the treatment of chronic HCV genotype 1 to 6 infection in adults in over 30 countries. SOF 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) in over 40 countries. Two INDs are in effect in the Division of Antiviral Products: INDs 125751 for SOF/VEL/VOX and 119926 for VOX. SOF/VEL/VOX was granted Breakthrough Therapy Designation on February 19, 2016.

The Applicant has requested a partial waiver for pediatric studies in subjects < 12 years of age and requested a deferral for pediatric studies in subjects 12 to < 18 years of age until additional safety and efficacy data have been collected from SOF/VEL studies in pediatric subjects 12 to < 18 years.

3.2 General Pharmacology and Pharmacokinetic Characteristics

Pharmacology	
Mechanism of Action	VOX: HCV NS3/4A protease inhibitor VEL: HCV NS5A inhibitor SOF: HCV NS5B polymerase inhibitor
Active Moieties	VOX: ~ 91% of circulating radioactivity in plasma VEL: > 98% of circulating radioactivity in plasma SOF and GS-331007: ~ 4% and > 90% of circulating radioactivity in plasma; pharmacologically active nucleoside analog triphosphate (GS-461203) is not measurable in plasma
QT Prolongation	VOX: no prolongation of the QTc interval after a single therapeutic dose of 300 mg or a supratherapeutic dose of 900 mg (43- and 31-fold of VOX C_{max} and AUC within SOF/VEL/VOX in Phase 2 and 3 studies), administered with food. Refer to QT interdisciplinary review team summary (IND 119926, 08/29/2016) for details. VEL: no prolongation of the QTc interval after a single therapeutic dose of 100 mg or a supratherapeutic dose of 500 mg (4.2- and 3.5-fold of VEL C_{max} and AUC within SOF/VEL/VOX in Phase 2 and 3 studies). Refer to QT interdisciplinary review team summary (IND 115670, 04/15/2015) for details. SOF: no prolongation of the QTc interval after a single therapeutic dose of 400 mg or a supratherapeutic dose of 1200 mg (3.3- and 1.5-

<table>
<tr><td></td><td>fold of SOF C_{max} and AUC and 2.8- and 2.2-fold of GS-331007 C_{max} and AUC in Phase 2 and 3 studies). Refer to QT interdisciplinary review team summary (IND 116739, 11/26/2012) for details.</td></tr>
<tr><td colspan="2">General Information</td></tr>
<tr><td>Bioanalysis</td><td>Validated HPLC/MS/MS methods were used to determine VOX, VEL, SOF, GS-331007, and coadministered drug concentrations in human plasma, urine or feces (refer to Appendix 4.1).</td></tr>
<tr><td>Patients vs. Healthy</td><td>VOX: HCV patients have 3 to 4-fold higher VOX exposure compared to healthy subjects under fasted and fed conditions.
VEL: HCV patients have 40% lower VEL exposure compared to healthy subjects under fed conditions.
SOF and GS-331007: HCV patients have similar exposure compared to healthy subjects for both SOF and GS-331007 under fed conditions.</td></tr>
<tr><td>Drug Exposure at Steady State following the Therapeutic Dosing Regimen</td><td>Exposure based on population PK modeling for HCV-infected patients in four Phase 2 and four Phase 3 studies:
<table>
<tr><th></th><th rowspan="2">N</th><th>AUC_{tau} (h*ng/mL)</th><th>C_{max} (ng/mL)</th></tr>
<tr><th></th><th>mean (% CV)</th><th>mean (% CV)</th></tr>
<tr><td>VOX</td><td>1591</td><td>2577 (73.7)</td><td>192 (85.8)</td></tr>
<tr><td>VEL</td><td>1595</td><td>4041 (48.6)</td><td>311 (56.1)</td></tr>
<tr><td>SOF</td><td>1038</td><td>1665 (30.1)</td><td>678 (35.4)</td></tr>
<tr><td>GS-331007</td><td>1593</td><td>12,834 (29.0)</td><td>744 (28.3)</td></tr>
</table>
Note: Agreed upon parameter values that are presented in Section 12 of the label.</td></tr>
<tr><td>Minimal Effective Dose or Exposure</td><td>Not tested.
Only one FDC dose with SOF/VEL/VOX at 400/100/100 mg was evaluated for efficacy and safety in Phase 2 and Phase 3 studies.</td></tr>
<tr><td>Maximal Tolerated Dose or Exposure</td><td>Not reached.
Only one FDC dose with SOF/VEL/VOX at 400/100/100 mg was evaluated for efficacy and safety in Phase 2 and Phase 3 studies.</td></tr>
<tr><td>Dose Proportionality</td><td>VOX: both C_{max} and AUC increased dose-proportionally from 30 to 300 mg under fasted conditions, while they increased much greater than dose-proportionally from 100 to 900 mg under fed conditions.
VEL: both C_{max} and AUC increased less than dose-proportionally from 50 to 450 mg.
SOF and GS-331007: both C_{max} and AUC of SOF and AUC of GS-331007 increased almost dose-proportionally, while C_{max} of GS-331007 increased less than dose-proportionally.</td></tr>
<tr><td>Accumulation</td><td>VOX ratio: 2-4
VEL ratio: < 2
SOF and GS-331007 ratio: ~ 1</td></tr>
<tr><td colspan="2">Absorption</td></tr>
<tr><td>Bioavailability</td><td>Absolute bioavailability has not been determined for VOX, VEL, or SOF.</td></tr>
<tr><td>T_{max}</td><td>Median 2-4 hours for all three components</td></tr>
</table>

<table>
<tr><td rowspan="5">Food Effect [AUC_{tau} % GLSM Ratio (90% CI)]</td><td></td><td>SOF</td><td>GS-331007</td><td>VEL</td><td>VOX</td></tr>
<tr><td>Light-fat meal vs. fasted</td><td>218 (151, 314)</td><td>↔</td><td>266 (161, 438)</td><td>212 (159, 282)</td></tr>
<tr><td>Moderate-fat meal vs. fasted</td><td>244 (169, 353)</td><td>↔</td><td>229 (134, 392)</td><td>285 (209, 387)</td></tr>
<tr><td>High-fat meal vs. fasted</td><td>164 (139, 192)</td><td>↔</td><td>140 (113, 175)</td><td>535 (428, 669)</td></tr>
<tr><td colspan="5">GLSM: geometric least-squares mean.
Light-fat meal = ~ 400 kcal, 10% fat; moderate-fat meal = ~600 kcal, 25-30% fat; high fat meal = ~1000 kcal, 45-55% fat.

SOF/VEL decreased VOX exposure (AUC 63% lower) under fasted conditions, which was mitigated when administered with food. Therefore, SOF/VEL/VOX was administered with food in Phase 2 and Phase 3 studies.</td></tr>
<tr><td colspan="6">Distribution</td></tr>
<tr><td>Volume of Distribution [inter-individual variability (%)]</td><td colspan="5">The volume of distribution related parameters were estimated based on population PK:

<table>
<tr><td></td><td>V_c/F (L)</td><td>V_p/F (L)</td></tr>
<tr><td>VOX</td><td>707 (109)</td><td>259 (NA)</td></tr>
<tr><td>VEL</td><td>286 (78)</td><td>135 (NA)</td></tr>
<tr><td>SOF</td><td>302 (34)</td><td>NA (NA)</td></tr>
<tr><td>GS-331007</td><td>207 (100)</td><td>804 (39)</td></tr>
</table>
NA: not available.</td></tr>
<tr><td>Plasma Protein Binding</td><td colspan="5">VOX: > 99%
VEL: > 99%
SOF: 61-65%
GS-331007: < 10%</td></tr>
<tr><td>Blood to Plasma Ratio</td><td colspan="5">VOX: 0.5-0.8
VEL:0.5-0.7
SOF/GS-331007: 0.7</td></tr>
<tr><td>Substrate of Transporter Systems [in vitro]</td><td colspan="5">VOX: P-gp, BCRP, OATP1B1, OATP1B3
VEL: P-gp, BCRP
SOF: P-gp, BCRP
GS-331007: none</td></tr>
<tr><td colspan="6">Elimination</td></tr>
<tr><td>Median (Q1, Q3) Terminal Elimination Half-life (h)</td><td colspan="5">VOX: 33 (28, 39)
VEL: 17 (14, 19)
SOF: 0.5 (0.43, 0.53)
GS-331007: 29 (27, 31)</td></tr>
<tr><td>Apparent Oral Clearance (CL/F) Estimate (L/h) [inter-individual variability (%)]</td><td colspan="5">The CL/F values were estimated based on population PK:
VOX: 66 (70)
VEL: 33 (55)
SOF: 273 (42)
GS-331007: 34 (25)</td></tr>
<tr><td colspan="6">Metabolism</td></tr>
</table>

Primary Metabolic Pathway(s) [*in vitro*]	VOX: CYP1A2, CYP 2C8, CYP3A4 VEL: CYP2B6, CYP2C8, CYP3A4 SOF: Cathepsin A, CES1, HINT1* GS-331007: none * CES1: carboxylesterase 1; HINT1:histidine triad nucleotide-binding protein 1			
Excretion				
Primary Excretion Pathways		SOF	VEL	VOX
	Major route	Renal excretion	Biliary excretion	Biliary excretion
	% Dose in urine	80%*	0.4%	none
	% Dose in feces	14%	94% (77% parent)	94% (40% parent)
	* Predominantly as GS-331007.			
In vitro Interaction Liability (Drug as Perpetrator)				
Inhibition/Induction of Metabolism	VOX, VEL, SOF, and GS-331007 did not inhibit or induce CYP enzymes or UGT1A1.			
Inhibition of Transporter Systems	VOX inhibited BCRP (IC_{50}: inhibit 6.7% at 10 µM), OATP1B1 (IC_{50}: 0.18 µM), OATP1B3 (IC_{50}: 0.70 µM), and BSEP (IC_{50}: 1.5 µM); VEL inhibited P-gp (IC_{50}: 20.6 µM), BCRP (IC_{50}: 0.23 µM) , OATP1B1 (IC_{50}: 1.5 µM), OATP1B3 (IC_{50}: 0.26 µM) , OATP2B1(IC_{50}: inhibit 30% at 10 µM), and BSEP (IC_{50}: 0.64 µM); SOF and GS-331007 showed little or no transporter inhibition.			

3.3 Clinical Pharmacology Review Questions

3.3.1 To what extent does the available clinical pharmacology information provide pivotal or supportive evidence of effectiveness?

The clinical pharmacology information provided supportive evidence of effectiveness. The exposure-response (E-R) relationship for efficacy was evaluated in HCV-infected subjects in four Phase 3 studies using VOX, VEL, SOF, and GS-331007 plasma exposure derived from population PK modeling (~ 1000 subjects for VOX, VEL, and GS-331007, and ~ 600 subjects for SOF). Exposure for all analytes in Phase 3 studies resides in the near-maximal portion of their respective monotherapy exposure-response curves (see 3.3.2 for details), with mean predicted maximal HCV RNA suppression (% of E_{max}) of a least 87% for all analytes. Consistent with this finding, an overall high response rate (> 95%) was observed in all four Phase 3 studies in both DAA-experienced and DAA-naïve patients.

Since SOF/VEL/VOX is indicated for DAA-experienced patients, without cirrhosis or with compensated cirrhosis, the E-R relationship for efficacy was examined in detail for DAA-experienced patients from two Phase 3 studies (GS-US-367-1171 (POLARIS-1, NS5A inhibitor-experienced) and GS-US-367-1170 (POLARIS-4, non-NS5A DAA-experienced)). No E-R relationships for efficacy were identified across exposure ranges for VOX, VEL, SOF, and GS-331007, though it should be noted that the Phase 3 studies evaluated only a single dose-level of SOF/VEL/VOX at 400/100/100 mg once daily. The SVR12 rates by population quartiles of VOX, VEL, SOF, and GS-331007 AUC_{tau} in DAA-experienced

subjects in study GS-US-367-1171 and study GS-US-367-1170 are summarized in **Table 3.3.1-1**. As indicated, the SVR12 rates in all quartiles of different AUC_{tau} range were high, at 92.9% to 100%. There was no significant exposure-SVR12 relationship in all DAA-experienced subjects or subjects with compensated cirrhosis. A futher evaluation of SVR12 rates by population quartiles of VOX, VEL, SOF, and GS-331007 AUC_{tau} and by HCV genotype did not reveal any E-R relationship either (**Table 3.3.1-2**).

Table 3.3.1-1: SVR12 Rate by Population Quartiles of VOX, VEL, SOF, and GS-331007 AUC_{tau} in DAA-Experienced Subjects in Study GS-US-367-1171 (POLARIS-1) or GS-US-367-1170 (POLARIS-4) (PK/PD Analysis Set)

	GS-US-367-1171					GS-US-367-1170				
	AUC_{tau} h*ng/mL (range)	All Subjects		Subjects with Cirrhosis		AUC_{tau} h*ng/mL (range)	All Subjects		Subjects with Cirrhosis	
		N	SVR12	N	SVR12		N	SVR12	N	SVR12
VOX										
Q4	3171.2 – 16,922.2	72	95.8%	54	94.4%	3158.9 – 9211.4	50	96.0%	36	94.4%
Q3	1993.3 – 3147.4	52	96.2%	27	96.3%	1992.5 – 3173.9	45	97.8%	25	96.0%
Q2	1238.7 – 1990.7	60	96.7%	19	89.5%	1212.0 – 1942.9	42	97.6%	13	100%
Q1	289.3 – 1202.3	77	97.4%	19	94.7%	289.7 – 1156.4	45	97.8%	10	100%
VEL										
Q4	5036.8 – 13,078.2	50	98.0%	20	95.0%	5059.2 – 11,991.4	33	100%	11	100%
Q3	3660.7 – 5016.6	55	98.2%	28	100%	3666.1 – 4953.2	44	97.7%	15	93.3%
Q2	2641.8 – 3645.9	70	92.9%	38	89.5%	2698.3 – 3644.6	46	93.5%	27	92.6%
Q1	613.7 – 2637.6	87	97.7%	34	94.1%	711.5 – 2636.6	58	98.3%	31	100%
SOF										
Q4	1949.3 – 3364.7	31	100%	14	100%	2062.8 – 2982.2	19	100%	6	100%
Q3	1572.1 – 1924.9	36	97.2%	25	96.0%	1567.8 – 1937.8	31	93.5%	16	93.8%
Q2	1327.3 – 1554.8	45	95.6%	18	88.9%	1342.1 – 1553.4	30	96.7%	16	93.8%
Q1	620.6 – 1317.6	55	98.2%	22	95.5%	778.0 – 1322.6	36	100%	19	100%
GS-331007										
Q4	14,641.8 – 31,920.8	47	93.6%	18	83.3%	14,816.5 – 23,445.3	31	100%	14	100%
Q3	12,238.4 – 14,622.6	58	98.3%	27	100%	12,246.6 – 14,636.5	47	93.6%	21	90.5%
Q2	10,443.3 – 12,202.6	74	98.6%	35	100%	10,436.2 – 12,224.6	47	100%	19	100%
Q1	46,16.2 – 10,376.7	82	95.1%	39	89.7%	5364.4 – 10,431.3	56	96.4%	29	96.6%

Table 3.3.1-2: SVR12 Rate by Population Quartiles of VOX, VEL, SOF, and GS-331007 AUC_{tau} and by HCV Genotype in DAA-Experienced Subjects in Study GS-US-367-1171 (POLARIS-1) or GS-US-367-1170 (POLARIS-4) (PK/PD Analysis Set)

GS-US-367-1171 (POLARIS-1)#				GS-US-367-1170(POLARIS-4)*				
VOX AUCtau (Range)	GT1 (N=149)	GT-3 (N=78)	GT-4 (N=21)	VOX AUCtau	GT-1 (N=78)	GT-2 (N=31)	GT-3 (N=54)	GT-4 (N=19)
Q4 (3171-16922)	34/34 (100%)	25/27 (92.6%)	7/8 (87.5%)	Q4 (3159-9211.4)	20/20 (100%)	5/5 (100%)	11/13 (84.6%)	12/12 (100%)
Q3 (1993.3-3147.4)	26/28 (92.9%)	16/16 (100%)	6/6 (100%)	Q3 (1992.-3137.9)	21/22 (95.5%)	4/4 (100%)	15/15 (100%)	4/4 (100%)
Q2 (1238.7-1990.7)	38/39 (97.4%)	13/14 (92.9%)	4/4 (100%)	Q2 (1212-1942.9)	16/17 (94.1%)	13/13 (100%)	12/12 (100%)	0/0

Q1 (289.3-1202.3)	47/48 (97.9%)	20/21 (95.2%)	3/3 (100%)	Q1 (289.7-1156.4)	19/19 (100%)	9/9 (100%)	13/14 (92.9%)	3/3 (100%)

[#] There were few genotype 2 (n=5), genotype 5 (n=1), and genotype 6 (n=6) subjects in GS-US-367-1171. The SVR12 rate was 100% in these subjects.

* No genotype 5 and 6 subjects were included in study GS-US-367-1170

3.3.2 Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

Yes, the proposed dosing regimen for SOF/VEL/VOX FDC at 400/100/100 mg taken orally once daily with food is acceptable for the general patient population with chronic HCV infection, without cirrhosis or with compensated cirrhosis, for genotype 1, 2, 3, 4, 5, or 6 in NS5A inhibitor-experienced patients, and for genotype 1a or 3 in NS5B inhibitor-experienced patients who are NS5A inhibitor-naïve (final indication wording is still under negotiation at the time of this review).

The dose regimen for SOF/VEL/VOX FDC at 400/100/100 mg once daily with food was used in both pivotal Phase 3 trials and sufficient effectiveness was demonstrated. In addition, the flat E-R relationship suggested that the exposure (for all three components) reached a plateau for efficacy (see 3.3.1 for details). In POLARIS-1 study, high SVR12 (> 91%) was observed across genotypes 1 to 6, without cirrhosis or with compensated cirrhosis, in NS5A inhibitor-experienced patients after 12 weeks treatment of SOF/VEL/VOX. In POLARIS-4 study, treatment with SOF/VEL/VOX for 12 weeks resulted in numerically higher SVR12 rates than treatment with SOF/VEL for 12 weeks in subjects with HCV genotype 1a and 3 infection. No additional benefit of SOF/VEL/VOX has been established over SOF/VEL for the treatment of HCV genotypes 1b, 2, 4, 5 and 6 in NS5B inhibitor-experienced adults who are NS5A inhibitor-naïve.

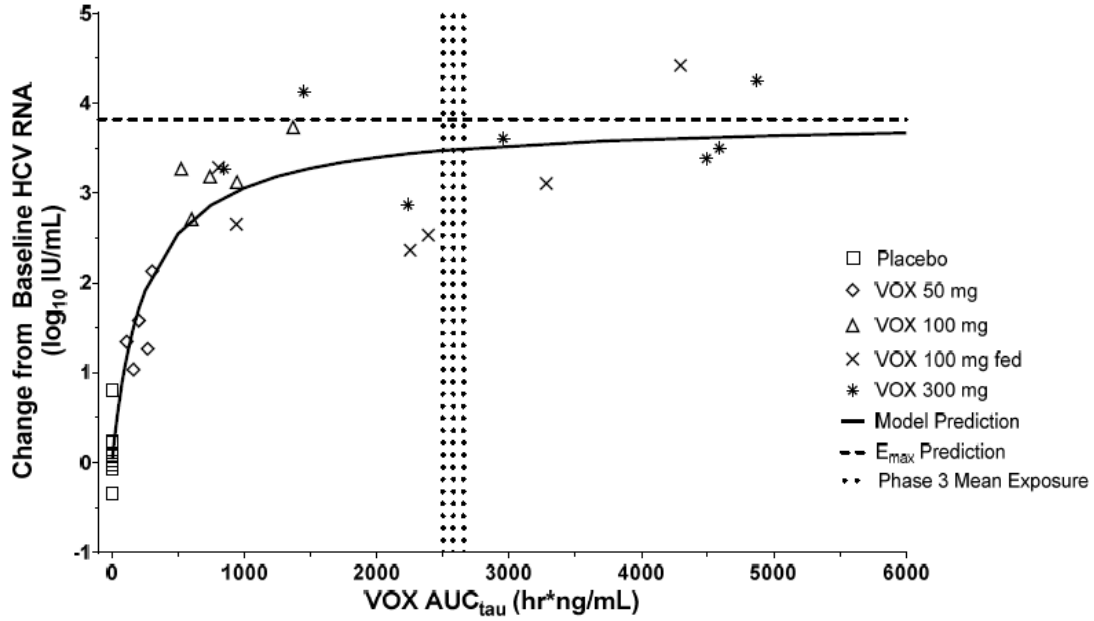
There was no relationship between exposure of VOX, VEL, or SOF and incidence of most frequently reported AEs (occurring in > 10% of subjects: headache, fatigue, diarrhea, and nausea). There was no association between exposure of SOF or VEL and laboratory measures such as lipase, creatine kinase, or total bilirubin. However, there was a linear relationship between VOX AUC_{tau} and maximum change from baseline in total bilirubin (mg/dL). The magnitude of increase in total bilirubin was not considered clinically significant, given the low grade of total bilirubin elevation (predominantly Grade 1) and its return to baseline after completion of treatment.

Initial Dose Selection

The SOF/VEL FDC dose of 400/100 mg is the approved dose of SOF/VEL (Epclusa®) for the treatment of HCV infection. As such, this dose was selected in the coformulation with VOX in a FDC tablet for evaluation in the SOF/VEL/VOX development program. The dose of VOX 100 mg was selected based on safety, antiviral activity, and PK data from Phase 1b dose-ranging finding study (Study GS-US-338-1121) and Phase 2 studies in HCV-infected subjects.

In the Phase 1b proof-of-concept study (Study GS-US-338-1121) for initial VOX dose-finding, similar median antiviral responses (reduction from baseline HCV RNA on Day 4) of > 3.0 log_{10} were observed in subjects with genotypes 1a, 1b, 2, and 4 HCV infection following VOX doses of 50, 100 or 300 mg under fasted conditions, yielding a flat VOX E-R relationship for anti-HCV activity. However, in subjects with genotype 3 HCV infection (50, 100, 300 mg under fasted conditions or 100 mg under fed conditions), an E-R relationship was observed for VOX and is presented in **Figure 3.3.2-1**. Based on these relationships, near maximal antiviral response (≥ 90% of E_{max}) was achieved at a VOX dose of 100 mg within SOF/VEL/VOX (mean VOX AUC: 2577 h*ng/mL) administered under fed conditions, and doses greater than 100 mg once daily are unlikely to result in further meaningful reductions in HCV RNA.

Figure 3.3.2-1 GS-US-338-1121: Observed Versus Predicted Exposure (VOX AUC_{tau} on Day 3) – Reponse (reduction from baseline HCV RNA on Day 4) for Genotype 3 HCV-infected subjects



"Phase 3 Mean Exposure" marker encompasses mean ± 90% CI

Based on a previously established E-R relationship for VEL and SOF (refer to Epclusa®, NDA 208341 for additional details), patients experience near maximal antiviral response (≥ 99.5% of E_{max} for VEL, and ≥ 90% of E_{max} for SOF) at a SOF/VEL dose of 400/100 mg within SOF/VEL/VOX FDC regimen (mean SOF AUC: 1,665 h*ng/mL; mean GS-331007 AUC: 12,834 h*ng/mL; mean VEL AUC: 4,041 h*ng/mL), and SOF/VEL doses greater than 400/100 mg once daily are unlikely to result in further meaningful reductions in HCV RNA.

Selection of Treatment Duration and Exclusion of Ribavirin

Four Phase 2 trials (Study GS-US-337-1468 [LEPTON, Cohorts 4 and 5], Study GS-US-367-1168, Study GS-US-367-1169, and Study GS-US-367-1871) evaluated the antiviral efficacy

for SOF/VEL 400/100 mg + VOX 100 mg ± RBV with food for 4, 6, 8 or 12 weeks in subjects with genotype 1, 2, 3, 4, 5, or 6 HCV infection, including DAA-experienced subjects and subjects with compensated cirrhosis. High SVR12 rates were achieved across all HCV genotypes in DAA-naive or DAA-experienced subjects receiving SOF/VEL 400/100 mg + VOX 100 mg for 8 or 12 weeks, respectively. Ribavirin did not improve efficacy in combination with SOF/VEL/VOX. Therefore, SOF/VEL/VOX FDC (no ribavirin) at 400/100/100 mg with food was selected for Phase 3 trials with 12 weeks treatment in DAA-experienced patients and 8 weeks in DAA-naïve patients.

3.3.3 Is an alternative dosing regimen or management strategy required for subpopulations based on intrinsic factors?

Hepatic Impairment

No dosage adjustment of SOF/VEL/VOX is required for patients with mild hepatic impairment, and SOF/VEL/VOX is not recommended in patients with moderate or severe hepatic impairment (CPT B and C). The recommendation for use of SOF/VEL/VOX in subjects with hepatic impairment is driven by its most restrictive component in the setting of hepatic impairment, VOX. The following summarizes the rationale in support of this recommendation.

SOF: the PK of SOF was previously evaluated following 7-day dosing of 400 mg SOF in HCV-infected subjects with moderate and severe hepatic impairment (CPT B and C). Relative to subjects with normal hepatic function, the SOF AUC_{0-24} was 126% and 143% higher in moderate and severe hepatic impairment, respectively, while the GS-331007 AUC_{0-24} was 18% and 9% higher, respectively. Population pharmacokinetic analysis in HCV-infected subjects indicated that cirrhosis (CPT A) had no clinically relevant effect on the exposure of SOF and GS-331007 (refer to Sovaldi®, NDA 204671 for additional details).

VEL: the PK of VEL was previously evaluated following a single dose of 100 mg VEL in HCV negative subjects with moderate and severe hepatic impairment (CPT B and C). VEL plasma exposure (AUC_{inf}) was similar in subjects with moderate hepatic impairment, severe hepatic impairment, and control subjects with normal hepatic function. Population pharmacokinetic analysis in HCV-infected subjects indicated that cirrhosis (CPT A) had no clinically relevant effect on the exposure of VEL (refer to Epclusa®, NDA 208341 for additional details).

VOX: the PK of VOX was studied following a single dose of 100 mg VOX in HCV-negative subjects with moderate and severe hepatic impairment (CPT B and C). Relative to subjects with normal hepatic function, VOX plasma exposures (AUC_{inf} and C_{max}) were 299% and 238% higher in subjects with moderate hepatic impairment, and 500% and 614% higher in subjects with severe hepatic impairment (**Table 3.3.3-1**). Changes in VOX exposure are likely attributable to changes in hepatic transport and metabolism as a result of hepatic impairment. VOX unbound fraction was similar in subjects with normal hepatic function and moderate hepatic impairment, and was approximately 2-fold higher in subjects with severe hepatic impairment (refer to **Appendix 4.4.5**).

Table 3.3.3-1 GS-US-338-1126: Effect of Moderate and Severe Hepatic Impairment on the PK of VOX in Healthy Subjects

VOX PK Parameter	Mean (%CV)		% GLSM Ratio (90% CI)
	Hepatic Impairment	Normal Hepatic Function	
Moderate Hepatic Impairment, (N = 10) vs Matched Normal Hepatic Function (N = 10)			
AUC_{inf} (h*ng/mL)	3021.9 (61.0)	673.0 (70.2)	399.24 (210.89, 755.81)
C_{max} (ng/mL)	304.8 (95.4)	63.7 (53.6)	338.41 (168.96, 677.79)
Severe Hepatic Impairment, (N = 9) vs Matched Normal Hepatic Function (N = 9)			
AUC_{inf} (h*ng/mL)	5066.1 (59.3)	810.6 (62.5)	599.63 (342.36, 1050.22)
C_{max} (ng/mL)	498.1 (66.7)	58.8 (57.6)	713.92 (384.07, 1327.06)

SOF/VEL/VOX: in addition to the dedicated hepatic impairment study, the effect of compensated cirrhosis (CPT A) was examined within the population PK analysis in Phase 2 and Phase 3 studies. The population PK analysis set included 672 (42.1%) subjects with compensated cirrhosis and 926 (57.9%) subjects without cirrhosis. Population PK modeling identified compensated cirrhosis to be the most siginificant covariate on the PK of VOX. **Table 3.3.3-2** presents VOX PK parameters following administration of SOF/VEL+VOX or SOF/VEL/VOX in HCV-infected subjects with or without cirrhosis. Voxilaprevir exposure parameters were increased (AUC_{tau} 73.3%, C_{max} 74.1%, and C_{tau} 83.0%) in subjects with compensated cirrhosis compared with subjects without cirrhosis. These modestly higher VOX exposures in HCV-infected subjects with compensated cirrhosis are consistent with the findings from the dedicated hepatic impairment study for VOX (**Table 3.3.3-1**). This magnitude of increase in VOX exposure does not warrant dose adjustment in patients with compensated cirrhosis (CPT A).

SOF, GS-331007, and VEL exposures were similar in HCV-infected subjects with or without compensated cirrhosis (refer to **Appendix 4.2** for additional details).

Table 3.3.3-2 VOX PK Parameters by Cirrhosis (Compensated) Status Following Administration of SOF/VEL+VOX or SOF/VEL/VOX in HCV-Infected Subjects

VOX PK Parameter	Mean (%CV)		% GLSM Ratio (90% CI)
	Cirrhosis (N = 672)[a]	No Cirrhosis (N = 926)[b]	
AUC_{tau} (h*ng/mL)	3412 (66.0)	1970 (65.6)	173.3 (164.3, 182.7)
C_{max} (ng/mL)	252 (77.8)	148 (80.6)	174.1 (163.6, 185.2)
C_{tau} (ng/mL)	64 (73.9)	34 (65.9)	183.0 (174.4, 192.0)
Supportive Demographics Summary			
Sex, N (%)	Female: 193 (28.7%) Male: 479 (71.3%)	Female: 374 (40.4%) Male: 552 (59.6%)	

Demographic Population PK Analysis Set included HCV-infected subjects who received SOF 400 mg, VEL 100 mg, and VOX 100 mg as SOF/VEL+VOX or SOF/VEL/VOX and had evaluable PK parameters in Study GS-US-337-1468, GS-US-367-1168, GS-US-367-1169, GS-US-367-1871, GS-US-367-1170, GS-US-367-1171, GS-US-367-1172, or GS-US-367-1173.

a Of the 672 subjects, 670 subjects had evaluable PK parameters

b Of the 926 subjects, 921 subjects had evaluable PK parameters

Renal Impairment

No dose adjustment of SOF/VEL/VOX is warranted for subjects with mild or moderate renal impairment (GFR ≥ 30 ml/min/1.73m^2) and no dosage recommendations can be made for patients with severe or end stage renal disease (GFR < 30 ml/min/1.73 m^2), due to the unknown safety of SOF/VEL/VOX in those patients. The recommendation for use of SOF/VEL/VOX in subjects with renal impairment is driven by its most restrictive component in the setting of renal impairment, GS-331007. The following summarizes the rationale in support of this recommendation.

SOF: the PK of SOF was previously studied in HCV negative subjects with mild, moderate, and severe renal impairment following a single dose of SOF 400 mg, and in 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. Compared with subjects with normal renal function, the SOF AUC_{inf} was approximately 61%, 107%, and 171% higher and the GS-331007 AUC_{inf} was approximately 55%, 88% and 451% higher in subjects with mild, moderate, and severe renal impairment, respectively. In subjects with ESRD, compared with subjects with normal renal function, SOF and GS-331007 AUC_{inf} was approximately 28% and 1283% higher when SOF was dosed 1 hour before hemodialysis compared with 60% and 2072% higher when SOF was dosed 1 hour after hemodialysis, respectively (refer to Sovaldi®, NDA 204671 for additional details).

VEL: the PK of VEL was previously studied following a single dose of 100 mg VEL in HCV negative subjects with severe renal impairment. No clinically relevant differences in VEL PK were observed between healthy subjects and subjects with severe renal impairment (refere to Epclusa®, NDA 208341).

VOX: the PK of VOX was studied with a single dose of 100 mg VOX in HCV negative subjects with severe renal impairment (reduced-design). VOX AUC and C_{max} were approximately 71% and 45% higher, respectively in subjects with severe renal impairment compared with subjects with normal renal function (**Table 3.3.3-3**). Protein binding was similar between groups (refer to **Appendix 4.4.4**). While VOX is eliminated in the feces with no detectable VOX parent or metabolite in the urine, results of the renal impairment study were consistent with literature data demonstrating that renal impairment may potentially alter the PK of compounds by changes in intestinal and hepatic metabolism and transport.

Table 3.3.3-3 GS-US-338-1125: Effect of Severe Renal Impairment on the PK of VOX in Healthy Subjects

VOX PK Parameter	Mean (%CV)		% GLSM Ratio (90% CI)
	Severe Renal Impairment (N = 10)	Normal Renal Function (N = 10)	
AUC_{inf} (h*ng/mL)	1033.7 (76.0)	525.8 (57.3)	171.27 (97.65, 300.38)
AUC_{last} (h*ng/mL)	910.8 (82.2)	444.9 (55.3)	172.92 (97.93, 305.33)
C_{max} (ng/mL)	68.4 (86.9)	38.4 (60.5)	145.43 (83.77, 252.48)

SOF/VEL/VOX: in addition to the dedicated renal impairment study, the impact of renal function was evaluated with population PK analysis in the Phase 2 and Phase 3 studies by categorizing subjects with varying degrees of renal impairment including normal, mild, and moderate renal impairment (refer to **Appendix 4.2**). The categories were based on subjects' estimated baseline CLcr (calculated by the Cockcroft-Gault equation) (Normal: CLcr ⩾ 90 mL/min; Mild: CLcr = 60-89 mL/min; and Moderate: CLcr = 30-59 mL/min) according to the FDA guidance on PK in patients with impaired renal function. In the population PK analysis set, 75.7% (N = 1210) of subjects had normal renal function, 22.6% (N = 361) of subjects had mild renal impairment, and 1.7% (N = 27) of subjects had moderate renal impairment.

Population PK modeling did not identify CLcr as a clinically relevant covariate for VOX PK. **Table 3.3.3-4** presents VOX PK parameters by degrees of renal impairment following administration of SOF/VEL+VOX or SOF/VEL/VOX in HCV-infected subjects. VOX exposures were modestly higher in subjects with moderate renal impairment, though this difference may be accounted for by the presence of a higher proportion of females, who have higher exposure than males. Overall, these findings are consistent with the modest effect of severe renal impairment on VOX exposure (**Table 3.3.3-3**).

Population PK modeling did not identify CLcr as a statistically significant covariate for VEL PK. VEL exposure was modestly higher in subjects with moderate renal impairment, though this difference may be accounted for by the presence of a higher proportion of females, which are demonstrated to have higher exposure than males. Overall, these findings are consistent with the modest effect of severe renal impairment on VEL exposure (refer to Epclusa®, NDA 208341 for additional details).

Population PK modeling identified baseline CLcr as a statistically significant covariate (albeit minor) for SOF PK, and the greatest contributor to the variability in GS-331007 exposure. SOF exposure was similar across degrees of renal impairment, and renal function was inversely correlated with GS-331007 AUC_{tau} and C_{max}, showing some differences between subjects with moderate renal impairment compared with subjects with normal renal function. These findings are consistent with the findings of the renal impairment study (refer to Sovaldi®, NDA 204671 for additional details).

Table 3.3.3-4 VOX PK Parameters across Degrees of Renal Impairment Following Administration of SOF/VEL+VOX or SOF/VEL/VOX in HCV-Infected Subjects

VOX PK Parameter	Mean (% CV)		
	Normal Renal Function (N = 1210) [a]	Mild Renal Impairment (N = 361) [b]	Moderate Renal Impairment (N = 27) [c]
AUC_{tau} (h*ng/mL)	2519 (75.6)	2710 (69.4)	3415 (49.8)
C_{max} (ng/mL)	186 (87.3)	207 (83.2)	248 (55.6)
C_{tau} (ng/mL)	46 (84.4)	49 (76.4)	64 (54.2)
Supportive Demographics Summary			
CL_{cr} (mL/min)[d]	[90.0, 122.4, 297.5]	[60.3, 79.8, 89.8]	[39.9, 54.5, 59.5]
Sex, N (%)	Female: 344 (28.4%) Male: 866 (71.6%)	Female: 200 (55.4%) Male: 161 (44.6%)	Female: 23 (85.2%) Male: 4 (14.8%)
Cirrhosis, N (%)	Yes: 551 (45.5%) No: 659 (54.5%)	Yes: 109 (30.2%) No: 252 (69.8%)	Yes: 12 (44.4%) No: 15 (55.6%)

Demographic Population PK Analysis Set included HCV-infected subjects who received SOF 400 mg, VEL 100 mg, and VOX 100 mg as SOF/VEL+VOX or SOF/VEL/VOX and had evaluable PK parameters in Study GS-US-337-1468, GS-US-367-1168, GS-US-367-1169, GS-US-367-1871, GS-US-367-1170, GS-US-367-1171, GS-US-367-1172, or GS-US-367-1173.

a Of the 1210 subjects, 1205 subjects had evaluable PK parameters
b Of the 361 subjects, 360 subjects had evaluable PK parameters
c Of the 27 subjects, 26 subjects had evaluable PK parameters
d CL_{cr} presented as [min, median, max]

Demographic Factors

No clinically relevant effect has been found for age, race, BMI or sex. Several demographic factors, such as age (18 to 85 years), sex, race, and body mass index (BMI), have been evaluated to determine if these factors have an effect on the PK of SOF, GS-331007, VEL and VOX. No effect has been found for age, race or BMI. Although sex was identified as a statistically significant covariate for SOF, GS-331007, VEL, and VOX PK (17 to 65% higher exposure in females), based on the population PK analyses (see **Appendix 4.2**), the increased exposure in females does not warrant dose adjustment based on sex.

3.3.4 Are there clinically relevant food-drug or drug-drug interactions, and what is the appropriate management strategy?

Food effect

The effect of food on the PK of SOF, VEL, and VOX was evaluated following administration of SOF/VEL+VOX with both a light-fat meal and a moderate-fat meal (Study GS-US-338-1130) and the proposed commercial formulation, SOF/VEL/VOX FDC, with a high-fat/high-calorie meal (Study GS-US-367-1176) (see **Appendix 4.4.6 and 4.4.7**). It is

notable that a DDI was identified between SOF/VEL and VOX in the fasted state, resulting in lower VOX exposure as compared to VOX alone (GS-US-338-1130). Administration of SOF/VEL+VOX or SOF/VEL/VOX with a range of meal types mitigated the interaction (**Table 3.3.4-1).** These collective findings form the basis for recommending that SOF/VEL/VOX be administered with food.

Table 3.3.4-1 GS-US-338-1130 and GS-US-367-1176: Effects of Coadministration of VOX, VEL, and SOF With or Without Food on the PK of SOF, GS-331007, VEL, and VOX

% GLSM Ratio *(90% CI)*	**GS-US-338-1130 (SOF/VEL+VOX in Combination vs Individual, Fasted)**			
	SOF	GS-331007	VEL	VOX
AUC_{tau}	↔	↔	↔	37 *(30, 45)*
C_{max}	↔	↔	↔	28 *(22, 36)*
	GS-US-338-1176 (SOF/VEL/VOX Fed High-fat/High-calorie vs Fasted)			
	SOF	GS-331007	VEL	VOX
AUC_{inf}	164 *(139, 192)*	↔	140 *(113, 175)*	535 *(428, 669)*
C_{max}	↔	65 *(60, 70)*	137 *(111, 170)*	780 *(616, 988)*
	GS-US-338-1130 (SOF/VEL+VOX Fed Moderate-fat Meal vs Fasted)			
	SOF	GS-331007	VEL	VOX
AUC_{inf}	244 *(169, 353)*	↔	229 *(134, 392)*	285 *(209, 387)*
C_{max}	176 *(123, 254)*	↔	246 *(130, 468)*	359 *(251, 514)*
	GS-US-338-1130 (SOF/VEL+VOX Fed Light-fat Meal vs Fasted)			
	SOF	GS-331007	VEL	VOX
AUC_{inf}	218 *(151, 314)*	↔	266 *(161, 438)*	212 *(159, 282)*
C_{max}	173 *(121, 247)*	↔	287 *(161, 513)*	247 *(190, 323)*

GLSM: geometric least-squares mean.
↔: 90% CIs of the GLSM ratio were within the predetermined lack of PK alteration boundaries of 70% to 143% for Studies GS-US-338-1130 and GS-US-367-1176.

The increase in VOX exposure when administered with food is the net effect of multiple factors, including increased solubility of VOX and mitigation of the DDI in which VOX exposure is decreased by SOF/VEL (mechanism unknown). SOF, GS-331007, and VEL exposures are not affected to a clinically relevant extent by the presence of food. Based on these data, SOF/VEL/VOX was administered with food in Phase 2 and Phase 3 studies.

It is noted that since a high-fat meal can increase VOX exposure to a greater extent than a moderate-fat meal (435% *vs.* 185% increase in AUC, 680% *vs.* 259% increase in C_{max}), there

is a concern for how a high-fat meal may affect data interpretation in DDI studies with SOF/VEL/VOX as perpetrator drugs, which were all performed under moderate-fat meal conditions.

The DDI perpetrator studies were designed to evaluate conditions where VOX exposure encompassed the clinical efficacious exposure of around 2577 h*ng/mL, based on population PK analysis. This exposure represents a mixture of patients who may have taken light-fat, moderate-fat, or high-fat meals. VOX exposure was found to be 3 to 4X higher in HCV patients *vs.* healthy subjects in early drug development, and thus the dose chosen for use in DDI perpetrator studies was SOF/VEL/VOX + VOX at 400/100/100 + 100 mg (total VOX dose of 200 mg) to cover the higher VOX exposure in HCV patients. However, the final VOX exposure in DDI perpetrator studies in healthy subjects ended up ranging from 4000 to 6000 h*ng/mL. These exposures were much higher than the clinical efficacious exposure of 2577 h*ng/mL and thus mitigates the concern for how a high-fat meal could impact DDI data interpretation.

Drug-drug interactions

As summarized in **Table 3.3.4-2**, SOF, VEL, and VOX are all substrates of drug transporters P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), while GS-331007 is not. VOX, and to a lesser extent VEL, are also substrates of OATP1B1 and OATP1B3. In addition, VEL is a substrate of CYP2B6, CYP2C8 and CYP3A4 with slow turnover, and VOX is a substrate of CYP1A2, CYP2C8, and primarily CYP3A4 with slow turnover (see **Appendix 4.4.13**). Drugs that are inducers of P-gp, and/or moderate to potent inducers of CYP2B6, CYP2C8, or CYP3A4 may decrease plasma concentrations of SOF, VEL, and/or VOX leading to reduced therapeutic effect of SOF/VEL/VOX. Co-administration with drugs that inhibit P-gp and/or BCRP may increase SOF, VEL, and/or VOX exposure. Co-administration with drugs that inhibit OATP may increase VOX exposure. Drugs that inhibit CYP2B6, CYP2C8, and CYP3A4 may increase plasma exposure of VEL and/or VOX.

SOF, GS-331007, VEL and VOX are not inhibitors or inducers of CYP or UGT1A1 enzymes, which is confirmed by clinical DDI studies with minimal effect on the following drugs: darunavir and rilpivirine (CYP3A4 substrates), efavirenz (CYP3A, CYP2B6 substrate), elvitegravir (CYP3A and UGT1A1/3 substrate). VEL and VOX are inhibitors of drug transporters P-gp, BCRP, OATP1B1 and OATP1B3, and co-administration of SOF/VEL/VOX with drugs that are substrates of these transporters may increase the exposure of such drugs. VEL and VOX are not inhibitors of renal transporters OCT2, OAT1, OAT3 or MATE 1 (see **Appendix 4.4.13**). SOF and GS-337001 showed minimal transporter inhibition.

Table 3.3.4-2 Substrate and Inhibition Profiles of VOX, VEL, SOF and GS-331007 for Enzymes and Transporters

		Substrate	Inhibition
VOX	Enzyme	CYP1A2, CYP2C8, CYP3A4	None
	Transporter	P-gp, BCRP, OATP1B1/3	P-gp, BCRP, BSEP, OATP1B1/3

VEL	Enzyme	CYP2B6, CYP2C8, CYP3A4	None
	Transporter	P-gp, BCRP, OATP1B1/3	P-gp, BCRP, BSEP, OATP1B1/3, OATP2B1
SOF	Enzyme	CatA/CES1, HINT1*	None
	Transporter	P-gp, BCRP	None
GS-331007	Enzyme	None	None
	Transporter	None	None

* CatA: cathepsin A; CES1: carboxylesterase 1; HINT1: histidine triad nucleotide-binding protein 1

An extensive battery of DDI studies has been conducted to evaluate possible drug interactions with SOF/VEL/VOX as a perpetrator or victim of interactions with frequently co-administered drugs in the HCV population, including acid reducing agents, antiarrhythmics, anticoagulants, anticonvulsants, antimycobacterials, antiretrovirals, herbal supplements, HMG-CoA reductase inhibitors, immunosuppressants, oral contraceptives, etc.

SOF/VEL/VOX or its component(s) as victim drug(s):

Table 3.3.4-3 shows statistically significant (90% CI is outside of 80 to 125%) changes in PK parameters of SOF, GS-331007, VEL, and VOX in the presence of the coadministered drug(s).

Table 3.3.4-3 Drug Interactions: Changes in Pharmacokinetic Parameters for Sofosbuvir, GS-331007, Velpatasvir, and Voxilaprevir in the Presence of the Coadministered Drug[a]

Co-administered Drug		Sofosbuvir (SOF)/ Velpatasvir (VEL)/ Voxilaprevir (VOX)			Geometric Mean Ratio (90% CI) of Sofosbuvir, GS-331007, Velpatasvir, and Voxilaprevir PK With/Without Coadministered Drug No Effect=1.00			
Drug	Dosage (mg)	Active Component	Dosage (mg)	N	Component	C_{max}	AUC	C_{min}
Atazanavir + ritonavir	300 + 100 single dose	SOF/VEL/ VOX	400/100/100 single dose	15	sofosbuvir	1.29 (1.09, 1.52)	1.40 (1.25, 1.57)	NA
					GS-331007	1.05 (0.99, 1.12)	1.25 (1.16, 1.36)	NA
					velpatasvir	1.29 (1.07, 1.56)	1.93 (1.58, 2.36)	NA
					voxilaprevir	4.42 (3.65, 5.35)	4.31 (3.76, 4.93)	NA
Cyclosporine	600 single dose	SOF	400 single dose	19	sofosbuvir	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
		VEL	100 single dose	12	velpatasvir	1.56 (1.22, 2.01)	2.03 (1.51, 2.71)	NA
		VOX	100 single dose	25	voxilaprevir	19.02 (14.12,	9.39 (7.37,	NA

Co-administered Drug		Sofosbuvir (SOF)/ Velpatasvir (VEL)/ Voxilaprevir (VOX)		N	Geometric Mean Ratio (90% CI) of Sofosbuvir, GS-331007, Velpatasvir, and Voxilaprevir PK With/Without Coadministered Drug No Effect=1.00			
Drug	Dosage (mg)	Active Component	Dosage (mg)		Component	C_{max}	AUC	C_{min}
						25.62)	11.96)	
Darunavir + ritonavir + emtricitabine/ tenofovir DF	800 + 100 + 200/300 once daily	SOF/VEL/ VOX + VOX	400/100/100 + 100 once daily	29	sofosbuvir	0.70 (0.62, 0.78)	0.78 (0.73, 0.83)	NA
					GS-331007	1.06 (1.01, 1.10)	1.15 (1.12, 1.19)	NA
					velpatasvir	0.78 (0.73, 0.84)	0.95 (0.88, 1.02)	1.16 (1.07, 1.26)
					voxilaprevir	1.72 (1.51, 1.97)	2.43 (2.15, 2.75)	4.00 (3.44, 4.65)
Efavirenz/ emtricitabine/ tenofovir DF[b]	600/200/300 once daily	SOF/VEL	400/100 once daily	14	sofosbuvir	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)
					velpatasvir	0.53 (0.43, 0.64)	0.47 (0.39, 0.57)	0.43 (0.36, 0.52)
Elvitegravir/ cobicistat/ emtricitabine/ tenofovir alafenamide[c]	150/150/200/ 10 once daily	SOF/VEL/ VOX + VOX	400/100/100 + 100 once daily	29	sofosbuvir	1.27 (1.09, 1.48)	1.22 (1.12, 1.32)	NA
					GS-331007	1.28 (1.25, 1.32)	1.43 (1.39, 1.47)	NA
					velpatasvir	0.96 (0.89, 1.04)	1.16 (1.06, 1.27)	1.46 (1.30, 1.64)
					voxilaprevir	1.92 (1.63, 2.26)	2.71 (2.30, 3.19)	4.50 (3.68, 5.50)
Ketoconazole	200 twice daily	VEL	100 single dose	12	velpatasvir	1.29 (1.02, 1.64)	1.71 (1.35, 2.18)	NA
Methadone	30 to 130 daily	SOF	400 once daily	14	sofosbuvir	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 2 hours prior to [TRADENAME]	SOF/VEL/ VOX	400/100/100 single dose	34	sofosbuvir	0.77 (0.65, 0.91)	0.73 (0.67, 0.79)	NA
					GS-331007	1.27 (1.20, 1.34)	0.97 (0.94, 1.01)	NA
					velpatasvir	0.43 (0.38, 0.49)	0.46 (0.41, 0.52)	NA
					voxilaprevir	0.76 (0.69, 0.85)	0.80 (0.74, 0.87)	NA
	20 once daily 4 hours after [TRADENAME]	SOF/VEL/ VOX	400/100/100 single dose	34	sofosbuvir	0.94 (0.83, 1.06)	0.82 (0.77, 0.87)	NA
					GS-331007	1.19 (1.13, 1.26)	0.99 (0.97, 1.01)	NA
					velpatasvir	0.49 (0.43, 0.55)	0.49 (0.43, 0.55)	NA
					voxilaprevir	1.08 (0.96, 1.22)	0.95 (0.88, 1.03)	NA
Rifampin	600 once daily	SOF	400 single dose	17	sofosbuvir	0.23 (0.19, 0.29)	0.28 (0.24, 0.32)	NA

Co-administered Drug		Sofosbuvir (SOF)/ Velpatasvir (VEL)/ Voxilaprevir (VOX)		N	Geometric Mean Ratio (90% CI) of Sofosbuvir, GS-331007, Velpatasvir, and Voxilaprevir PK With/Without Coadministered Drug No Effect=1.00			
Drug	Dosage (mg)	Active Component	Dosage (mg)		Component	C_{max}	AUC	C_{min}
					GS-331007	1.23 (1.14, 1.34)	0.95 (0.88, 1.03)	NA
		VEL	100 single dose	12	velpatasvir	0.29 (0.23, 0.37)	0.18 (0.15, 0.22)	NA
		VOX	100 single dose	24	voxilaprevir	0.91 (0.76, 1.10)	0.27 (0.23, 0.31)	NA
	600 single dose	VEL	100 single dose	12	velpatasvir	1.28 (1.05, 1.56)	1.46 (1.17, 1.83)	NA
		VOX	100 single dose	24	voxilaprevir	11.10 (8.23,14.98)	7.91 (6.20,10.09)	NA
Tacrolimus	5 single dose	SOF	400 single dose	16	sofosbuvir	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
Voriconazole	200 twice daily	VOX	100 single dose	24	voxilaprevir	1.13 (0.98, 1.31)	1.84 (1.66, 2.03)	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).

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

SOF/VEL/VOX or its component(s) as perpetrator drug(s):

Table 3.3.4-4 shows statistically significant (90% CI is outside of 80 to 125%) changes in PK parameters of coadministered drugs in the presence of SOF/VEL/VOX or its component(s).

Table 3.3.4-4 Changes in Pharmacokinetic Parameters for Coadministered Drug in the Presence of SOF/VEL/VOX or its component(s)[a]

Co-administered Drug		Sofosbuvir (SOF)/ Velpatasvir (VEL)/ Voxilaprevir (VOX)		N	Geometric Mean Ratio (90% CI) of Coadministered Drug PK With/Without SOF/VEL/VOX or its components No Effect=1.00		
Drug	Dosage (mg)	Active Component	Dosage (mg)		C_{max}	AUC	C_{min}
Cyclosporine	600 single dose	SOF	400 single dose	19	1.06 (0.94, 1.18)	0.98 (0.85, 1.14)	NA
		VEL	100 single dose	12	0.92 (0.82, 1.02)	0.88 (0.78, 1.00)	NA

Co-administered Drug		Sofosbuvir (SOF)/ Velpatasvir (VEL)/ Voxilaprevir (VOX)		N	Geometric Mean Ratio (90% CI) of Coadministered Drug PK With/Without SOF/VEL/VOX or its components No Effect=1.00		
Drug	Dosage (mg)	Active Component	Dosage (mg)		C_{max}	AUC	C_{min}
		VOX	100 single dose	24	0.95 (0.88, 1.03)	0.94 (0.84, 1.06)	NA
Dabigatran etexilate	75 single dose	SOF/VEL/ VOX + VOX	400/100/100 + 100 once daily	36	2.87 (2.61, 3.15)	2.61 (2.41, 2.82)	NA
Darunavir + ritonavir + emtricitabine/ tenofovir DF[b]	darunavir 800 once daily	SOF/VEL/ VOX + VOX	400/100/100 + 100 once daily	29	0.89 (0.85, 0.94)	0.86 (0.81, 0.91)	0.66 (0.58, 0.74)
	ritonavir 100 once daily				1.60 (1.47, 1.75)	1.45 (1.35, 1.57)	0.80 (0.72, 0.89)
	emtricitabine 200 once daily				0.88 (0.82, 0.94)	0.99 (0.96, 1.03)	1.20 (1.15, 1.26)
	tenofovir DF 300 once daily				1.48 (1.36, 1.61)	1.39 (1.32, 1.46)	1.47 (1.38, 1.56)
Digoxin	0.25 single dose	VEL	100 once daily	21	1.88 (1.71, 2.08)	1.34 (1.13, 1.60)	NA
Efavirenz/ emtricitabine/ tenofovir DF[c]	efavirenz 600 once daily	SOF/VEL	400/100 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	elvitegravir 150 once daily	SOF/VEL/ VOX + VOX	400/100/100 + 100 once daily	29	0.79 (0.75, 0.85)	0.94 (0.88, 1.00)	1.32 (1.17, 1.49)
	cobicistat 150 once daily				1.23 (1.18, 1.28)	1.50 (1.44, 1.58)	3.50 (3.01, 4.07)
	emtricitabine 200 once daily				0.87 (0.84, 0.91)	0.96 (0.94, 0.99)	1.14 (1.09, 1.20)
	tenofovir alafenamide 10 once daily				0.79 (0.68, 0.92)	0.93 (0.85, 1.01)	NA
Emtricitabine/ rilpivirine/tenofovir alafenamide[e]	emtricitabine 200 once daily	SOF/VEL/ VOX + VOX	400/100/100 + 100 once daily	30	0.88 (0.83, 0.93)	0.93 (0.90, 0.96)	1.07 (1.01, 1.14)
	rilpivirine 25 once daily				0.79 (0.74, 0.84)	0.80 (0.76, 0.85)	0.82 (0.77, 0.87)
	tenofovir alafenamide 25 once daily				1.32 (1.17, 1.48)	1.52 (1.43, 1.61)	NA
Pravastatin	pravastatin 40 single dose	SOF/VEL/ VOX + VOX	400/100/100 + 100 once daily	19	1.89 (1.53, 2.34)	2.16 (1.79, 2.60)	NA
Rosuvastatin	rosuvastatin 10 single dose	SOF/VEL/ VOX + VOX	400/100/100 + 100 once daily	19	18.88 (16.23, 21.96)	7.39 (6.68 8.18)	NA
Raltegravir + emtricitabine/ tenofovir DF	emtricitabine 200 once daily	SOF/VEL	400/100 once daily	30	1.08 (1.04, 1.12)	1.05 (1.03, 1.07)	1.02 (0.97, 1.08)

Co-administered Drug		Sofosbuvir (SOF)/ Velpatasvir (VEL)/ Voxilaprevir (VOX)		N	Geometric Mean Ratio (90% CI) of Coadministered Drug PK With/Without SOF/VEL/VOX or its components No Effect=1.00		
Drug	Dosage (mg)	Active Component	Dosage (mg)		C_{max}	AUC	C_{min}
	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	SOF	400 once daily	16	0.73 (0.59, 0.90)	1.09 (0.84, 1.40)	NA

NA = not available/not applicable
a. All interaction studies conducted in healthy volunteers
b. Comparison based on exposures when administered as darunavir + ritonavir + emtricitabine/tenofovir DF.
c. Administered as ATRIPLA (efavirenz, emtricitabine and tenofovir DF fixed-dose combination).
d. Administered as GENVOYA (elvitegravir, cobicistat, emtricitabine and tenofovir alafenamide fixed-dose combination).
e. Administered as ODEFSEY (emtricitabine, rilpivirine, and tenofovir alafenamide fixed-dose combination).

No effect on the pharmacokinetic parameters of the following coadministered drugs was observed with SOF/VEL/VOX (ethinyl estradiol/norgestimate) or its components SOF/VEL (dolutegravir) or SOF (methadone).

Management strategy for clinically significant DDIs

The proposed management strategy for clinically important DDIs is generally acceptable. The potentially or established clinically important DDIs for specific drugs and their management strategy are summarized in **Table 3.3.4-5**, including the Agency's recommendations to be incorporated in the labeling. An alteration in dose or regimen may be recommended based on drug interaction studies or a mechanistically predicted interaction.

- The reviewers agree with the following DDI management strategy proposed by the Applicant:
  1. P-gp inducers and/or moderate to potent CYP inducers (e.g., St. John's wort, carbamazepine) may decrease concentrations of SOF, VEL, and/or VOX, and are **NOT** recommended for co-administration.
  2. Inhibitors of OATP may increase VOX exposure substantially, and are **NOT** recommended for co-administration.
  3. SOF/VEL/VOX may be coadministered with P-gp, BCRP, and CYP450 inhibitors due to the lack of clinicaly relevant changes in exposure of VOX, VEL, and SOF.

- The reviewers have the following recommendations for DDI management:
  1. Omeprazole 20 mg can be coadministered with SOF/VEL/VOX as Applicant proposes, however, use with other PPIs have not been studied.
  2. Rifampin decreases exposure of SOF, VEL, and VOX following multiple doses and significantly increases exposure of VOX following a single dose, and thus the coadministration is **CONTRAINDICATED**.

3. SOF/VEL/VOX FDC tablets may increase the exposure of BCRP substrates (e.g., methotrexate, mitoxantrone, imatinib, irinotecan, lapatinib, rosuvastatin, sulfasalazine, topotecan) substantially, and thus are **NOT** recommended for co-administration with BCRP substrates.
4. SOF/VEL/VOX FDC tablets may increase the exposure of statins substantially, which are substrates of P-gp, BCRP, and/or OATP (e.g., atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin). For pravastatin, the dose should not exceed 40 mg. For rosuvastatin, the coadministration is not recommended. For pitavastatin, the coadministration is not recommended due to an increased risk of severe myopathy at > 4 mg in premarketing clinical studies. For other unstudied statins, including atorvastatin, fluvastatin, lovastatin, simvastatin, the lowest necessary dose should be used.

- Key review questions relating to DDIs:

1. Coadministration with PPIs:

PPIs are not recommended for coadministration with SOF/VEL due to a ~50% decrease in VEL exposure and the risk in compromising SOF/VEL efficacy, especially for HCV genotype 3 (refer to Epclusa®, NDA 208341 for additional details). A similar DDI result (~ 50% VEL exposure decrease) was observed when dosing omeprazole at 20 mg 2 h before or 4 h after administration of SOF/VEL/VOX at 400/100/100 mg, with no effect on the exposure of SOF, GS-331007, and VOX.

To support the proposal for coadministration of omeprazole with SOF/VEL/VOX, the Applicant claims:

1) Decreases in VEL exposure in the presence of omeprazole were not uniform across the study population. Those with highest exposure at baseline exhibited the largest decreases in VEL exposure, while those with lower exposure at baseline were minimally affected.
2) ~ 50% decrease of VEL exposure is not expected to impact the efficacy of SOF/VEL/VOX.

Regarding the first claim, the DDI should be assessed by evaluating the AUC_{test} to $AUC_{reference}$ ratios, not by the absolute difference between the AUC_{test} and $AUC_{reference}$. There was no trend suggesting that subjects with lower $AUC_{reference}$ had a lower degree of interaction when assessing AUC_{test} to $AUC_{reference}$ ratios (see **Appendix 4.4.10**).

Regarding the second claim, the reviewer evaluated the SVR12 rate in Phase 3 studies and found high SVR12 (> 95%) was achieved across the entire VEL exposure range (AUC range: 600 to 12,000 h*ng/mL), even for genotype 3 in both DAA-experienced and DAA-naïve patients (mean AUC: ~4000 h*ng/mL). VEL exposure from SOF/VEL/VOX at 400/100/100 mg with food was ~ 2-fold higher than VEL exposure from Epclusa, due to a combination of food effect on VEL absorption and a greater degree of intestinal P-gp and BCRP inhibition by VOX when administered with food. With a ~50% decrease in VEL AUC when coadministered with omeprazole, predicted

mean VEL exposure for patients would still be high at ~ 2000 h*ng/mL, which is similar to VEL exposure when HCV patients are administered Epclusa, and the mean percent of maximal antiviral activity of VEL is maintained at 99% based on the E-R relationship for VEL monotherapy. Moreover, only VEL exposure is decreased, while the exposure and thus viral suppression from SOF and VOX should not be affected. In summary, the impact of 50% lower VEL exposures in the context of SOF/VEL/VOX dosing is unlikely to be meaningful. Thus, it is acceptable to allow co-administration of omeprazole (20 mg) with SOF/VEL/VOX.

2. Statin coadministration:

The applicant conducted DDI studies with two statins: pravastatin and rosuvastatin. A siginificant DDI was observed with both pravastatin (OATP substrate) and rosuvastatin (BCRP and OATP substrate). Currently, there are five other marketed statins which were not studied: atorvastatin, fluvastatin, lovastatin, pitavastatin, and simvastatin. All these statins are known substrates of OATP, and P-gp and BCRP may also be involved in their absorption and excretion based on the most recent research (*Clin Pharmacol Ther. 2010; 87: 130-133*).

Cyclosporine (CsA), an inhibitor of P-gp, BCRP, OATP, MRP2, and a weak inhibitor of CYP3A4, has similar transporter inhibition mechanisms as SOF/VEL/VOX, where VEL and VOX are inhibitors of P-gp, BCRP, OATP, BSEP. In addition, CsA has caused comparable increases in rosuvastatin and pravastatin exposures as SOF/VEL/VOX. CsA increases the exposure of statins significantly with AUC and C_{max} ratios ranging from 5 to 12 for pitavastatin, atorvastatin, simvastatin, and lovastatin. Given the similarity in pravastatin and rosuvastatin results, the impact of CsA on the rest of the statins suggests the potential for a significant DDI with statins when coadministered with SOF/VEL/VOX.

High exposure to statins has been associated with serious myopathy, including rhabdomyolysis. For pitavastatin, doses greater than 4 mg have been associated with an increased risk for severe myopathy in premarketing clinical studies. For simvastatin, the myopathy rate is 0.02%/0.08%/0.53% at 20/40/80 mg, respectively. In addition, serious safety events (rhabdomyolysis) have been reported with the use of various DAAs with atorvastatin, rosuvastatin, and simvastatin.

Based on this information, for pitavastatin, coadministration is not recommended due to an increased risk of severe myopathy at > 4 mg in premarketing clinical studies. For other unstudied statins, including atorvastatin, fluvastatin, lovastatin, simvastatin, the lowest necessary dose should be used, due to the potential for increased risk of myopathy, including rhabdomyolysis associated with higher exposure of statins. The applicant's strategy that pravastatin can be coadministered at doses ≤ 40 mg, and "coadministration with rosuvastatin is not recommended" is acceptable, based on clinical DDI studies for these two statins. The recommendations for all statins were agreed upon in a consult with the metabolic products review team in OCP.

Table 3.3.4-5 Established and Potentially Significant Drug Interactions[c]

Concomitant Drug Class: Drug Name	Effect on Concentration[a]	Clinical Effect/Recommendation
Acid Reducing Agents:		
	↓ velpatasvir	Velpatasvir solubility decreases as pH increases. Drugs that increase gastric pH are expected to decrease concentration of velpatasvir.
Antacids (e.g., aluminum and magnesium hydroxide)		Separate antacid and Vosevi administration by 4 hours.
H_2-receptor antagonists (e.g., famotidine)[b]		H_2-receptor antagonists may be administered simultaneously with or staggered from Vosevi at a dose that does not exceed doses comparable with famotidine 40 mg twice daily.
Proton-pump inhibitors (e.g., omeprazole)[b]		Omeprazole 20 mg can be administered with Vosevi. Use with other proton pump-inhibitors has not been studied.
Antiarrhythmics:		
amiodarone	Effect on amiodarone, sofosbuvir, velpatasvir, and voxilaprevir concentrations unknown	Coadministration of amiodarone with Vosevi may result in serious symptomatic bradycardia. The mechanism of this effect is unknown. Coadministration of amiodarone with Vosevi is not recommended; if coadministration is required, cardiac monitoring is recommended *[see Warnings and Precautions (5.2)]*.
digoxin[b]	↑ digoxin	Therapeutic concentration monitoring of digoxin is recommended when coadministered with Vosevi. Refer to digoxin prescribing information for monitoring and dose modification recommendations for concentration increases with unclear magnitude.
Anticoagulants:		
dabigatran etexilate[b]	↑ dabigatran	No dose adjustment of dabigatran is required when coadministered with Vosevi in patients with normal renal function and mild (50–80 mL/min) renal impairment. In patients with moderate (30–50 mL/min) renal impairment, reduce dose of dabigatran to 75 mg twice-daily. Vosevi is not recommended for severe renal impairment or ESRD.
Anticonvulsants:		
carbamazepine phenytoin phenobarbital oxcarbazepine	↓ sofosbuvir ↓ velpatasvir ↓ voxilaprevir	Coadministration is not recommended.

Antimycobacterials:		
rifampin[b]	↓ sofosbuvir ↓ velpatasvir ↑ voxilaprevir (single dose), ↓ voxilaprevir (multiple dose)	**Coadministration with rifampin is contraindicated [see Contraindications (4)]**
rifabutin rifapentine	↓ sofosbuvir ↓ velpatasvir ↓ voxilaprevir	Coadministration is not recommended.
Antiretrovirals:		
atazanavir[b] lopinavir	↑ voxilaprevir	Coadministration of Vosevi with atazanavir- or lopinavir-containing regimens is not recommended.
tipranavir/ritonavir	↓ sofosbuvir ↓ velpatasvir	Coadministration is not recommended. The effect on voxilaprevir is unknown.
efavirenz[b]	↓ velpatasvir ↓ voxilaprevir	Coadministration of Vosevi with efavirenz-containing regimens is not recommended.
tenofovir disoproxil fumarate (tenofovir DF)[b]	↑ tenofovir	Monitor for tenofovir-associated adverse reactions in patients receiving Vosevi concomitantly with a regimen containing tenofovir DF. Refer to the prescribing information of the tenofovir DF-containing product for recommendations on renal function monitoring.
Herbal Supplements:		
St. John's wort	↓ sofosbuvir ↓ velpatasvir ↓ voxilaprevir	Coadministration is not recommended.
HMG-CoA Reductase Inhibitors:		
pravastatin[b]	↑ pravastatin	Coadministration of Vosevi with pravastatin has been shown to increase the concentration of pravastatin, which is associated with increased risk of myopathy, including rhabdomyolysis. Pravastatin may be administered with Vosevi at a dose that does not exceed pravastatin 40 mg.

rosuvastatin[b]	↑ rosuvastatin	Coadministration of Vosevi with rosuvastatin may significantly increase the concentration of rosuvastatin which is associated with increased risk of myopathy, including rhabdomyolysis. Coadministration of Vosevi with rosuvastatin is not recommended.
Pitavastatin	↑ pitavastatin	Coadministration with Vosevi may increase pitavastatin concentrations and is not recommended, due to an increased risk of severe myopathy.
atorvastatin fluvastatin lovastatin simvastatin	↑ atorvastatin ↑ fluvastatin ↑ lovastatin ↑ simvastatin	Coadministration with Vosevi may increase the concentrations of atorvastatin, fluvastatin, lovastatin, and simvastatin, which is associated with increased risk of myopathy, including rhabdomyolysis. The lowest necessary dose should be used for those statins.
Immunosuppresants:		
cyclosporine[b]	↑ voxilaprevir	Coadministration of voxilaprevir with cyclosporine has been shown to substantially increase the plasma concentration of voxilaprevir, the safety of which has not been established. Coadministration of Vosevi with cyclosporine is not recommended.

a. ↓ = decrease, ↑ = increase
b. These interactions have been studied in healthy adults.
c. For all other drugs except those marked as b, the interactions are speculative prediction.

4 Appendix

4.1 Summary of Bioanalytical Method Validation

Multiple validated assays were used for quantification of SOF, GS-331007, VEL, VOX and coadministered drugs in various matrices for VOX and SOF/VEL/VOX clinical studies.

Three bioanalytical labs have been used for quantification of SOF, GS-331007, VEL, VOX and coadministered drugs:

- [redacted] (b) (4) for VOX and cyclosporine
- [redacted] (b) (4) for dabigatran [total (unconjugated and dabigatran glucuronide) and free (unconjugated dabigatran)]
- [redacted] (b) (4) for SOF, GS-331007, VEL and all other coadministered drugs

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.

Standards, quality control solutions, blank matrix, and study samples were prepared according to the validated methods. All samples were analyzed within the time frame supported by long-term storage stability data. The standard curve and QC data indicated that the plasma and urine assay methods for SOF, GS-331007, VEL, VOX, and coadministered drugs were precise and accurate. The bioanalytical method and the validation parameters for determination of SOF, GS-331007, VEL and VOX are summarized below, and the details for coadministered drugs are included in individual clinical pharmacology report reviews (refer to **Appendix 4.4**).

The bioanalytical method for determination of SOF, GS-566500 and GS-331007, in human plasma was developed and validated (b) (4) (GS-566500 is an unstable intermediate of SOF and very closely mimics the PK profile of SOF. Thus only SOF and GS-331007 are included in the PK summaries and discussion in this review). This method involved protein precipitation extraction of SOF, GS-566500, and GS-331007 and internal standards (SOF-d_4, GS-566500-$^{13}C_1{}^2H_3$, and GS-331007-$^{13}C_1$ $^2H_3$) from human plasma followed by LC-MS/MS with negative ionization. Bioanalytical method validation parameters are summarized in **Table 4.1-1**.

Table 4.1-1 Bioanalytical Method Validation for SOF, GS-566500 and GS-331007 in Human Plasma (b) (4) 60-1323)

Parameter	SOF	GS-566500	GS-331007
Calibrated range (ng/mL)	5 to 2500	10 to 5000	10 to 5000
Interassay precision range (%CV)	2.4 to 9.7	5.1 to 7.7	2.5 to 7.2
Interassay accuracy range (%RE)	−5.1 to 3.4	−2.2 to 2.9	−1.0 to 2.5
Studies Supported	GS-US-342-1137, GS-US-342-1138, GS-US-342-1139, GS-US-342-1140, GS-US-342-1167, GS-US-342-1326, GS-US-342-1346, GS-US-342-1709, GS-US-337-1468, GS-US-338-1121, GS-US-338-1130, GS-US-367-1168, GS-US-367-1169, GS-US-367-1170, GS-US-367-1171, GS-US-367-1172, GS-US-367-1173, GS-US-367-1176, GS-US-367-1657, GS-US-367-1726, GS-US-367-1727, GS-US-367-1871, GS-US-367-1905, GS-US-367-1909, GS-US-380-1999		

The bioanalytical method for determination of VEL in human plasma was developed and validated (b) (4) This method involved the liquid-liquid extraction of VEL and its internal standard (VEL-$^{13}C_6$) from human plasma followed by LC-MS/MS with positive ionization. Bioanalytical method validation parameters are summarized in **Table 4.1-2**.

Table 4.1-2 Bioanalytical Method Validation for VEL in Human Plasma [redacted] 60-1393)

	VEL
Calibrated Range (ng/mL)	1 to 1000
Interassay Precision Range (%CV)	1.6 to 3.8
Interassay Accuracy Range (%RE)	-2.0 to 4.5
Studies Supported	GS-US-281-1054, GS-US-281-1055, GS-US-281-1056, GS-US-281-1058, GS-US-342-1137, GS-US-342-1138, GS-US-342-1139, GS-US-342-1140, GS-US-342-1326, GS-US-342-1346, GS-US-342-1709, GS-US-337-1468, GS-US-338-1121, GS-US-338-1130, GS-US-367-1168, GS-US-367-1169, GS-US-367-1170, GS-US-367-1171, GS-US-367-1172, GS-US-367-1173, GS-US-367-1176, GS-US-367-1657, GS-US-367-1726, GS-US-367-1727, GS-US-367-1871, GS-US-367-1905, GS-US-367-1909, GS-US-380-1999

The bioanalytical method for quantitation of VOX in human plasma was developed and validated [redacted] The method involved protein precipitation extraction of VOX and internal standard GS-646906 (D_9-VOX) from human plasma followed by LC-MS/MS with negative ionization. Bioanalytical method validation parameters are summarized in **Table 4.1-3**.

Table 4.1-3 Bioanalytical Method Validation for VOX in Human Plasma [redacted] 8109.123113.1)

	VOX
Calibrated Range (ng/mL)	0.5 to 1000
Interassay Precision Range (%CV)	5.10 to 9.29
Interassay Accuracy Range (%RE)	4.00 to 5.33
Studies Supported	GS-US-337-1468, GS-US-338-1120, GS-US-338-1121, GS-US-338-1123, GS-US-338-1124, GS-US-338-1125, GS-US-338-1126, GS-US-338-1130, GS-US-338-1417, GS-US-367-1168, GS-US-367-1169, GS-US-367-1170, GS-US-367-1171, GS-US-367-1172, GS-US-367-1173, GS-US-367-1176, GS-US-367-1657, GS-US-367-1726, GS-US-367-1727, GS-US-367-1871, GS-US-367-1905, GS-US-367-1909, GS-US-380-1999

The bioanalytical method for quantitation of VOX in human urine was developed and validated [redacted] The method involved protein precipitation extraction of VOX and internal standard GS-646906 (D_9-VOX) from human urine followed by LC-MS/MS with negative ionization. Bioanalytical method validation parameters are summarized in **Table 4.1-4**.

Table 4.1-4 Bioanalytical Method Validation for VOX in Human Urine (b) (4) 8207.061515)

	VOX
Calibrated Range (ng/mL)	50.0 to 50,000
Interassay Precision Range (%CV)	5.99 to 6.56
Interassay Accuracy Range (%RE)	-0.667 to 3.47
Studies Supported	GS-US-338-1124, GS-US-338-1125

4.2 Population PK Analysis

Population PK Analysis: Voxilaprevir

The Applicant performed population pharmacokinetic analysis of voxilaprevir (VOX, GS-9857) using the data collected from 4 Phase 2 (GS-US-337-1468, GS-US-367-1168, GS-US-367-1169, GS-US-367-1871) and 4 Phase 3 (GS-US-367-1170, GS-US-367-1171, GS-US-367-1172, and GS-US-367-1173) clinical studies.

Objectives: The objective of the analysis was to develop a model to characterize VOX PK and the effects of demographic, pathophysiologic, and HCV disease-related covariates on the PK of VOX. Model predicted individual VOX PK parameters and exposure were also used for additional exposure-response analysis

Data: Data for the population PK analysis were pooled from the 8 clinical studies mentioned above. A total of 9991 data points from 1591 subjects were included in the model development dataset. A summary of the subjects included in the population PK analysis is provided in Table 4.2-1.

Table 4.2-1: Summary of Subjects Included in the Population PK Analysis

Study Number	Phase	Study Drug Administration	Number of subjects		
			Total	Sparse	Intensive PK Substudy
337-1468	2	Cohort 4: VOX, then SOF/VEL+VOX 6 weeks	15	All	15
		Cohort 5: SOF/VEL+VOX 4, 6, or 8 weeks	146	All	57
367-1168	2	SOF/VEL + VOX 6, 8, or 12 weeks	205	All	29
367-1169	2	SOF/VEL + VOX 6, 8, or 12 weeks	128	All	24
367-1871	2	SOF/VEL + VOX ± RBV 12 weeks	49	All	N/A
367-1170	3	SOF/VEL/VOX 8 weeks	182	All	N/A
367-1171	3	SOF/VEL/VOX 12 weeks	262	All	37
367-1172	3	SOF/VEL/VOX 12 weeks	500	All	N/A
367-1173	3	SOF/VEL/VOX 8 weeks	110	All	N/A

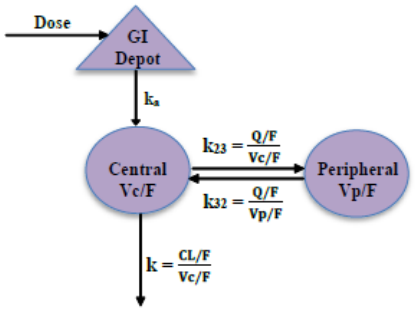
Source: Table 1 on page 17 of Applicant's population PK report for VOX

Population PK Model Development

Base Model: The selected base model that best described the VOX 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 cirrhosis on CL/F and Vc/F were also included in the base model. The scheme of the model is depicted in Figure 4.2-1.

Figure 4.2-1: Two-compartment PK Model with First Order Absorption



k_a = absorption rate constant
k = elimination rate constant
k_{23} = rate constant from central to peripheral
k_{32} = rate constant from peripheral to central
CL/F = apparent oral clearance
Q/F = apparent inter-compartmental clearance
Vc/F = apparent central volume
Vp/F = apparent peripheral volume

The system was described by the following first-order differential equations:

$$\frac{dA_2}{dt} = ka \cdot A_1 - (k_{20} + k_{23}) \cdot A_2 + k_{32} \cdot A_3$$

$$\frac{dA_3}{dt} = k_{23} \cdot A_2 - k_{32} \cdot A_3$$

Source: Figure 4 on page 30 of Applicant's population PK report for VOX

Final Model

The full population PK Model was constructed via forward inclusion of covariates of interest and then 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 ($p < 0.001$). All covariates in the full model, including cirrhosis, sex, and concomitant calcium channel blockers on CL/F, and cirrhosis on Vc/F were significant and retained in the final model.

The final model includes the following parameter-covariate relationships:

$$CL_i = exp(\theta_1 + \theta_7 \cdot Cirrhosis + \theta_9 \cdot female + \theta_{10} \cdot CMCACH) + \eta_{CL,i})$$

$$Vc_i = exp(\theta_2 + \theta_8 \cdot Cirrhosis + \eta_{Vc,i})$$

$$Q_i = \exp(\theta_3)$$

$$Vp_i = \exp(\theta_4)$$

$$Ka_i = \exp(\theta_5 + \eta_{ka,i})$$

$$ALAG_i = \exp(\theta_6)$$

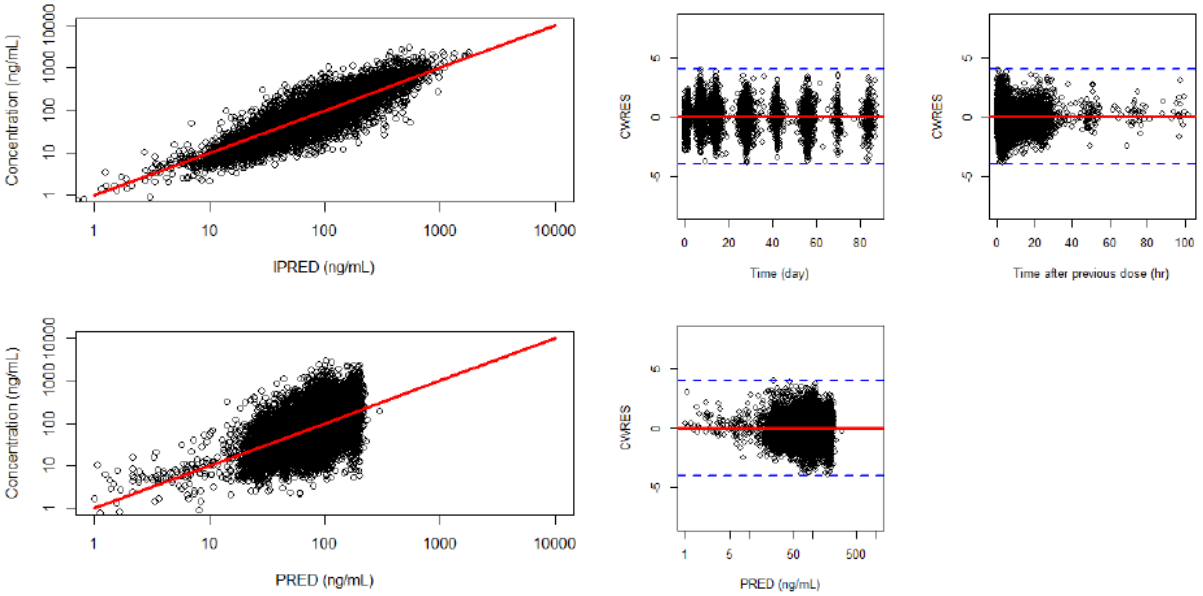
The parameter estimates of the final model are summarized in Table 4.2-2. The model performance was evaluated by goodness-of-fit plots and prediction corrected visual prediction checks (pcVPC) as demonstrated in Figure 4.2-2 and Figure 4.2-3, respectively.

Table 4.2-2: Parameter Estimates for VOX Final Model

Parameter	Parameter Description		Population Estimate (RSE%)	Change from Typical	Inter-Individual Variability (RSE%)
$exp(\theta_1)$	Apparent oral clearance CL/F (L/hr)	Male HCV-infected non-cirrhotic subject without CMCACH	65.8 (3.1%)	-	70.1 (4.63%)
$exp(\theta_1+\theta_7)$		Male HCV-infected cirrhotic subject without CMCACH	37.8	-42.6%	
$exp(\theta_1+\theta_9)$		Female HCV-infected non-cirrhotic subject without CMCACH	58.8	-10.6%	
$exp(\theta_1+\theta_{10})$		Male HCV-infected non-cirrhotic subject with CMCACH	56.7	-13.8%	
$exp(\theta_1+\theta_7+\theta_9)$		Female HCV-infected cirrhotic subject without CMCACH	33.8	-48.6%	
$exp(\theta_1+\theta_7+\theta_{10})$		Female HCV-infected non-cirrhotic subject with CMCACH	32.6	-50.5%	
$exp(\theta_1+\theta_9+\theta_{10})$		Male HCV-infected cirrhotic subject with CMCACH	50.7	-22.9%	
$exp(\theta_1+\theta_7+\theta_9+\theta_{10})$		Female HCV-infected cirrhotic subject with CMCACH	29.2	-55.6%	
$exp(\theta_2)$	Apparent central volume, Vc/F (L)	HCV-infected non-cirrhotic subject	707 (7.29%)	-	109.0 (8.16%)
$exp(\theta_2+\theta_8)$		HCV-infected cirrhotic subject	391.4	-44.7%	
$exp(\theta_3)$	Inter-compartment clearance Q, (L/hr)		11.0 (13.3%)		
$exp(\theta_4)$	Peripheral volume Vp (L)		259 (10.4%)		
$exp(\theta_5)$	Absorption rate constant Ka (1/hr)		0.401 (7.9%)		83.6 (29.9%)
$exp(\theta_6)$	Absorption lag time Tlag, (hr)		0.462 (1.8%)		
$\omega^2_{CL/F,Vc/F}$	Interaction of CL/F and Vc/F		0.720 (5.25%)		
σ	Residual error (%)		60.2 (3.19%)		

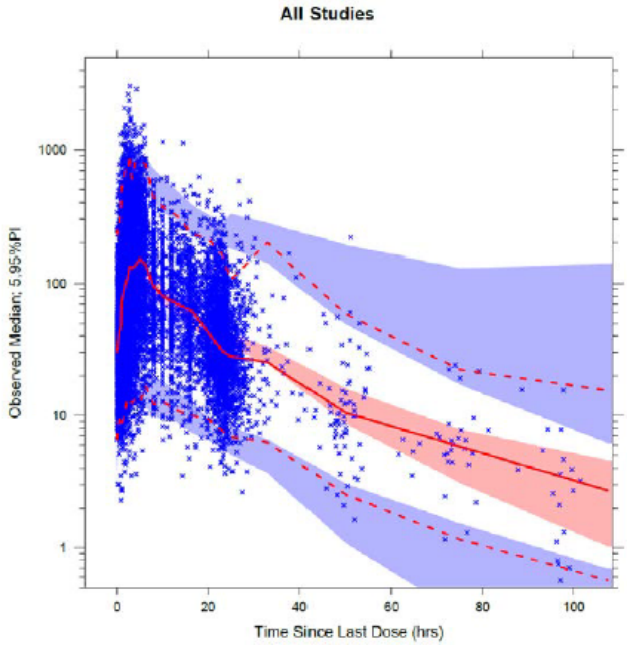
Source: Table 6 on page 32 of Applicant's population PK report for VOX

Figure 4.2-2: Goodness-of-Fit Plots for the Final Model of VOX



Source: Adapted from Figure 5 and Figure 6 on page 34 and 35 of Applicant's population PK report for VOX, respectively.

Figure 4.2-3: pcVPC of VOX Plasma Concentration-Time Profiles



Circles are observed VOX plasma concentrations, solid red line represents the median observed value, and dashed lines represent 2.5%tile and 97.5% tile of the observed values. Red shaded areas represent the spread of the median predicted values (5% tile to 95% tile), and blue shaded areas represent the spread (5%tile and 95%tile) of the 5th and 95th predicted percentile concentrations

Source: Figure 14 on page 41 of Applicant's population PK report for VOX

Effect of Covariates on VOX Pharmacokinetics

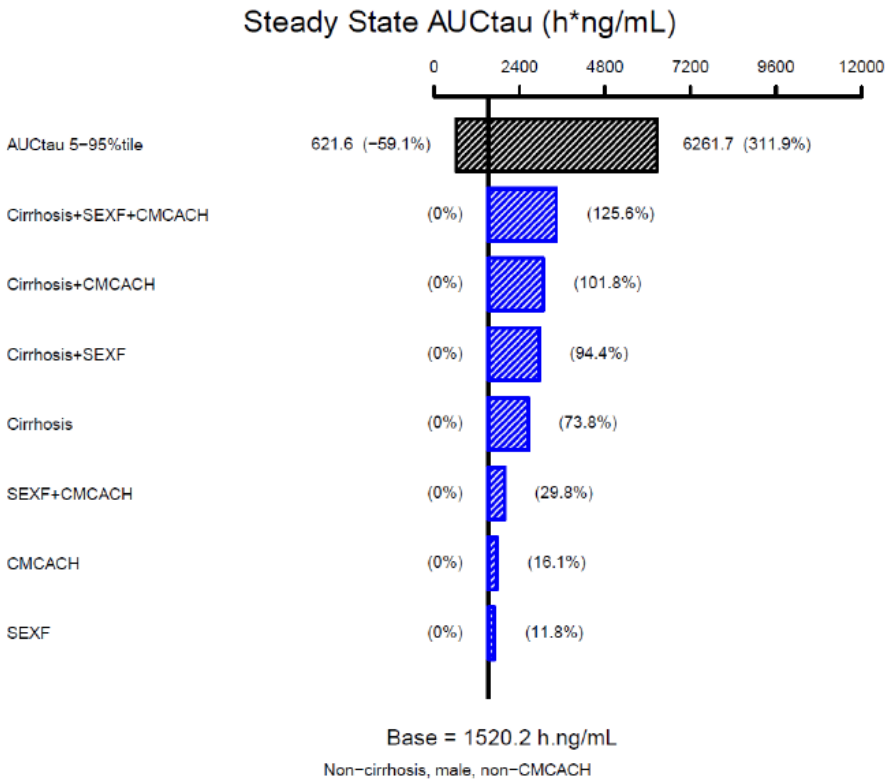
The effect of covariates on VOX PK and exposure is summarized in Table 4.2-3 and demonstrated in Figure 4.2-4. Tested covarites included demographics (baseline age, body weight, sex, race), pathophysiological covariates (baseline CLCR, cirrhosis status, and concomitant medications such as anti-coagulants, statins, selective serotonin reuptake inhibitors, calcium channel blockers, H2-receptor antagonists, diuretics, proton pump inhibitors, and P-gp inhibitors). Covariates identified to have statistically significant influence on VOX pharmacokinetics were cirrhosis, sex, and concomitant use of calcium channel blockers. The effect of cirrhosis on VOX CL/F and Vc/F was the most influential and resulted in 73.8% higher AUC_{tau}, 76% higher C_{max} and 79.1% higher C_{tau} in subjects with cirrhosis (CPT-A) compared to subjects without cirrhosis. Females subjects had 11.8% higher AUC_{tau}, 7% higher C_{max}, and 24.2% higher C_{tau} than male subjects; Subjects with calcium channel blocker usage had 16.1% higher AUC_{tau}, 9.5% higher C_{max} and 33.1% higher C_{tau} than subjects without calcium channel blocker usage. The combination of these covariate effects (cirrhosis, calcium channel blocker usage and female sex) resulted in VOX steady-state AUC_{tau}, C_{max}, and C_{tau} that were 125.6%, 107%, and 187% higher, respectively, than the typical subject (non-cirrhotic male without calcium channel blocker usage). However, the difference in AUC caused by these factors is not clinically significant and no dose adjustments based on these factors are necessary.

Table 4.2-3: Effect of Covariates on Key PK Parameters of Voxilaprevir

PK Parameters and Baseline Covariates	Estimate	Change from Typical (%)	Inter-Individual Variability
Typical CL/F (L/hr, Non-cirrhotic, male subject without CMCACH)	65.8		70.1%
Cirrhosis	37.8	-42.6	
Female	58.8	-10.6	
CMCACH	56.7	-13.8	
Typical Vc/F (L)	707		109.0%
Cirrhosis	393	-44.7	
Typical Q/F (L/hr)	11.0		
Typical Vp/F (L)	259		
Typical ka (1/hr)	0.401		83.6%
Typical lag time (hr)	0.462		
Residual variability as coefficient of variation (%)	60.2		

Source: Table A on page 12 of applicant's population PK report for voxilaprevir

Figure 4.2-4: Effect of Covariates on Voxilaprevir Steady-State Exposure (AUC_{tau})



Source: Figure 4 on page 128 of Applicant's summary of clinical pharmacology studies

Reviewer's Comment: The Applicant's population PK analysis for VOX is acceptable to the reviewer. The observed VOX concentrations were captured by the final population PK model. The fitting of the model seems adequate and the estimated PK parameters appear reasonable. The predictive performance of the model as indicated in the pcVPC is acceptable.

Population PK Analysis: Velpatasvir

The Applicant performed population pharmacokinetic analysis of GS-5816 (velpatasvir) using the data collected from 8 clinical studies (GS-US-337-1468, GS-US-367-1168, GS-US-367-1169, GS-US-367-1871, GS-US-367-1170, GSUS-367-1171, GS-US-367-1172, and GS-US-367-1173).

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 the PK of velpatasvir. Model predicted individual velpatasvir PK parameters were also estimated for additional exposure-response safety and efficacy analyses for velpatasvir.

Data: Data for the population PK analysis were pooled from the 8 clinical studies mentioned above. A total of 9754 data points from 1595 subjects were included in the model development dataset. A summary of subjects included in the population PK analysis of VEL is shown below in Table 4.2-4 and Table 4.2-5.

Table 4.2-4: Baseline Population Characteristics in Phase II Studies in the PopPK Model Development Dataset

Characteristics	337-1468	367-1168	367-1169	367-1871
No. of subjects	161	204	127	49
No. of samples	2107	1562	1077	236
Continuous Covariates				
Covariates	**Median [min, max]**	**Median [min, max]**	**Median [min, max]**	**Median [min, max]**
Age (yr)	57 [24, 73]	59 [19, 73]	58 [18, 77]	57 [18, 75]
Body Weight (kg)	78.6 [51.2, 141.4]	82.8 [48.7, 153.5]	83.0 [42.6, 146.9]	87.4 [51.1, 160.0]
BMI (kg/m^2)	26.7 [19.1, 45.1]	27.3 [18.4, 51.7]	27.7 [18.4, 52.6]	29.3 [19.5, 55.4]
BSA (m^2)	1.9 [1.5, 2.6]	2.0 [1.5, 2.8]	2.0 [1.3, 2.7]	2.0 [1.5, 2.7]
CLCR (mL/min)	109.5 [59.5, 256]	108.9 [58.9, 287.1]	114 [55.3, 253.6]	120.4 [69.7, 280.3]
eGFR ($mL/min/1.73m^2$)	89.4 [49.5, 171.3]	87.6 [49.8, 169.6]	90.1 [31.1, 161.7]	90.9 [40.4, 141.5]
Categorical Covariates				
Covariates	**N**	**N**	**N**	**N**
SEXF (0/1)	113/48	133/71	79/48	32/17
RACE (1/2/3/4)	140/0/6/15	174/29/0/1	102/7/12/6	39/10/0/0
ETH (Missing/1/2)	0/1/160	0/42/162	0/17/110	0/16/33
PAT (0/1/2)	115/0/40/0/0/6	204/0/0/0/0/0	1/33/73/17/0/3/0	49/0/0/0/0/0
HCVGT (0/1/2/3/4/5/6)	0/55/89/17	4/51/109/40	2/47/61/17	0/7/23/19
IL28B (Missing/0/1/2/3)	161/0	204/0	126/1	49/0
FORM (0/1)	0/161	0/204	0/127	49/0
RBV (0/1)	161/0	173/31	127/0	24/25
CIRRHOS (0/1)	74/87	108/96	68/59	24/25
PRTEXPN (1/2)	63/98	134/70	63/64	0/49
PRDAA (0/1/2)	99/7/55	140/30/34	89/6/32	0/20/29
CMCOAG (0/1)	154/7	177/27	110/17	39/10
CMSSRI (0/1)	142/19	181/23	118/9	43/6
CMSTAT (0/1)	155/6	190/14	120/7	40/9
CMCACH (0/1)	154/7	177/27	112/15	43/6
CMH2RA (0/1)	150/11	203/1	119/8	40/9
CMDUR (0/1)	160/1	184/20	123/4	39/10
CMPPI (0/1)	144/17	182/22	110/17	33/16
CMPGP (0/1)	154/7	192/12	123/4	47/2

Source: Table 4 on page 24 of Applicant's population PK report for VEL

Table 4.2-5: **Baseline Population Characteristics in Phase III Studies in the PopPK Model Development Dataset**

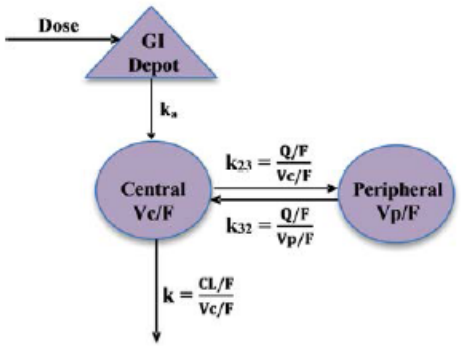
Characteristics	367-1170	367-1171	367-1172	367-1173
No. of subjects	181	262	501	110
No. of samples	732	1680	1947	413
Continuous Covariates				
Covariates	**Median [min, max]**	**Median [min, max]**	**Median [min, max]**	**Median [min, max]**
Age (yr)	58 [24, 85]	59 [27, 84]	55 [18, 78]	55 [25, 75]
Body Weight (kg)	87.0 [46.0, 156.1]	85.7 [48.1, 177.1]	75.0 [34.7, 144.7]	80.7 [49.4, 153.0]
BMI (kg/m^2)	27.8 [18.0, 45.4]	27.8 [18.4, 66.7]	25.9 [16.9, 57.3]	26.7 [19.6, 50.4]
BSA (m^2)	2.1 [1.4, 2.9]	2.0 [1.4, 2.9]	1.9 [1.1, 2.7]	2.0 [1.5, 2.8]
CLCR (mL/min)	118.8 [53.4, 275.7]	118.2 [39.9, 229.2]	105.6 [42.6, 286.1]	115.4 [47.4, 297.5]
eGFR ($mL/min/1.73m^2$)	93.6 [39.8, 187.4]	91.9 [35.1, 178.2]	89.6 [28.3, 189.4]	94.7 [62.8, 171.0]
Categorical Covariates				
Covariates	**N**	**N**	**N**	**N**
SEXF (0/1)	142/39	200/62	255/246	74/36
RACE (1/2/3/4)	158/16/2/5	210/36/8/8	386/47/51/17	100/0/8/2
ETH (Missing/1/2)	0/19/162	2/15/245	8/31/462	1/9/100
PAT (0/1/2)	68/29/51/17/1/0/15	151/4/76/17/2/0/12	243/55/88/56/20/0/39	0/0/110/0/0/0/0
HCVGT (0/1/2/3/4/5/6)	0/33/107/41	0/47/165/50	0/166/253/82	0/41/57/12
IL28B (Missing/0/1/2/3)	181/0	257/5	498/3	108/2
FORM (0/1)	181/0	262/0	501/0	110/0
RBV (0/1)	181/0	262/0	501/0	110/0
CIRRHOS (0/1)	97/84	142/120	411/90	0/110
PRTEXPN (1/2)	0/181	0/262	382/119	75/35
PRDAA (0/1/2)	0/0/181	1/260/1	501/0/0	110/0/0
CMCOAG (0/1)	154/27	227/35	458/43	97/13
CMSSRI (0/1)	164/17	237/25	449/52	100/10
CMSTAT (0/1)	173/8	247/15	482/19	104/6
CMCACH (0/1)	157/24	221/41	456/45	101/9
CMH2RA (0/1)	157/24	235/27	455/46	93/17
CMDUR (0/1)	160/21	237/25	466/35	97/13
CMPPI (0/1)	154/27	219/43	443/58	90/20
CMPGP (0/1)	174/7	248/14	483/18	103/7

Source: Table 5 on page 25 of Applicant's population PK report for VEL

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 scheme of the model is depicted in Figure 4.2-5.

Figure 4.2-5: Two-compartment PK Model with First Order Absorption for VEL



k_a = absorption rate constant
k = elimination rate constant
k_{23} = rate constant from central to peripheral
k_{32} = rate constant from peripheral to central
CL/F = apparent oral clearance
Q/F = apparent inter-compartmental clearance
Vc/F = apparent central volume
Vp/F = apparent peripheral volume

The system was described by the following first-order differential equations:

$$\frac{dA_2}{dt} = ka \cdot A_1 - (k + k_{23}) \cdot A_2 + k_{32} \cdot A_3$$

$$\frac{dA_3}{dt} = k_{23} \cdot A_2 - k_{32} \cdot A_3$$

Source: Figure 4 on page 28 of Applicant's population PK report for VEL

Final Model

The full population PK Model was constructed via forward inclusion of covariates of interest and then a reduction step by 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:

$$CL_i = \exp(\theta_1 + \theta_7 \cdot \boldsymbol{female} + \eta_{CL,i})$$

$$Vc_i = exp(\theta_2 + \theta_8 \cdot \boldsymbol{female} + \theta_9 \cdot \boldsymbol{Cirrhosis} + \eta_{Vc,i})$$

$$Q_i = exp(\theta_3)$$

$$Vp_i = exp(\theta_4)$$

$$Ka_i = exp(\theta_5 + \eta_{Ka,i})$$

$$ALAG_i = exp(\theta_6)$$

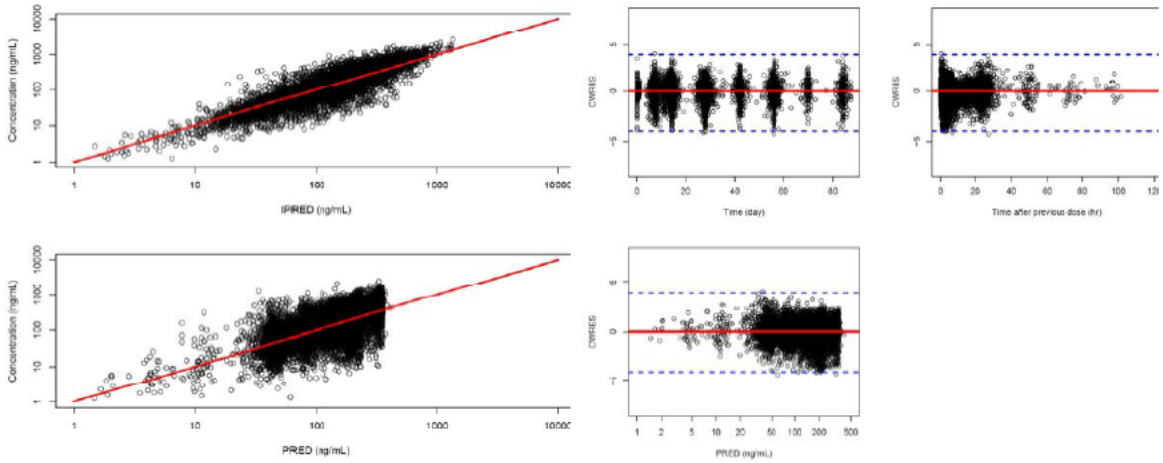
The parameter estimates of the final model are summarized in Table 4.2-6. The model was evaluated by goodness-of-fit plots and pcVPC as demonstrated in Figure 4.2-6 and Figure 4.2-7.

Table 4.2-6: Parameter Estimates for Velpatasvir Final Model and Bootstrap Results

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)	32.83 (31.34, 34.4)	32.94 (31.47, 34.72)
θ_7	Influence of sex on CL/F	-0.440 (-0.508, -0.371)	-0.439 (-0.504, -0.373)
$exp(\theta_2)$	Apparent central volume, Vc/F (L)	286.3 (251.0, 326.6)	298.2 (252.1, 344.9)
θ_8	Influence of sex on Vc/F	-0.376 (-0.511, -0.241)	-0.383 (-0.504, -0.240)
θ_9	Influence of cirrhosis on Vc/F	0.176 (0.096, 0.256)	0.172 (0.091, 0.256)
$exp(\theta_3)$	Apparent inter-compartmental clearance, Q/F (L/hr)	6.726 (5.232, 8.647)	6.783 (4.996, 8.636)
$exp(\theta_4)$	Apparent peripheral volume, Vp/F (L)	134.6 (112.9, 160.5)	133.5 (107.9, 161.3)
$exp(\theta_5)$	Absorption rate constant, Ka (1/hr)	0.424 (0.368, 0.488)	0.455 (0.364, 0.553)
$exp(\theta_6)$	Lag time (hr)	0.697 (0.681, 0.712)	0.697 (0.678, 0.716)
Inter-individual variability (%)	CL/F	54.70 (51.20, 57.99)	54.60 (51.34, 58.09)
	V/F	77.76 (69.34, 85.36)	78.98 (70.41, 87.65)
	Ka	110.3 (97.94, 121.4)	108.7 (91.99, 122.9)
$\omega_{(CL,Vc)}$	Covariance between CL/F and Vc/F	0.391 (0.331, 0.450)	0.390 (0.332, 0.452)
σ	Residual error	57.61 (55.80, 59.36)	57.52 (55.67, 59.41)

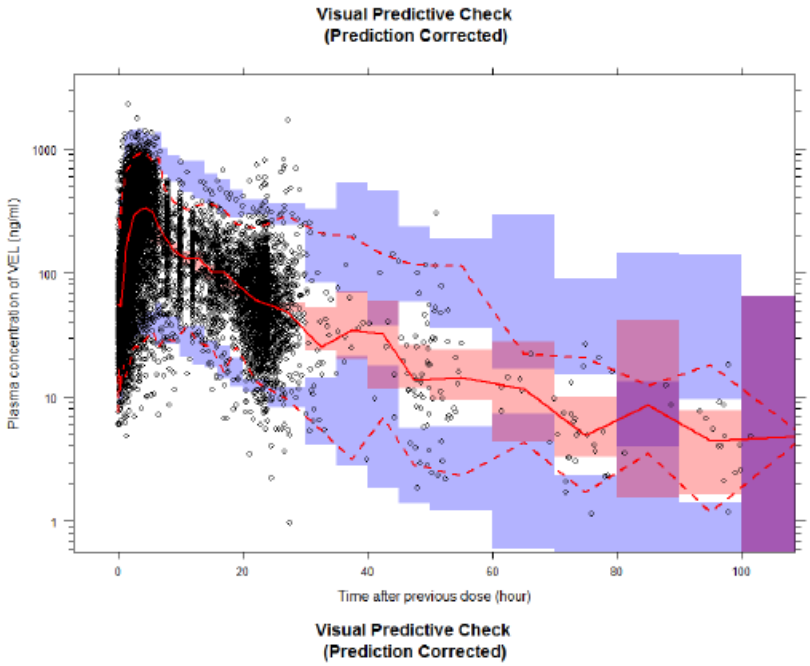
Source: Table 7 on page 31 of Applicant's population PK report for velpatasvir

Figure 4.2-6: Goodness-of-Fit Plots for the Final Model of Velpatasvir



Source: Adapted from Figure 5 and Figure 6 on page 32 of Applicant's population PK report for VEL

Figure 4.2-7: pcVPC of Velpatasvir Plasma Concentration-Time Profile



Circles are observed VEL plasma concentrations, solid red line represents the median observed value, and dashed lines represent 2.5%tile and 97.5% tile of the observed values. Red shaded areas represent the spread of the median predicted values (5% tile to 95% tile), and blue shaded areas represent the spread (5%tile and 95%tile) of the 5th and 95th predicted percentile concentrations

Source: Adapted from Figure 12 on page 37 of Applicant's population PK report for velpatasvir

Effect of Covariates on VEL Pharmacokinetics

The effect of covariates on VEL PK and exposure is summarized in Table 4.2-7 and Figure 4.2-8. The covariate identified to have statistically significant influence on VEL oral clearance (CL/F) was sex. Covariates influencing Vc/F were sex and cirrhosis. Estimated Vc/F was 19.3% higher for cirrhotic (CPT -A) compared to non-cirrhotic subjects. Inter-individual variability on CL/F, Vc/F, and Ka were 54.7%, 77.8% and 110.3%, respectively.

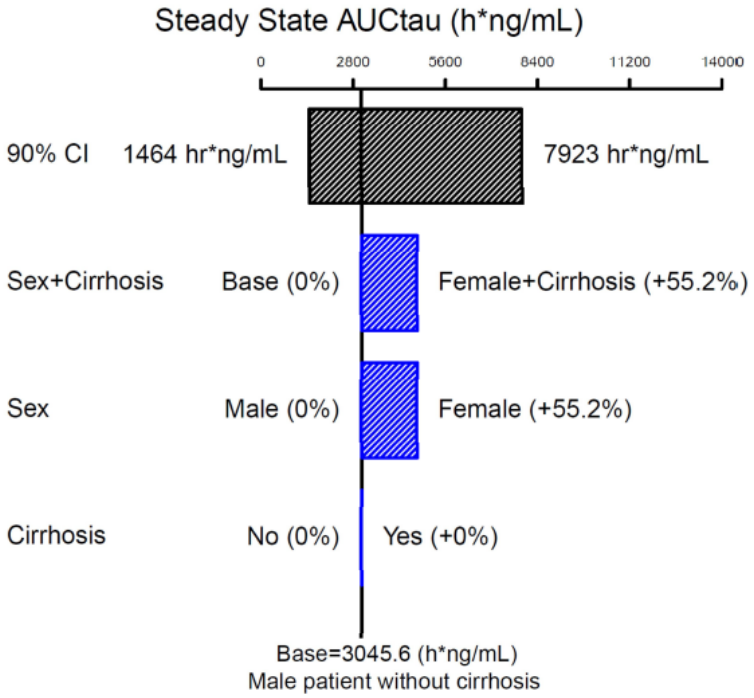
The covariate effect of sex on VEL CL/F and Vc/F resulted in 55.2% higher AUC_{tau}, 50.4% higher C_{max} and 79.0% higher C_{tau} in female subjects compared to males. The covariate effect of cirrhosis on VEL Vc/F resulted in 7.6% lower C_{max} and 18.3% higher C_{tau} in subjects with cirrhosis (CPT-A) compared to subjects without cirrhosis. The combination of these covariate effects (female, cirrhosis), resulted in VEL steady-state AUC_{tau},C_{max}, and C_{tau} that were 55.2%, 39.5%, and 105% higher, respectively, than the typical subject (male, noncirrhotic). Due to lack of clinically significant exposure-response relationships for safety or efficacy, the difference in VEL exposure is not considered clinically significant, and no dose adjustments for VEL based on these factors are necessary.

Table 4.2-7: **Effect of Covariates on Key PK Parameters of VEL**

PK Parameters and Baseline Covariates	Estimate	Change from Typical (%)	Inter-Individual Variability
Typical CL/F (L/hr, Male patient with normal hepatic function)	32.83	-	54.7%
Female	21.16	-35.6	-
Typical Vc/F (L)	286.3	-	77.8%
Female	196.6	-31.3	-
Cirrhosis (CPT-A)	341.5	19.3	-
Typical Q/F (L/hr)	6.726	-	-
Typical Vp/F (L)	134.6	-	-
Typical ka (1/hr)	0.424	-	110.3%
Typical lag time (hr)	0.697	-	-
Residual variability as coefficient of variation (%)	57.61	-	-

Source: Table A on page 11 of applicant's population PK report for VEL

Figure 4.2-8: **Effect of Covariates on VEL Steady-State Exposure (AUC_{tau})**



Source: Figure 5 on page 130 of Applicant's summary of clinical pharmacology studies

Reviewer's Comment: The Applicant's population PK analysis for velpatasvir is acceptable to the reviewer. The observed velpatasvir concentrations were captured by the final population PK model. The fitting of the model seems adequate and the estimated PK parameters appear reasonable. The predictive performance of the model, as indicated in the pcVPC, is acceptable.

Population PK Analysis: Sofosbuvir

The Applicant performed population pharmacokinetic analysis of GS-7977 (sofosbuvir) using the data collected from 8 clinical studies (GS-US-337-1468, GS-US-367-1168, GS-US-367-1169, GS-US-367-1871, GS-US-367-1170, GS-US-367-1171, GS-US-367-1172, and GS-US-367-1173). The studies included patients treated with SOV/VEL+VOX or SOF/VEL/VOX up to 12 weeks.

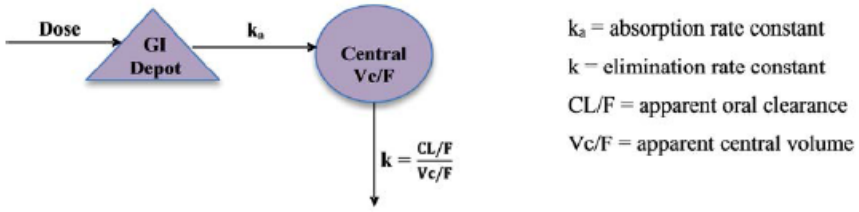
Objectives: The objective of the analysis was to develop a model to characterize sofosbuvir PK and evaluate the effects of demographic, pathophysiologic, and HCV disease-related covariates on sofosbuvir PK parameters. Model predicted individual sofosbuvir PK parameters were also estimated for exposure-response safety and efficacy analyses.

Data: Data for population PK analysis were pooled from the 8 clinical studies mentioned above. A total of 20.7% (1,141/5,515) data points were BLQs because sofosbuvir is a prodrug which has low systemic exposure and a short terminal elimination half-life of ~0.5 to 1 hr. A total of 4374 data points from 1041 subjects were included in the model development dataset. The censored-data likelihood method (M3 method in NONMEM) was employed to examine the final PopPK model.

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, and first-order elimination. The PK model was parameterized with clearance (CL), volume of distribution (V), absorption rate constant (Ka) and a lag time (T_{lag}). The scheme of the model is depicted in Figure 4.2-9.

Figure 4.2-9: One-compartment PK Model with First Order Absorption



Source: Figure 4 on page 30 of Applicant's population PK report for SOF

Final Model

The full population PK Model was constructed via forward inclusion of covariates of interest and then via a reduction step by removing covariates using a stepwise backward elimination method. The criterion for retention was a change in likelihood ratio > 10.98 for 1 parameter ($p< 0.001$).

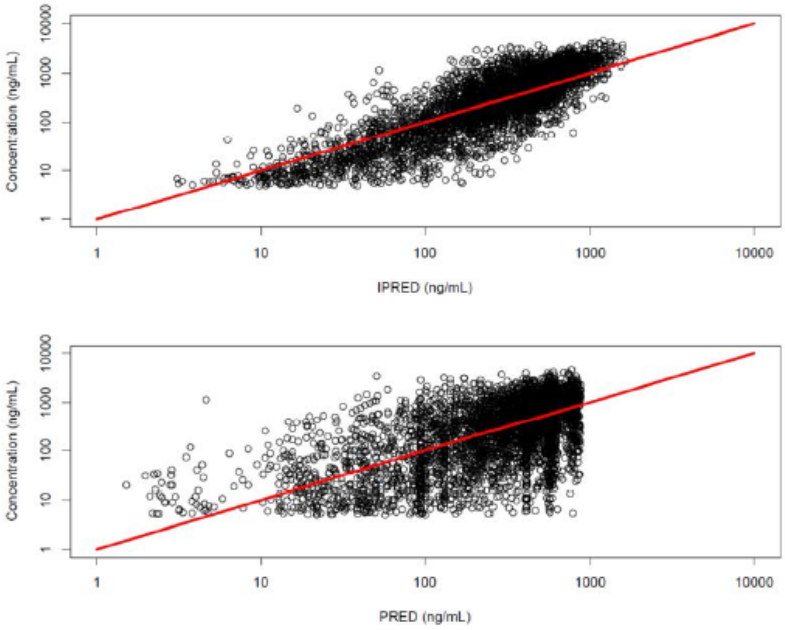
The parameter estimates of the final model are summarized in Table 4.2-8. The model was evaluated by goodness-of-fit plots and pcVPC as shown in Figure 4.2-10 and Figure 4.2-11.

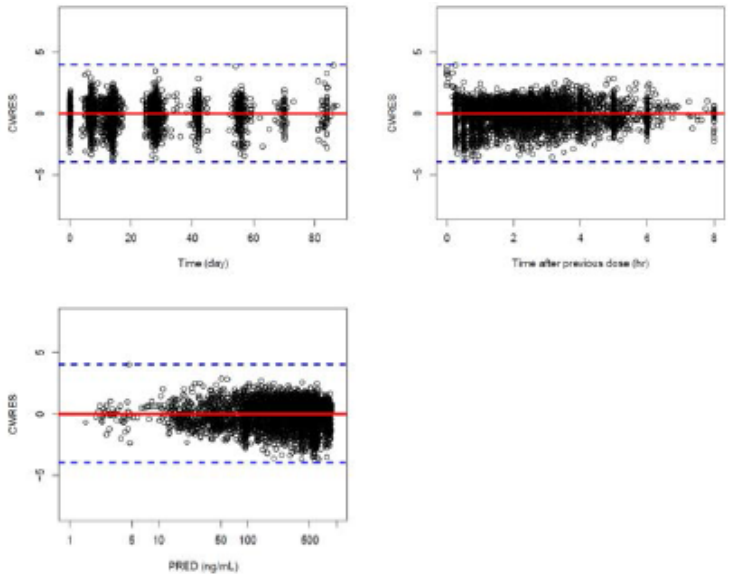
Table 4.2-8: Population PK Parameters for Sofosbuvir (Final PopPK Model)

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)	273.1 (257.2, 290.0)	272.5 (257.1, 290.1)
θ_5	Influence of CLCR on CL/F	0.2146 (0.1070, 0.3221)	0.2119 (0.1009, 0.3273)
θ_6	Influence of sex on CL/F	-0.2797 (-0.3819, -0.1776)	-0.2838 (-0.3763, -0.1836)
$exp(\theta_2)$	Apparent central volume, V_c/F (L)	302.2 (275.9, 331.0)	301.2 (275.0, 328.7)
θ_7	Influence of sex on V_c/F	-0.3369 (-0.4872, -0.1866)	-0.3407 (-0.4749, -0.1857)
$exp(\theta_3)$	Absorption rate constant, k_a (1/hr)	1.448 (1.335, 1.572)	1.475 (1.281, 1.723)
$exp(\theta_4)$	Lag time (hr)	0.1976 (0.1934, 0.2018)	0.1978 (0.1935, 0.2329)
Inter-individual variability (%)	CL/F	41.92 (34.84, 47.97)	42.01 (35.86, 48.72)
	V_c/F	34.20 (20.09, 43.99)	34.72 (24.14, 45.37)
	k_a	131.0 (118.0, 142.8)	131.7 (117.3, 147.8)
$\omega_{(CL,Vc)}$	Covariance between CL/F and V_c/F	0.1224 (0.0621, 0.1827)	0.1231 (0.0734, 0.1939)
σ	Residual error	97.16 (93.39, 100.8)	96.54 (92.52, 100.5)

Source: Table 7 on page 33 of Applicant's population PK report for sofosbuvir

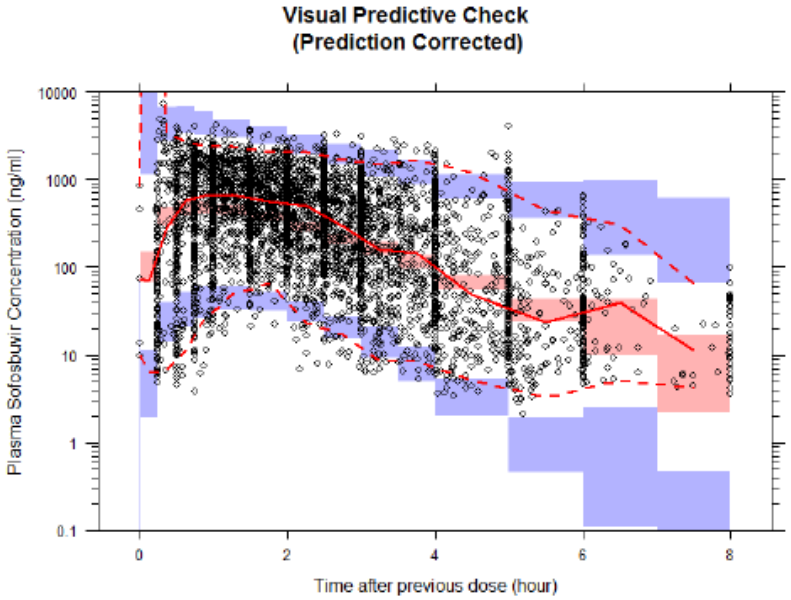
Figure 4.2-10: Goodness-of-Fit Plots for the Final Model of Sofosbuvir





Source: Adapted from Figure 5 and Figure 6 on page 34 of Applicant's population PK report for sofosbuvir

Figure 4.2-11: **pcVPC of Sofosbuvir Plasma Concentration-Time Profiles across All Studies**



Circles are observed SOF plasma concentrations, solid red line represents the median observed value, and dashed lines represent 2.5%tile and 97.5% tile of the observed values. Red shaded areas represent the spread of the median predicted values (5% tile to 95% tile), and blue shaded areas represent the spread (5%tile and 95%tile) of the 5th and 95th predicted percentile concentrations

Source: Figure 13 on page 38 of Applicant's population PK report for sofosbuvir

Effect of Covariates on SOF Pharmacokinetics

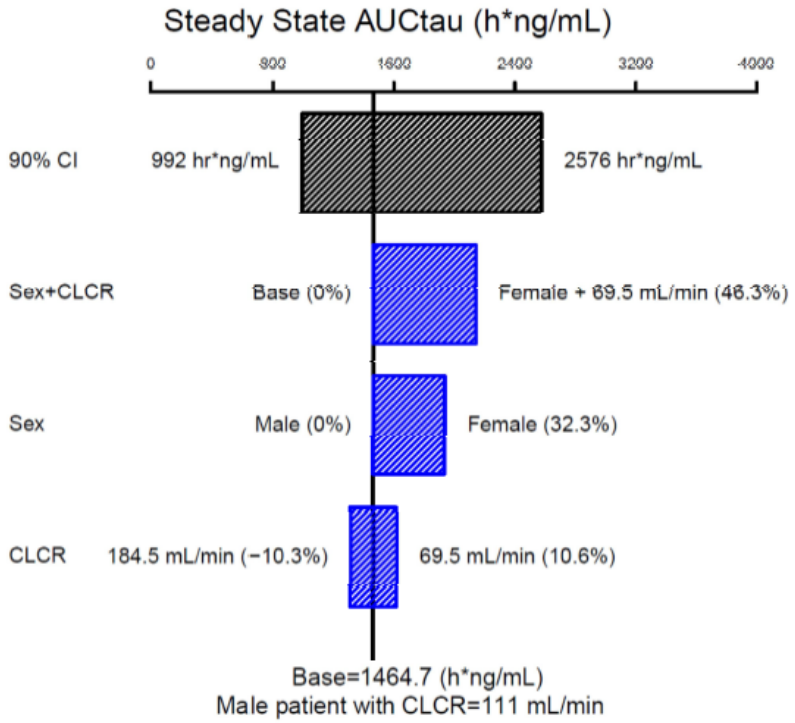
The effect of covariates on SOF pharmacokinetics and exposure is summarized in Table 4.2-9 and demonstrated in Figure 4.2-12. Covariates identified to have statistically significant influence on sofosbuvir oral clearance (CL/F) were sex and CLCR. The effect of sex on SOF CL/F and Vc/F resulted in 32.3% higher AUC_{tau}, and 36.7% higher C_{max} in female subjects compared to males. Baseline CLCR was also a significant covariate for SOF exposure. A CLCR decrease from 184.5 to 69.5 mL/min (90^{th} and 10^{th} percentile range of CLCR) is expected to results in a 20.8% increase in SOF exposure. Compared to a male with CLCR of 111 mL/min, SOF AUC_{tau} is 10.3% lower in a male with CRCL of 184.5 mL/min. Similarly, SOF AUC_{tau} would be 10.6% higher in a male with CRCL of 69.5 mL/min compared to a male with a CLCR of 111 mL/min (Figure 4.2-12). The combination of the sex and CLCR covariate effects (female with CLCR at the 5%tile of the population; 69.5 mL/min), resulted in SOF steady-state AUC_{tau}, and C_{max} that were 46.3%, and 42.6% higher, respectively, than the typical subject. However, as the difference in AUC caused by these factors did not translate into difference in efficacy (SVR12 rate), the difference in SOF exposure is not considered clinically significant, and no dose adjustments for sofosbuvir based on these factors are necessary.

Table 4.2-9: Effect of Covariates on Key PK Parameters of SOF

PK Parameters and Baseline Covariates	Estimate	Change from Typical (%)	Inter-individual Variability (%)
Typical CL/F (L/hr)	273.1	—	41.9
Creatinine clearance			
High (95%)	304.5	11.5	—
Low (5%)	247.1	-9.50	—
Female	206.5	-24.4	—
Typical V_c/F (L)	302.2	—	34.2
Female	215.8	-28.6	—
Typical k_a (1/hr)	1.448	—	131.0
Typical lag time (hr)	0.198	—	—
Residual variability as coefficient of variation (%)	97.16	—	—

Source: Table A on page 13 of applicant's population PK report for sofosbuvir

Figure 4.2-12: Effect of Covariates on SOF Steady-State Exposure (AUC_{tau})



Source: Figure 6 on page 131 of Applicant's summary of clinical pharmacology studies

Reviewer's Comment: The Applicant's population PK analysis for SOF is acceptable to the reviewer. The observed SOF concentrations were captured by the final population PK model. The fitting of the model seems adequate and the estimated PK parameters appear reasonable. The predictive performance of the model as indicated in the pcVPC plots is acceptable.

Population PK Analysis: GS-331007

The applicant performed population pharmacokinetic analysis of GS-331007 (sofosbuvir metabolite) using the data collected from 4 Phase 2 (GS-US-337-1468, GS-US-367-1168, GS-US-367-1169, GS-US-367-1871) and 4 Phase 3 (GS-US-367-1170, GS-US-367-1171, GS-US-367-1172, and GS-US-367-1173) clinical studies.

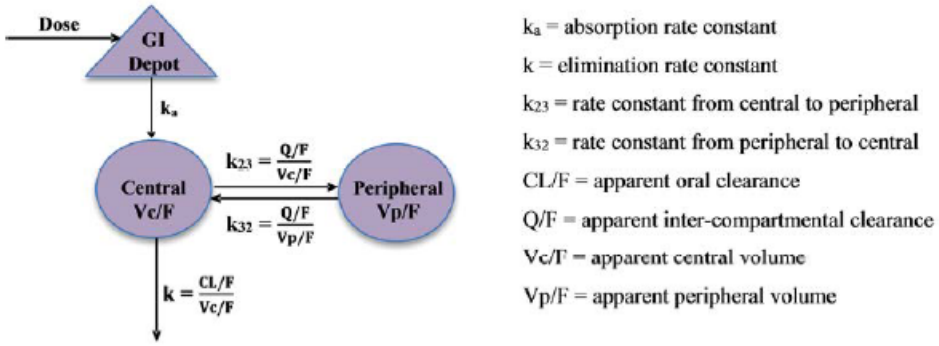
Objectives: The objective of the analysis was to develop a model to characterize GS-331007 pharmacokinetics following the combination treatment with with SOV/VEL+VOX or SOF/VEL/VOX up to 12 weeks. The effects of demographic, pathophysiologic, and HCV disease-related covariates on GS-331007 PK parameters were also explored. Model predicted individual GS-331007 exposure was generated for additional exposure-response analysis for safety and efficacy.

Data: Data for population PK analysis were pooled from 8 clinical studies as mentioned above. A total of 9721 samples from 1593 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 an absorption lag time, as illustrated in Figuer 4.2-13. 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 4.2-13: Two-compartment Model Describing Plasma GS-331007 Concentration Time Course Data Following an Oral Dose



The system was described by the following first-order differential equations:

$$\frac{dA_2}{dt} = ka \cdot A_1 - (k + k_{23}) \cdot A_2 + k_{32} \cdot A_3$$

$$\frac{dA_3}{dt} = k_{23} \cdot A_2 - k_{32} \cdot A_3$$

Source: Figure 4 on page 32 of Applicant's population PK report for GS-331007

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 age, body weight, sex, race, ethnicity, CLCR, cirrhosis, ribavirin usage, formulation, and concomitant medications. The final model only contained covariates that met the pre-defined statistical criteria.

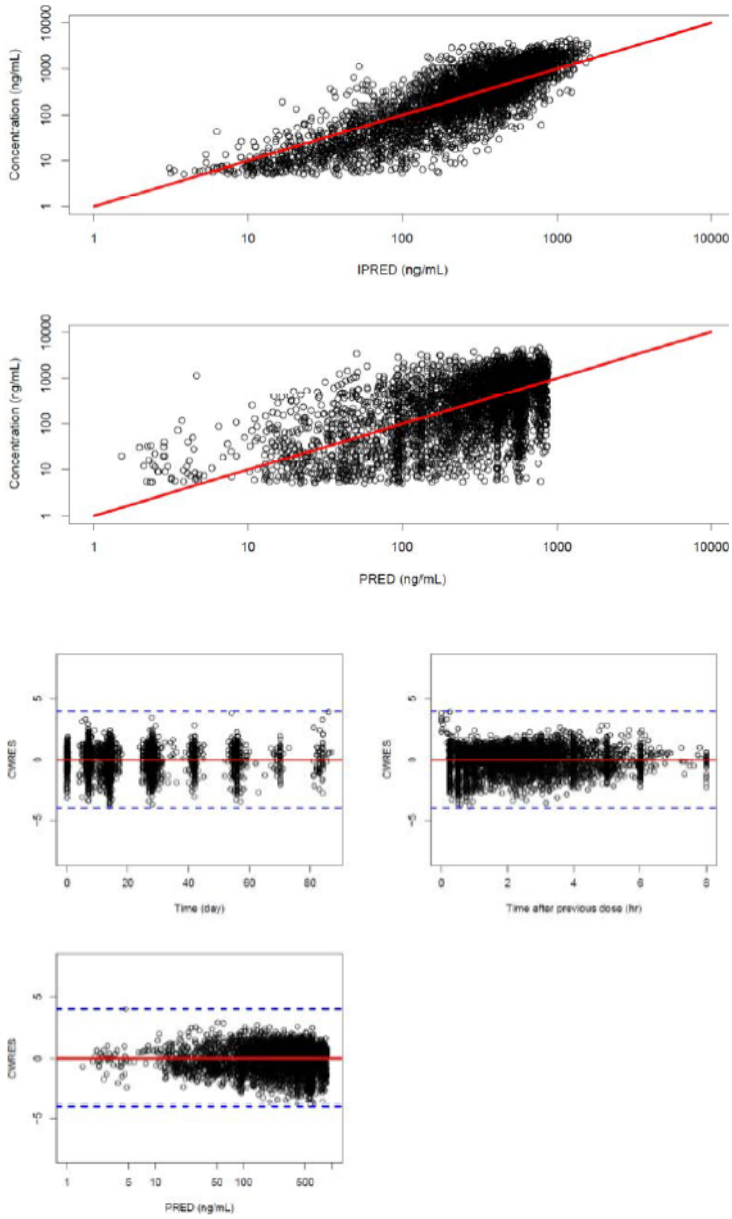
The parameter estimates of the final model are summarized below in Table 4.2-10. The model goodness-of-fit plots and pcVPC plots are shown in Figure 4.2-14 and Figure 4.2-15, respectively.

Table 4.2-10: 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)	33.69 (32.97, 34.43)	33.68 (32.93, 34.46)
θ_{11}	Influence of CLCR on CL/F	0.445 (0.353, 0.536)	0.446 (0.396, 0.499)
θ_{13}	Influence of sex on CL/F	-0.184 (-0.216, -0.153)	-0.184 (-0.212, -0.152)
θ_{17}	Influence of RBV on CL/F	0.182 (0.107, 0.256)	0.180 (0.104, 0.258)
θ_{20}	Influence of non-White on CL/F	0.100 (0.0621, 0.139)	0.101 (0.0606, 0.140)
$exp(\theta_2)$	Apparent central volume, V_c/F (L)	207.4 (167.7, 256.6)	205.1 (160.1, 254.6)
θ_{10}	Influence of weight on V_c/F	0.741 (0.408, 1.07)	0.7247 (0.3695, 1.057)
θ_{18}	Influence of RBV on V_c/F	1.050 (0.651, 1.45)	1.051 (0.5873, 1.541)
$exp(\theta_3)$	Apparent inter-compartmental clearance, Q/F (L/hr)	42.52 (34.81, 51.93)	42.50 (34.21, 51.16)
$exp(\theta_4)$	Apparent peripheral volume, V_p/F (L)	804.1 (725.3, 891.5)	804.5 (729.3, 887.3)
$exp(\theta_5)$	Absorption rate constant, k_a (1/hr)	0.1470 (0.1224, 0.1764)	0.1459 (0.1201, 0.1783)
$exp(\theta_6)$	Lag time (hr)	0.4544 (0.4357, 0.4739)	0.4549 (0.4384, 0.6182)
Inter-individual variability (%)	CL/F	24.96 (23.27, 26.53)	24.90 (23.55, 26.39)
	V_c/F	100.5 (78.19, 118.7)	100.3 (88.34, 114.3)
	Q/F	36.51 (11.52, 50.34)	35.84 (26.01, 43.76)
	V_p/F	39.00 (21.37, 50.85)	38.76 (26.41, 54.36)
	k_a	13.33 (0, 37.91)	16.35 (0.7152, 28.02)
$\omega_{CL/F,Vc/F}$	Covariance between CL/F and V_c/F	0.08433 (0.01371, 0.1549)	0.08385 (0.05730, 0.1109)
$\omega_{Q/F,Vp/F}$	Covariance between Q/F and V_p/F	0.06046 (-0.02257, 0.1435)	0.05665 (-0.008537, 0.1216)
σ	Residual error (%)	25.71 (24.84, 26.54)	25.65 (24.84, 26.51)

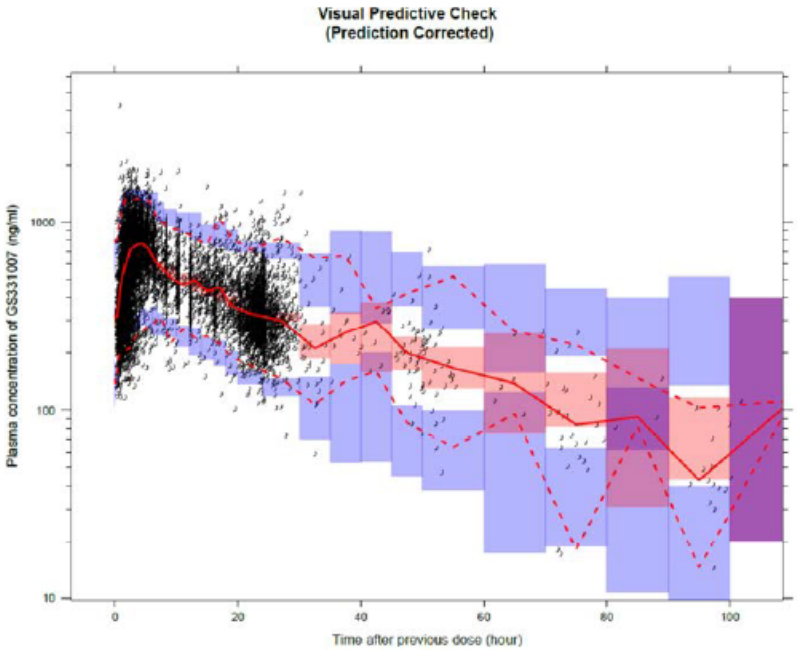
Source: Table 7 on page 38 of applicant's population PK report for GS-331007

Figure 4.2-14: Goodness-of-Fit Plots for the Final Model of GS-331007



Source: Adapted from Figure 5 on page 39 and 40 of applicant's population PK report for GS-331007

Figure 4.2-15: **pcVPC of GS-331007 Plasma Concentration-Time across All Studies**



Circles are observed GS-331007 plasma concentrations, solid red line represents the median observed value, and dashed lines represent 2.5%tile and 97.5% tile of the observed values. Red shaded areas represent the spread of the median predicted values (5% tile to 95% tile), and blue shaded areas represent the spread (5%tile and 95%tile) of the 5th and 95th predicted percentile concentrations

Source: Figure 12 on page 46 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 4.2-11 and Figure 4.2-16. A 10% decrease in weight resulted in 7.5% decreased V_c/F (e.g., a 10% decrease of 80 kg resulted in V_c/F of 191.8 L). A 10% decrease in CLCR resulted in 4.6% decreased CL/F (e.g., a 10% decrease from 111 mL/min resulted in CL/F of 32.14 L/hr). Subjects using RBV exhibited 19.9% higher CL/F and 185% higher V_c/F than subjects not using RBV. Interindividual variability on CL/F, V_c/F, Q/F, V_p/F, and k_a were 25.0%, 101%, 36.5%, 39.0%, and 13.3%, respectively. The typical elimination half-life was calculated as 32.2 hr.

The covariate effect of CLCR on GS-331007 CL/F was the most influential covariate contributing to the variability of GS-331007 exposure. GS-331007 exposure was 43.3% for an individual with CLCR of 69.5 mL/min compared to an individual with CLCR of 184.5 mL/min (10th percentile to the 90th percentile range of CLCR). For an individual with median CLCR of 111 mL/min (80 kg white male), GS-331007 AUC_{tau} is 20.2% higher compared to a CLCR of 184.5 mL/min and 23.1% lower compared to a CLCR of 69.5 mL/min (Figure 4.2-11)

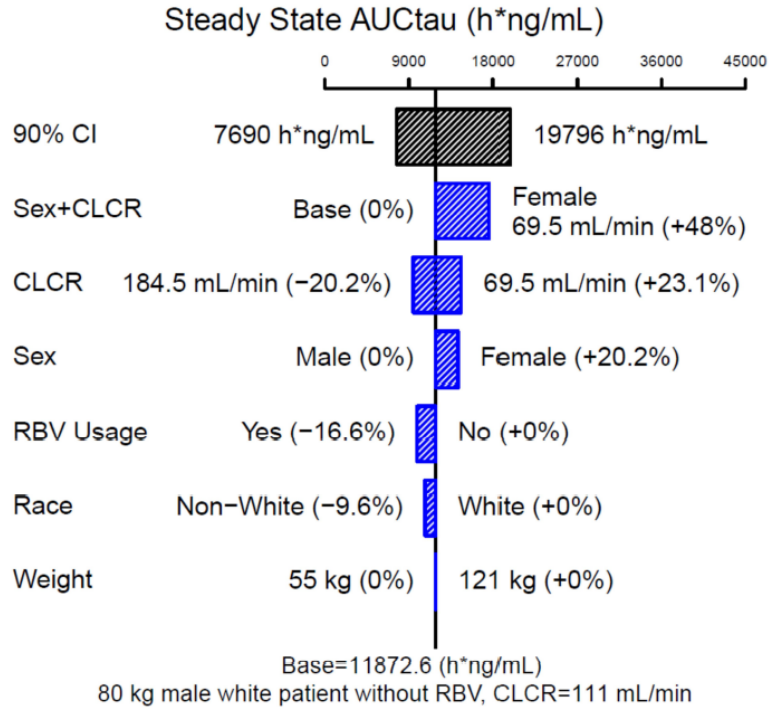
The covariate effect of sex on GS-331007 CL/F resulted in 20.2% higher AUC_{tau}, 14.6% higher C_{max} and 28.1% higher C_{tau} in female subjects compared to male subjects. RBV usage

resulted in 16.6% lower GS-331007 AUC_{tau}, 28.5% lower C_{max} and 3.4% lower C_{tau}. Race, and body weight had more modest impact on GS-331007 exposure (<13%). The combination of the CLCR and sex covariate effects (female with CLCR at the 5thtile of the population; 22.8 mL/min), resulted in GS-331007 steady-state AUC_{tau}, C_{max}, and C_{tau} that were 48.0%, 33.8%, and 67.0% higher, respectively, than the typical subject. This magnitude of change is not considered as clinically significant based on exposure-response relationships for safety or efficacy observed in the Phase 3 program.

Table 4.2-11: **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/F (L/hr, male, white, non-RBV)		33.69	—	24.96
CLCR (mL/min)	*5%tile*	27.36	-18.8	—
	95%tile	42.23	25.3	—
Sex	*Female*	28.02	-16.8	—
RBV	*Yes*	40.41	19.9	—
Race	*Non-White*	37.25	10.6	—
Typical V_c/F (L, non-RBV)		207.4	—	100.5
Body weight (kg)	*5%tile*	157.1	-24.2	—
	95%tile	281.8	35.9	—
RBV	*Yes*	591.7	185	—
Typical Q/F (L/hr)		42,52	—	36.51
Typical V_p/F (L)		804.1	—	39.00
Typical k_a (1/hr)		0.147	—	13.33
Typical Lag time (hr)		0.454	—	—
Intra-individual variability (%)		25.71	—	—

Figure 4.2-16: **Sensitive Plot Comparing the Effect of Covariates on GS-331007 Steady State Exposure (AUC_{tau})**



Source: Figure 7 on page 133 of Applicant's summary of clinical pharmacology studies

Reviewer's Comment: The applicant's population PK analysis for GS-331007 was acceptable. The model adequately characterized the PK profile of GS-331007. The inter-individual variability expressed as CV% was modest for clearance (25%) suggesting that the key PK parameter (CL/F) was 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 SOF/VEL, NDA208341). No new findings or conclusions were derived from the analysis.

Since SOF, a component of various HCV treatment regimens, has slightly different exposures depending on the regimen despite always being administered as 400 mg, the reviewer collected information from different labels and population PK reports to permit a side-by-side comparison of these exposures. SOF regimens considered in this summary were SOF SOF with ledipasvir, SOF/VEL, and SOF/VEL/VOX. Regimens containing ledipasvir, VEL, or VOX contain 90 mg, 100 mg, and 100 mg of the compounds, respectively. A comparison of clearance (CL/F) estimate is shown below in Table 4.2-12. VEL and ledipasvir appear to decrease SOF clearance significantly, while VOX decreases the clearance of both SOF and VEL.

Table 4.2-12: **Comparison of Clearance Estimate Mean (CV%) by Population PK Models under Various Combinations**

Combination		*CL/F (L/h)*			
	SOF	*GS-331007*	*LDV*	*VEL*	*VOX*
SOF	*652(71.2%)*	*39.5(33.2%)*	-	-	-
SOF/Ledipasvir	*304.9(40.2%)*	*31.8(21.9%)*	*13.1(47.6%)*	-	-
SOF/VEL	*352.4(48.2%)*	*30.3(23.9%)*	-	*46.5(50.8%)*	-
SOF/VEL/VOX	*273.1(41.9%)*	*33.69(25.0%)*	-	*32.83(54.7%)*	*65.8(70.1%)*
	AUC_{tau} (ng.hr/mL)				
SOF	*860(36.3%)*	*7200(30.5%)*	-	-	-
SOF/Ledipasvir	*1380(34.0%)*	*12500(29.2%)*	*8530(60.8%)*	-	-
SOF/VEL	*1268(38.5%)*	*14372(28.0%)*	-	*2980(51.3%)*	-
SOF/VEL/VOX	*1665(30.1%)*	*12834(29.0%)*	-	*4041(48.6%)*	*2577(73.7%)*

4.3 Exposure-response Analysis

Exposure-Response Analysis for Efficacy

The PK/PD relationship for SOF/VEL/VOX was evaluated in HCV-infected subjects in Phase 3 studies (GS-US-367-1171 and GS-US-367-1170) using VOX, VEL, SOF, and GS-331007 plasma exposures derived from population PK analyses. The primary efficacy endpoint for the SOF/VEL/VOX Phase 3 studies was SVR12.

No exposure-response relationships for efficacy were identified in DAA-experienced subjects across exposure ranges for VOX, VEL, SOF, and GS-331007 observed in the Phase 3 studies, though it should be noted that the Phase 3 studies evaluated only a single dose-level of the three compounds (400/100/100 mg QD). The SVR12 rates were high in all groups. The SVR12 rate by population quartiles of VOX, VEL, SOF, and GS-331007 AUC_{tau} in DAA-experienced subjects in study GS-US-367-1171 and study GS-US-367-1170 is summarized in Table 4.3-1. As indicated, the SVR12 rates by population quartiles of VOX, VEL, SOF, and GS-331007 AUC_{tau} in all subjects ranged from 92.9% to 100%. There was no significant exposure-SVR12 relationship in all DAA-experienced subjects or subjects with compensated cirrhosis. A summary of SVR12 rates by population quartiles of VOX, VEL, SOF, and GS-331007 AUC_{tau} and by HCV genotype did not reveal any exposure-response relationship either (Table 4.3-1).

Table 4.3-1: SVR12 Rate by Population Quartiles of VOX, VEL, SOF, and GS-331007 AUC_{tau} in DAA-Experienced Subjects in Study GS-US-367-1171 (POLARIS-1) or GS-US-367-1170 (POLARIS-4) (PK/PD Analysis Set)

	GS-US-367-1171					GS-US-367-1170				
	AUC_{tau} h*ng/mL (range)	All Subjects		Subjects with Cirrhosis		AUC_{tau} h*ng/mL (range)	All Subjects		Subjects with Cirrhosis	
		N	SVR12	N	SVR12		N	SVR12	N	SVR12
VOX										
Q4	3171.2 – 16,922.2	72	95.8%	54	94.4%	3158.9 – 9211.4	50	96.0%	36	94.4%
Q3	1993.3 – 3147.4	52	96.2%	27	96.3%	1992.5 – 3173.9	45	97.8%	25	96.0%
Q2	1238.7 – 1990.7	60	96.7%	19	89.5%	1212.0 – 1942.9	42	97.6%	13	100%
Q1	289.3 – 1202.3	77	97.4%	19	94.7%	289.7 – 1156.4	45	97.8%	10	100%
VEL										
Q4	5036.8 – 13,078.2	50	98.0%	20	95.0%	5059.2 – 11,991.4	33	100%	11	100%
Q3	3660.7 – 5016.6	55	98.2%	28	100%	3666.1 – 4953.2	44	97.7%	15	93.3%
Q2	2641.8 – 3645.9	70	92.9%	38	89.5%	2698.3 – 3644.6	46	93.5%	27	92.6%
Q1	613.7 – 2637.6	87	97.7%	34	94.1%	711.5 – 2636.6	58	98.3%	31	100%
SOF										
Q4	1949.3 – 3364.7	31	100%	14	100%	2062.8 – 2982.2	19	100%	6	100%
Q3	1572.1 – 1924.9	36	97.2%	25	96.0%	1567.8 – 1937.8	31	93.5%	16	93.8%
Q2	1327.3 – 1554.8	45	95.6%	18	88.9%	1342.1 – 1553.4	30	96.7%	16	93.8%
Q1	620.6 – 1317.6	55	98.2%	22	95.5%	778.0 – 1322.6	36	100%	19	100%
GS-331007										
Q4	14,641.8 – 31,920.8	47	93.6%	18	83.3%	14,816.5 – 23,445.3	31	100%	14	100%
Q3	12,238.4 – 14,622.6	58	98.3%	27	100%	12,246.6 – 14,636.5	47	93.6%	21	90.5%
Q2	10,443.3 – 12,202.6	74	98.6%	35	100%	10,436.2 – 12,224.6	47	100%	19	100%
Q1	46,16.2 – 10,376.7	82	95.1%	39	89.7%	5364.4 – 10,431.3	56	96.4%	29	96.6%

Source: Table 51 on page 179 of Summary of Clinical Pharmacology Studies

Table 4.3-2: SVR12 Rate by Population Quartiles of VOX, VEL, SOF, and GS-331007 AUC_{tau} and by HCV Genotype in DAA-Experienced Subjects in Study GS-US-367-1171 (POLARIS-1) or GS-US-367-1170 (POLARIS-4) (PK/PD Analysis Set)

GS-US-367-1171 (POLARIS-1)[#]				GS-US-367-1170(POLARIS-4)*				
VOX AUCtau (Range)	GT1 (N=149)	GT-3 (N=78)	GT-4 (N=21)	VOX AUCtau	GT-1 (N=78)	GT-2 (N=31)	GT-3 (N=54)	GT-4 (N=19)
Q4 (3171-16922)	34/34 (100%)	25/27 (92.6%)	7/8 (87.5%)	Q4 (3159-9211.4)	20/20 (100%)	5/5 (100%)	11/13 (84.6%)	12/12 (100%)
Q3 (1993.3-3147.4)	26/28 (92.9%)	16/16 (100%)	6/6 (100%)	Q3 (1992.-3137.9)	21/22 (95.5%)	4/4 (100%)	15/15 (100%)	4/4 (100%)
Q2 (1238.7-1990.7)	38/39 (97.4%)	13/14 (92.9%)	4/4 (100%)	Q2 (1212-1942.9)	16/17 (94.1%)	13/13 (100%)	12/12 (100%)	0/0
Q1 (289.3-1202.3)	47/48 (97.9%)	20/21 (95.2%)	3/3 (100%)	Q1 (289.7-1156.4)	19/19 (100%)	9/9 (100%)	13/14 (92.9%)	3/3 (100%)

[#]Note: There were few genotype 2 (n=5), genotype 5 (n=1), and genotype 6 (n=6) subjects in GS-US-367-1171. The SVR12 rate was 100% in these subjects.

*Note: No genotype 5 and 6 subjects were included in study GS-US-367-1170

Source: Adapted from Table 2.4.1 of PK-PD Tables Figures and Listings

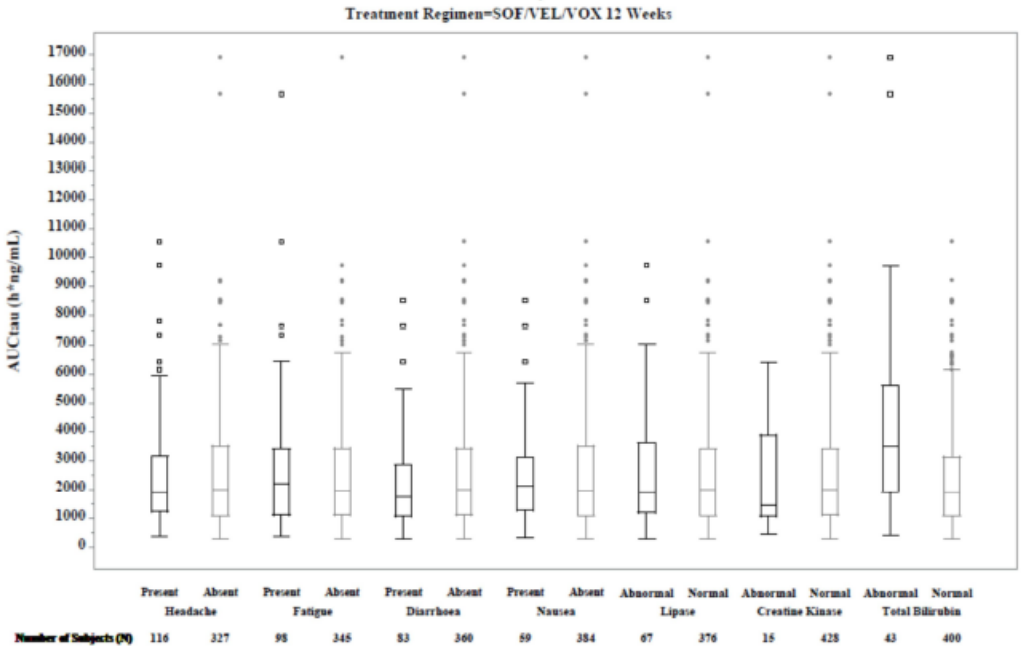
Exposure-Response Analysis for Safety

The safety PK/PD dataset included HCV-infected subjects who received SOF/VEL/VOX and had evaluable PK parameters in four Phase 3 studies (GS-US-367-1170, GS-US-367-1171, GS-US-367-1172, and GS-US-367-1173). The PK parameters were derived from the population PK analysis. The treatment duration in GS-US-367-1172 and GS-US-367-1173 was 8-weeks instead of 12-weeks as was evaluated in GS-US-367-1170, GS-US-367-1171. Separate analyses were conducted based on treatment duration.

VOX: There was no duration dependent relationship and incidence of the four most frequently reported AEs (occurring in > 10% of subjects) observed in pooled SOF/VEL/VOX Phase 3 studies by treatment duration (SOF/VEL/VOX 12-weeks or 8-weeks). These AEs (incidences) were headache (26.1%, 26.4%), fatigue (22.2%, 21.9%), diarrhea (18.7%, 17.2%), and nausea (13.3%, 16.9%), respectively. No difference in event rate was identified based on treatment duration of 12-weeks versus 8-weeks.

Further exploring the data, there was no relationship between VOX exposure and incidence of abnormal lipase (all graded abnormalities), creatine kinase (all graded abnormalities). In addition, there was no clear association between VOX exposure and findings of headache, fatigue, diarrhea, and nausea. However, higher VOX AUC_{tau} was correlated with maximum change from baseline in total bilirubin (mg/dL) (Figure 4.3-1).

Figure 4.3-1: Box Plots of VOX AUCtau by Presence or Absence of Selected Adverse Events and Laboratory Abnormalities in SOF/VEL/VOX Phase 3 Studies (PK/PD Analysis Set)

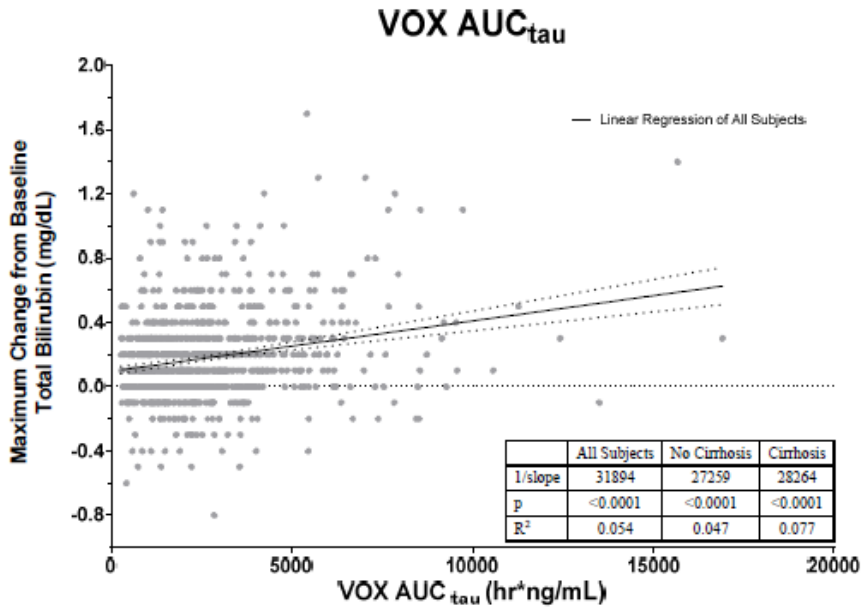


Source: Figure 11 on page 184 of Summary of Clinical Pharmacology Studies

The Applicant further explored this observation using maximum on treatment change in total bilirubin. Linear regression analysis indicated that increases of VOX AUC_{tau} by

approximately 3200 h*ng/mL (i.e., an approximate doubling of exposure observed in Phase 2 and 3 studies) were associated with an increase in total bilirubin of 0.1 mg/dL (Figure 4.3-2). This magnitude of increase in total bilirubin was considered to not be clinically relevant given the small magnitude of the change and resolution of elevated total bilirubin after completion of treatment with SOF/VEL/VOX.

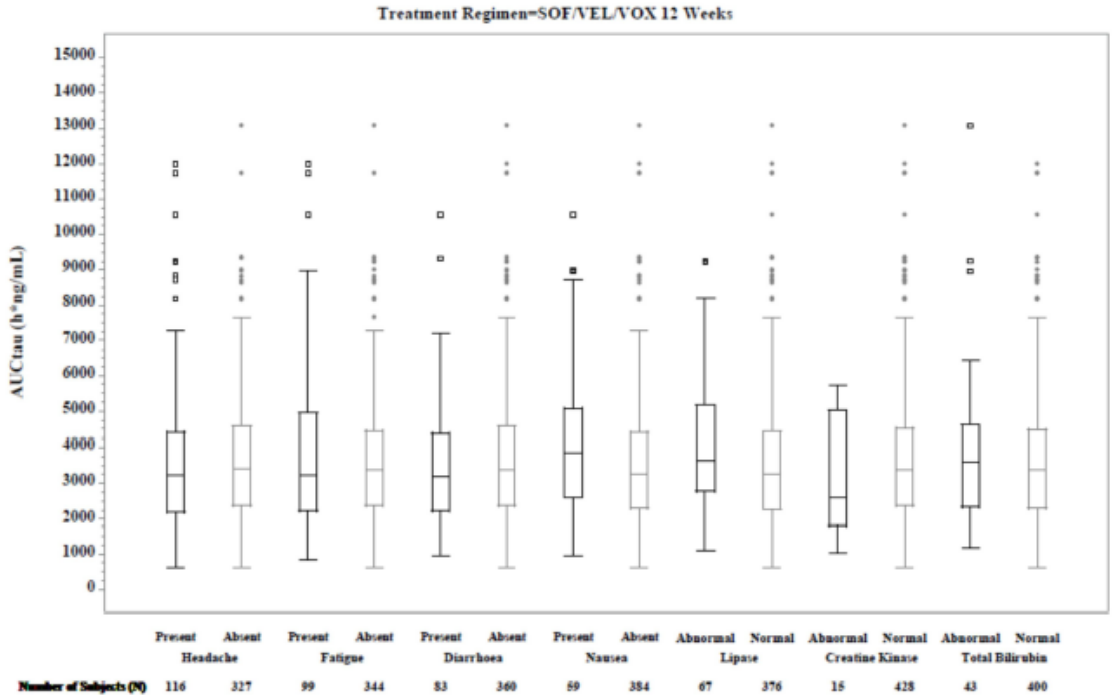
Figure 4.3-2: **Correlations of VOX AUC_{tau} with Maximum on Treatment Change from Baseline in Total Bilirubin (PK/PD Analysis Set)**



Source: Adapted from Figure 13 on page 186 of the Summary of Clinical Pharmacology Studies

VEL and SOF: Relationship between VEL exposure and the most frequent AEs were also evaluated with the PK/PD safety dataset. As shown in Figure 4.3-3, there was no evident association between VEL exposure and incidence rate of headache, fatigue, diarrhea, and nausea or abnormalities in lipase, creatine kinase, or total bilirubin.

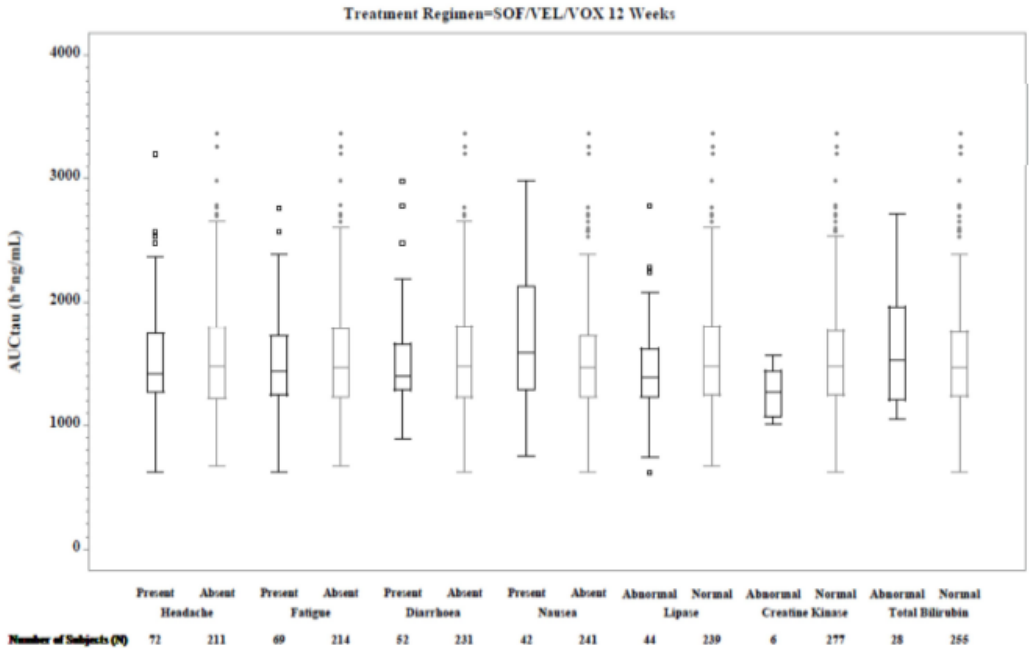
Figure 4.3-3: Box Plots of VEL AUC_{tau} by Presence or Absence of Selected Adverse Events and Laboratory Abnormalities in SOF/VEL/VOX Phase 3 Studies (PK/PD Analysis Set)



Source: Figure 14 on page 188 of Summary of Clinical Pharmacology Studies

Similarly, there was no relationship between SOF (Figure 4.3-4) or GS-331007 exposure and incidence rate of headache, fatigue, diarrhea, and nausea or abnormalities in lipase, creatine kinase, or total bilirubin.

Figure 4.3-4: Box Plots of SOF AUC_{tau} by Presence or Absence of Selected Adverse Events and Laboratory Abnormalities in SOF/VEL/VOX Phase 3 Studies (PK/PD Analysis Set)



Source: Adapted from Figure 15 on page 190 of Summary of Clinical Pharmacology Studies

Oral Conraceptives and ALT Increase

In the four Phase 3 studies, a total of 4 subjects (GS-US-367-1170-05969-27808, GS-US-367-1171-00381-24106, GS-US-367-1171-01981-24096GS-US-367-1172-04251-26934 GS-US-367-1172-04251-26934) were identified as having ALT Grade 3 event. The relationship between ALT increase and use of oral contraceptives (OC) was raised by the team and explored by the reviewer. Of the twelve and four subjects with ALT Grade 2 and Grade 3 elevations, respectively, none of the subjects were taking oral contraceptives as a concomitant medication.

In the four Phase 3 studies, fourteen subjects were identified as having an on-treatment OC listed as a concomitant medication. Of these subjects, 13 were on an ethinyl estradiol (EE) containing OC. None of these subjects had a Grade 2 or 3 ALT elevation. Of the subjects on an OC, only 7 had exposure data. In subjects on an OC, 50% higher VEL AUC_{tau} was observed compared to non-OC subjects, but SOF and VOX exposure were comparable.

While the above data are imited, the data do not suggest that ALT elevations observed in the Phase 3 study were associated with concomitant OC use.

Table 4.3-1: Summary of AUC_{tau} in HCV-infected Subjcts With or Without On-Treatment Concomitant Oral Contraceptive Use

		AUC_{tau} (ng.hr/mL)				
		n	Mean	SD	Min	Max
No OC Subjects (N=1048)	SOF	596	1641.3	478.7	620.6	3759.4
	VEL	1047	4014.4	2026.7	613.7	14395.3
	VOX	1045	2481.2	1872.3	268.8	16922.2
OC Subjects (N=7)	SOF	5	1771.2	283.5	1347.4	2131.6
	VEL	7	6271.3	1534.8	5036.8	8706.3
	VOX	7	2439.9	1599.2	983.5	5174.9

4.4 Individual Clinical Pharmacology Report Reviews

Table 4.4-1 List of Individual Study Reports Reviewed

#	Study No.	Study information
4.4.1	GS-US-338-1120	SAD and MAD PK of VOX
4.4.2	GS-US-338-1121	MAD and Monotherapy Efficacy for VOX
4.4.3	GS-US-338-1124	Mass Balance Study for VOX
4.4.4	GS-US-338-1125	Renal Impairment Study
4.4.5	GS-US-338-1126	Hepatic Impairment Study
4.4.6	GS-US-338-1130	DDI, BA, FE, SAD, Steady State PK
4.4.7	GS-US-367-1176	Relative BA and High-fat Meal FE
4.4.8	GS-US-367-1417	DDI Enzyme/Transporter Inhibition/Induction on VOX
4.4.9	GS-US-367-1657	DDI ARVs
4.4.10	GS-US-367-1726	DDI with H2-Receptor Antagonist or Proton Pump Inhibitor
4.4.11	GS-US-367-1727	DDI with Transporter Substrates (Statins and Dabigatran) and ARVs
4.4.12	GS-US-367-1909	DDI with a hormonal contraceptive
4.4.13		In Vitro Study Reports

4.4.1 GS-US-338-1120 SAD and MAD PK of VOX

1. Title

A Phase 1 Study in Healthy Volunteers to Evaluate the Safety, Tolerability, and Pharmacokinetics of GS-9857, and the Effect of Food and Solution Formulation on GS-9857 Pharmacokinetics

2. Information Regarding the Clinical Trial Site and Duration of the Trial

The trial was conducted (b) (4) from March 13, 2014 to June 25, 2014, with the final report date of January 5, 2015.

3. Objectives

- To evaluate the safety and tolerability of escalating single and multiple oral doses of GS-9857
- To characterize the single- and multiple-dose pharmacokinetics (PK) of GS-9857
- To perform a preliminary evaluation of the effect of concomitant food intake and solution formulation on GS-9857 PK

4. Trial Design

The study proceeded in 2 parts (Parts A and B).

Part A: Single- and Multiple-Ascending Doses (Cohorts 1 through 6):
Part A consisted of 6 randomized, double-blind cohorts of single- and multiple-ascending doses of GS-9857 including 3 staggered prespecified cohorts (Cohort 1: 30 mg, Cohort 2: 100 mg, and Cohort 3: 300 mg) and 3 adaptive cohorts (Cohorts 4 through 6: between 10 and 600 mg). Within each cohort, unique subjects were randomized to receive either active GS-9857 (N = 12) or matching placebo (N = 3). Subjects received a single dose of GS-9857 or placebo in the fasted state on Day 1, followed by a 6-day washout, and then once-daily doses of GS-9857 or placebo in the fasted state on Days 8 to 17. After review of safety and available PK data from Cohorts 1 through 3, Cohorts 4 through 6 were not initiated.

Part B: Food and Formulation Effect (Cohorts 7 through 9):
Part B consisted of 3 open-label, parallel cohorts, including a fasted/light breakfast cohort (Cohort 7), a fasted/high-fat breakfast cohort (Cohort 8), and a fasted/solution formulation dosing cohort (Cohort 9). Based on available data from Part A, the 100-mg dose of GS-9857 was selected for evaluation in Part B. Within each cohort, 12 subjects received a single dose of GS-9857 in the fasted state on Day 1, followed by a 6-day washout, and then a second single dose of GS-9857 in the fed state (Cohort 7: light breakfast; Cohort 8: high-fat breakfast) or fasted state (Cohort 9: solution formulation dosing) on Day 8. A light breakfast contained approximately 400 kcal and 10% fat; a high-fat/high-calorie breakfast contained approximately 1000 kcal and 50% fat. On the evening prior to PK assessment days, subjects underwent an overnight fast (e.g., no foods or liquids, except water, for at least 10 hours) prior to study drug administration (in the fed state as described above) the next day.

In Cohorts 1 through 3, GS-9857 was administered as a single oral dose in the fasted state on Day 1 and as once-daily doses in the fasted state for 10 days from Days 8 to 17, as follows:

- Cohort 1: GS-9857 30 mg (3 × 10-mg tablets)

- Cohort 2: GS-9857 100 mg (2 × 50-mg tablets)
- Cohort 3: GS-9857 300 mg (6 × 50-mg tablets)

In Cohorts 7 and 8, GS-9857 was administered as a single oral dose in the fasted state on Day 1 and as a single dose in the fed state (light breakfast or high-fat breakfast) on Day 8, as follows:

- Cohort 7: GS-9857 100 mg (10 × 10-mg tablets)
- Cohort 8: GS-9857 100 mg (10 × 10-mg tablets)

In Cohort 9, GS-9857 was administered as a single oral dose in the fasted state on Day 1 and as a single oral dose in solution in the fasted state on Day 8, as follows:

- Cohort 9: GS-9857 100 mg (10 × 10-mg tablets on Day 1) and 100 mg (100 mL solution on Day 8)

Eligible subjects were healthy males and nonpregnant, nonlactating females, 18 to 45 years of age (inclusive), with a body mass index (BMI) of 19 to 30 kg/m^2 (inclusive), normal 12-lead electrocardiograms (ECGs) (or one with abnormalities considered clinically insignificant), normal renal function with an estimated creatinine clearance (CLcr) (calculated using the Cockcroft-Gault equation) ≥ 80 mL/min, no significant medical history, and in good general health.

5. Excluded Medications and Restrictions

- Any prescription medications and OTC medications including herbal products and antacids with the exception of vitamins, and/or acetaminophen and/or ibuprofen and/or hormonal contraceptive medications. However, the short-term use of topical hydrocortisone cream or A&D ointment to treat minor skin irritation due to ECG leads was allowed.
- Smokers, use of nicotine or nicotine-containing products within 90 days prior to the first dose of study drug and/or a urine cotinine ≥ 500 ng/mL at screening and clinic admission
- Alcohol or substance abuse as assessed by the investigator
- Had previously participated in an investigational trial involving administration of any investigational compound within 30 days prior to the study dose
- Had taken systemic steroids, immunosuppressant therapies or chemotherapeutic agents within 3 months of study screening or expected to receive these agents during the study (e.g., corticosteroids, immunoglobulins, and other immune- or cytokine-based therapies)

6. Rationale for Doses Used in the Trial

VOX: 100 mg is the dose co-administered with SOF/VEL FDC at 400/100 mg in most Phase 1 studies and also the dose in Phase 2 and 3 studies.

7. Drugs Used in the Trial

VOX conventional 10 mg tablets – Lot Number(s): DY1401B1 (Manufactured by (b) (4)

VOX conventional 50 mg tablets – Lot Number(s): DY1403B1 (Manufactured by (b) (4)

[redacted]

VOX placebo 10 mg tablets – Lot Number(s): DY1402B1 (Manufactured by [redacted]

VOX placebo 50 mg tablets – Lot Number(s): DY1405B1 (Manufactured by [redacted]

VOX 100 mg solution – Lot Number(s): DY1406A (for GS-9857 oral solution dosing, GS-9857 drug substance (in powder form) was provided to the investigator or qualified designee for compounding use only)

8. Sample Collection, Bioanalysis, Pharmacokinetic Assessments, and Statistical Analysis

Sample Collection

Part A: Single- and Multiple-Ascending Doses (Cohorts 1 through 3)

For subjects enrolled in Cohorts 1 through 3, serial blood samples were collected for analysis of GS-9857 (and metabolites, if appropriate) plasma concentrations on Days 1 and 17 relative to study drug administration in the morning at the following time points: predose (within 5 minutes), 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48, 72, and 96 hours postdose. In addition, single predose trough samples were collected on Days 13, 14, 15, and 16.

Part B: Food and Formulation Effect (Cohorts 7 through 9)

For subjects enrolled in Cohorts 7 through 9, serial blood samples were collected for analysis of GS-9857 plasma concentrations on Days 1 and 8 relative to study drug administration in the morning at the following time points: predose (within 5 minutes), 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48, 72, and 96 hours postdose.

Bioanalytical method

- Validation and Bioanalytical Report:

Analyte	Matrix	Validation Report	Bioanalytical Report
GS-9857 (VOX)	Plasma	[redacted] 8109.123113	[redacted] 8220.093014

- Bioanalytical Method:

Method type: LC/MS/MS; Matrix: K_2EDTA plasma

Analyte	Range (ng/mL)	Stability in frozen matrix (days)
GS-9857 (VOX)	0.5 to 1000	42 at -20ºC and 38 at -70ºC

Validation	▪ Method validated prior to use	☒ Yes ☐ No ☐ NA
	▪ Method validation acceptable	☒ Yes ☐ No ☐ NA
Study Samples Analysis	▪ Samples analyzed within the established stability period	☒ Yes ☐ No
	▪ Quality control samples range acceptable	☒ Yes ☐ No
	▪ Chromatograms provided	☒ Yes ☐ No
	▪ Accuracy and precision of the calibration curve	☒ Yes ☐ No

	acceptable	
	▪ Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	▪ Incurred samples analysis is acceptable	☒ Yes ☐ No
	▪ Overall performance acceptable	☒ Yes ☐ No
Inspection	▪ Will the bioanalytical site be inspected	☐ Yes ☒ No

Pharmacokinetic Assessments
The following single- or multiple-dose plasma PK parameters of GS-9857 were calculated as appropriate: AUC_{last}, AUC_{inf} (single dose), AUC_{tau} (multiple dose), % AUC_{exp} (single dose), C_{max}, T_{max}, C_{last}, T_{last}, C_{tau} (multiple dose), λ_z, CL/F, and $t_{1/2}$.

Statistical Analysis
Part A: Single- and Multiple-Ascending Doses (Cohorts 1 through 3):
Dose proportionality was obtained by comparing PK parameters of GS-9857 across evaluated dose levels. The primary method for evaluation of dose proportionality was based on AUC_{last}, AUC_{inf} (single dose), AUC_{tau} (multiple doses), and C_{max} using a power model that was fitted using all fasted doses across cohorts.

Part B: Food and Formulation Effect (Cohorts 7 through 9):
The effect of food (Cohorts 7 and 8) or formulation (Cohort 9) on PK of GS-9857 was assessed by using a linear mixed-effects model with a fixed effect of food or formulation, as appropriate, and a random effect of subject. The geometric least-squares means ratios (i.e., fed/fasted for Cohorts 7 and 8, or test/reference formulations for Cohort 9) and their 90% CIs were constructed for the natural log-transformed primary PK parameters AUC_{last}, AUC_{inf}, and C_{max}.

9. Results

9.1 Subject Demographics and Disposition
A total of 81 subjects were enrolled into the study across 6 cohorts, and all subjects completed the study (Part A: 45 subjects (12 active and 3 placebo subjects per cohort), Part B: 36 subjects (12 subjects per cohort)).

Overall, the majority of subjects were male (60.5%), white (53.1%), and Hispanic or Latino (56.8%). Subjects had a mean (SD) age of 32 (7.2) years (range: 19 to 45 years), a mean (SD) BMI of 25.2 (2.59) kg/m^2, and a mean (SD) CLcr of 118.99 (20.088) mL/min.

9.2 Pharmacokinetic and Statistical Analysis
The summary of PK parameters and statistical analysis for VOX in Part A and B are presented in **Table 1** and **Table 2**, respectively.

Half-life estimates after single-dose administration of GS-9857 30 mg were significantly shorter (9.4 hours) than multiple-dose (27-40 hours) as a result of concentrations falling

below the lower limit of quantitation during the distribution phase. Steady-state concentrations of GS-9857 were achieved after 7 days of once daily dosing across all dose levels. GS-9857 exhibited linear PK ($AUC_{last,\ inf,\ or\ tau}$ and C_{max}) across the dose range evaluated (30 to 300 mg) after single and multiple oral doses. Consistent with the long half-life of GS-9857 and once-daily dosing, accumulation ratios of 3.6, 2.8, and 3.4 were observed for AUC_{tau}, C_{max}, and C_{tau}, respectively.

Administration of GS-9857 with food (light- or high-calorie/high-fat breakfast) increased the extent of absorption of GS-9857 approximately 2-fold with comparable inter-individual variability and no effect on the terminal half-life.

Table 1: Plasma PK Parameters and Statistical Analysis for VOX in Part A

Single-Dose GS-9857 PK Parameters			
GS-9857 PK Parameter	**GS-9857 30 mg (N = 12)**	**GS-9857 100 mg (N = 48)**	**GS-9857 300 mg (N = 12)**
AUC_{last} (ng•h/mL)	33.0 (90.5)	188.3 (64.1)	425.3 (62.8)
AUC_{inf} (ng•h/mL)	56.9 (107.2)	245.0 (79.8)	484.9 (58.1)
C_{max} (ng/mL)	3.7 (56.9)	17.6 (64.0)	34.5 (54.2)
C_{last} (ng/mL)	0.67 (16.3)	1.09 (191.6)	1.13 (42.1)
T_{max} (h)	4.00 (2.50, 5.00)	3.00 (1.75, 4.00)	2.00 (1.50, 3.00)
T_{last} (h)	18.00 (12.00, 36.00)	96.00 (48.00, 96.00)	96.00 (96.00, 96.00)
$t_{1/2}$ (h)	9.37, 4.33, 35.69)	36.67 (22.42, 49.20)	35.49 (28.83, 46.27)
CL/F (mL/h)	1,291,406.1 (84.5)	741,586.5 (105.8)	821,466.9 (51.2)
Multiple-Dose GS-9857 PK Parameters			
GS-9857 PK Parameter	**GS-9857 30 mg (N = 12)**	**GS-9857 100 mg (N = 12)**	**GS-9857 300 mg (N = 12)**
AUC_{tau} (ng•h/mL)	74.2 (40.7)	364.4 (81.3)	1030.5 (77.3)
C_{max} (ng/mL)	7.3 (57.6)	46.0 (126.1)	155.0 (99.6)
C_{tau} (ng/mL)	1.9 (50.6)	5.7 (44.9)	10.0 (56.2)
T_{max} (h)	5.00 (3.50, 5.00)	5.00 (1.50, 5.00)	3.50 (2.00, 4.51)
T_{last} (h)	96.00 (72.00, 96.00)	96.00 (96.00, 96.00)	96.00 (96.00, 96.00)
$t_{1/2}$ (h)	40.95 (30.07, 46.47)	30.49 (27.25, 31.95)	27.61 (25.12, 34.14)
CL_{ss}/F (mL/h)	464,375.7 (36.6)	429,788.6 (63.8)	432,361.9 (55.7)

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

GS-9857 PK Parameters	ANOVA Analysis %GLSM Ratio (Dose/100 mg) (90%CI)		Power Model Analysis Slope (90% CI)
	GS-9857 30 mg (N = 12)	GS-9857 300 mg (N = 12)	GS-9857 Across All Doses (N = 36)
Single-Dose Administration			
AUC_{0-24} (ng•h/mL)	99.27 (62.77, 157.00)	95.02 (60.08, 150.27)	0.98 (0.79, 1.18)
AUC_{inf} (ng•h/mL)	88.03 (48.19, 160.80)	104.97 (57.47, 191.74)	1.08 (0.82, 1.33)
C_{max} (ng/mL)	110.03 (71.48, 169.36)	103.90 (67.50, 159.93)	0.97 (0.79, 1.16)
Multiple-Dose Administration			
AUC_{tau} (ng•h/mL)	80.63 (53.14, 122.34)	96.61 (63.67, 146.58)	1.08 (0.90, 1.26)
C_{max} (ng/mL)	76.83 (45.46, 129.85)	129.56 (76.66, 218.96)	1.23 (1.00, 1.45)
C_{tau} (ng/mL)	111.89 (78.74, 158.99)	56.17 (39.53, 79.82)	0.70 (0.55, 0.86)

For ANOVA analysis all PK parameters were dose-normalized to 100 mg.

GS-9857 PK Parameter	% GLSM Ratio (Multiple-Dose/ Single-Dose) (90% CI)			
	GS-9857 30 mg (N = 12)	GS-9857 100 mg (N = 12)	GS-9857 300 mg (N = 12)	GS-9857 All Doses (N = 36)
AUC_{tau} (ng•h/mL)	310.71 (246.47, 391.70)	382.68 (303.56, 482.42)	388.95 (308.53, 490.32)	358.95 (314.02, 410.30)
C_{max} (ng/mL)	206.04 (156.50, 271.28)	295.07 (224.12, 388.49)	367.94 (279.47, 484.43)	281.77 (240.39, 330.26)
C_{tau} (ng/mL)	398.15 (295.50, 536.45)	422.92 (313.89, 569.82)	238.98 (177.37, 322.00)	342.68 (288.49, 407.05)

Table 2: Plasma PK Parameters and Statistical Analysis for VOX in Part B

GS-9857 PK Parameter	GLSM		% GLSM Ratio (Fed/Fasted)	90% CI
	GS-9857 100 mg Fed (N = 12)	GS-9857 100 mg Fasted (N = 12)		
Light Breakfast				
AUC_{last} (ng•h/mL)	377.27	172.43	218.79	(179.88, 266.11)
AUC_{inf} (ng•h/mL)	427.51	216.82	197.17	(167.48, 232.13)
C_{max} (ng/mL)	42.46	15.80	268.78	(210.16, 343.76)
High-fat Breakfast				
AUC_{last} (ng•h/mL)	423.42	199.22	212.54	(162.32, 278.28)
AUC_{inf} (ng•h/mL)	486.47	258.83	187.95	(137.68, 256.56)
C_{max} (ng/mL)	38.73	18.38	210.71	(149.35, 297.28)

GS-9857 PK Parameter	GLSM		% GLSM Ratio (Solution/Tablet)	90% CI
	GS-9857 100 mg Solution Fasted (N = 12)	GS-9857 100 mg Tablet Fasted (N = 12)		
AUC_{last} (ng•h/mL)	400.87	132.32	302.96	(218.25, 420.56)
AUC_{inf} (ng•h/mL)	462.64	169.81	272.45	(197.38, 376.07)
C_{max} (ng/mL)	59.16	14.56	406.37	(314.17, 525.63)

9.3 Safety Analysis

No Grade 2, 3, or 4 AEs, serious adverse events, or AEs leading to permanent discontinuation of study drug were reported in any cohort.

10. Sponsor's Conclusions

- GS-9857 exhibited linear PK across the evaluated single- or multiple-dose range. Consistent with the long half-life (28 to 41 hours) and once-daily dosing, accumulation ratios of 2.8 to 3.6 were observed for AUC_{tau}, C_{max}, and C_{tau}.
- Food increased the extent of GS-9857 absorption approximately 2-fold for the conventional tablets at 10 mg.
- A solution formulation of GS-9857 substantially increased GS-9857 exposure from conventional tablets at 10 mg (approximately 3-fold) while decreasing inter-individual variability.

11. Reviewer's Assessment

The study design is reasonable for both Part A and B and the sponsor's conclusions are valid. The food effect is also evaluated in other studies with SOF/VEL+VOX (modified formulation at 100 mg) (Study GS-US-338-1130) and SOF/VEL/VOX FDC at 400/100/100 mg (Study GS-US-367-1176), which is more relevant to the to-be-marketed formulation.

In this study, food effect was evaluated for VOX at 100 mg (10 × 10-mg tablets) in Cohort 7 (light breakfast *vs.* fasted) and Cohort 8 (high-fat meal *vs.* fasted). Light-fat meal increased VOX exposure by ~ 100%, similar as results from SOF/VEL+VOX (modified formulation at 100 mg) (Study GS-US-338-1130). However, high-fat meal increased VOX exposure by ~ 100%, compared to ~ 400% from SOF/VEL/VOX FDC at 400/100/100 mg (Study GS-US-367-1176). The fat components are similar between these studies, and the difference for the food effect may be caused by different formulations.

4.4.2 GS-US-338-1121 MAD and Monotherapy Efficacy for VOX

1. Title

Phase 1b, Randomized, Double-Blind, Multiple-Dose Ranging Study Evaluating the Safety, Tolerability, Pharmacokinetics, and Antiviral Activity of GS-9857 in Subjects with Chronic Hepatitis C Virus Infection

2. Information Regarding the Clinical Trial Site and Duration of the Trial

The trial was conducted (b) (4) from June 13, 2014 to September 28, 2015, with the final report date of January 25, 2016.

3. Objectives

Primary objectives:

- To evaluate safety and tolerability of multiple oral doses of GS-9857 alone or with sofosbuvir (SOF)/velpatasvir (VEL) fixed-dose combination (FDC) in subjects with hepatitis C virus (HCV) infection
- To evaluate antiviral activity of GS-9857 in subjects with genotype 1 to 4 HCV infection

Secondary objectives:

- To evaluate the pharmacokinetics (PK) and pharmacodynamics (PD) of GS-9857
- To evaluate the PK of GS-9857+SOF/VEL
- To characterize the kinetics of circulating HCV RNA during treatment and after treatment discontinuation
- To characterize sequence changes in the nonstructural protein (NS)3/4A coding region of HCV following multiple-dose administration of GS-9857 and for up to 48 weeks after discontinuation of study treatment

Exploratory objective:

- To identify or validate genetic markers that may have been predictive of the natural history of disease, response to therapy, and/or tolerability of medical therapies through genetic discovery research (e.g., pharmacogenomics) in subjects who provided their separate and specific consent

4. Trial Design

This was a double-blind, multicenter, randomized, placebo-controlled study of GS-9857 in subjects infected with HCV. Dosing was planned in up to 10 unique dosing cohorts. Cohorts 4 through 10 were adaptive cohorts. Actual enrollment and doses administered are presented below. In Cohorts 1, 2, and 3, the dose administered was blinded. All subjects in Cohorts 1, 2, and 3 received the same total number of tablets (up to 16 tablets per dose). Adaptive cohorts (Cohorts 4 through 10) were dosed in either a fasted or fed state and did not include placebo treatment. Cohorts 7, 8, and 9 were not conducted.

Cohort	Genotype	Group	N	Drugs Administered (Dosing Days 1–3)
Cohort 1 (N=28)	1a	1	8	GS-9857 50 mg ± placebo QD for 3 days Fasted
		2	8	GS-9857 100 mg ± placebo QD for 3 days Fasted
		3	8	GS-9857 300 mg ± placebo QD for 3 days Fasted
		4	4	GS-9857 matching placebo QD for 3 days Fasted
Cohort 2 (N=21)	3	1	6	GS-9857 50 mg ± placebo QD for 3 days Fasted
		2	6	GS-9857 100 mg ± placebo QD for 3 days Fasted
		3	7	GS-9857 300 mg ± placebo QD for 3 days Fasted
		4	2	GS-9857 matching placebo QD for 3 days Fasted
Cohort 3 (N=8)	2	1	6	GS-9857 100 mg QD for 3 days Fasted
		2	2	GS-9857 matching placebo QD for 3 days Fasted
Cohort 4	4		4	GS-9857 100 mg QD for 3 days Fasted
Cohort 5	1b		6	GS-9857 100 mg QD for 3 days Fasted
Cohort 6	3a		6	GS-9857 100 mg QD for 3 days Fed
Cohort 10 (N=16)	any genotype	1	8	Day 1: GS-9857 100 mg after moderate fat meal; Day 2: GS-9857 100 mg + SOF/VEL FDC (400/100 mg) after moderate fat meal; Day 3: GS-9857 100 mg + SOF/VEL FDC (400/100 mg) after light meal
		2	8	Day 1: GS-9857 100 mg after moderate fat meal; Day 2: GS-9857 100 mg + SOF/VEL FDC (400/100 mg) after moderate fat meal; Day 3: GS-9857 100 mg + SOF/VEL FDC (400/100 mg) after moderate fat meal

Subjects enrolled in this study were males and non-pregnant females 18 to 65 years old (inclusive), non-cirrhotic, and had a body mass index (BMI) 19 to 34 kg/m^2 (inclusive). Cohorts 1 through 9 had chronic genotype 1a, 1b, 2, 3, or 4, HCV infection with a screening plasma HCV RNA level ≥ 5 $\log_{10}$ IU/mL, and were naive to HCV NS3/4A protease inhibitor (PI) antiviral treatment. Cohort 10 included any genotype.

5. Excluded Medications and Restrictions

Concomitant use of some medications and herbal/natural supplements with GS-9857 may have resulted in PK interactions resulting in increases or decreases in the exposure of GS-9857. Examples of medications which were prohibited 30 days prior to the first dose of study

drug, during the confinement period and through Day 10 are provided below:

Drug Class	Agents Disallowed
Antibiotics	Azithromycin, Clarithromycin, Erythromycin
Acid Reducing Agents	Proton-Pump Inhibitors, H2-Receptor Antagonists, Antacids
Anticonvulsants	Phenobarbital, Phenytoin, Carbamazepine, Oxcarbazepine
Antifungals	Itraconazole, Ketoconazole,
Antimycobacterials	Rifamycins, Isoniazid
Cardiac Medications	Amiodarone, Dronedarone, Felodipine, Verapamil, Quinidine, Ranolazine, Bosentan, Olmesartan, Telmisartan, Valsartan
Herbal/Natural Supplements	St. John's Wort, Echinaccea. Milk thistle (i.e. silymarin), Chinese herb sho-saiko-to (or Xiao-Shai-Hu-Tang)
HMG-CoA Reductase Inhibitors	fluvastatin, lovastatin, pitavastatin , pravastatin, rosuvastatin
Selective Serotonin Reuptake Inhibitors	Fluvoxamine
Other	Modafinil

Vitamins, acetaminophen, ibuprofen, and hormonal contraceptive medications were allowed during the study period. The short term use of topical hydrocortisone cream or A&D ointment to treat minor skin irritation due to ECG leads was allowed.

6. Rationale for Doses Used in the Trial

The doses evaluated in Cohorts 1 through 3 were determined based on the safety and available PK data from study GS-US-338-1120.

7. Drugs Used in the Trial

VOX conventional 10 mg tablets – Batch Number(s): DY1401B1 (Manufactured by (b) (4)

VOX conventional 50 mg tablets – Batch Number(s): DY1403B1 (Manufactured by (b) (4)

VOX placebo 10 or 50 mg tablets – Batch Number(s): DY1405B1 (Manufactured by (b) (4)

VOX modified 100 mg tablets – Batch Number(s): DY1407B1 (Manufactured by (b) (4)

SOF/VEL 400/100 FDC tablets– Batch Number(s): DU1404B1 (Manufactured by Gilead Sciences)

8. Sample Collection, Bioanalysis, Pharmacokinetic Assessments, and Statistical Analysis

Sample Collection
Blood samples were collected after the first and last (third) dose at approximately the following time-points: 0 (pre-dose), 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16 and 24 hours.

Additional blood samples will be collected at approximately 48 (Day 5), 72 (Day 6), 96 (Day 7), 120 (Day 8) and 168 (Day 10) hours post the third dose.

Bioanalytical method

- Validation and Bioanalytical Report:

Analyte	Matrix	Validation Report	Bioanalytical Report
GS-7977 (SOF), GS-331007	Plasma	(b) (4) 60-1323 Amendment 2	(b) (4) 60-1486B
GS-5816 (VEL)	Plasma	(b) (4) 60-1393 Amendment 1	60-1486A
GS-9857 (VOX)	Plasma	(b) (4) 8109.123113.1	(b) (4) 8370.051515

- Bioanalytical Method:

Method type: LC/MS/MS; Matrix: K_2EDTA plasma

Analyte	Range (ng/mL)	Stability in frozen matrix (days)
GS-7977 (SOF)	5 to 2500	174 at −20°C and 813 at −70°C
GS-331007	10 to 5000	174 at −20°C and 813 at −70°C
GS-5816 (VEL)	1 to 1000	161 at −20°C and 570 at −70°C
GS-9857 (VOX)	0.5 to 1000	420 at −20ºC and at −70ºC

Validation	▪ Method validated prior to use	☒ Yes ☐ No ☐ NA
	▪ Method validation acceptable	☒ Yes ☐ No ☐ NA
Study Samples Analysis	▪ Samples analyzed within the established stability period	☒ Yes ☐ No
	▪ Quality control samples range acceptable	☒ Yes ☐ No
	▪ Chromatograms provided	☒ Yes ☐ No
	▪ Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	▪ Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	▪ Incurred samples analysis is acceptable	☒ Yes ☐ No
	▪ Overall performance acceptable	☒ Yes ☐ No
Inspection	▪ Will the bioanalytical site be inspected	☐ Yes ☒ No

Pharmacokinetic Assessments

The following plasma PK parameters were calculated for GS-9857, VEL, SOF, and GS-331007 (as appropriate): C_{max}, T_{max}, C_{last}, T_{last}, C_{24}, λ_z, AUC_{last}, AUC_{inf}, $\%AUC_{exp}$, AUC_{0-24}, CL/F, and $t_{1/2}$.

Statistical Analysis

Dose and exposure-response for GS-9857 across all active doses were evaluated as appropriate. Relationships between GS-9857 plasma PK and antiviral activity (PK/PD) were explored.

9. Results

9.1 Subject Demographics and Disposition
Of 101 subjects who were enrolled in the study, 89 received treatment. A total of 87 subjects completed the short-term follow-up period (through Day 10).

Baseline demographics were generally similar across treatment groups. The mean age of subjects who participated in the study was 51 years, ranging from 30 to 64 years. The majority of subjects across treatments were male (67.4%), white (75.3%), and not Hispanic or Latino (50.6%). The mean BMI was 27.5 kg/m^2 (range: 19.4 to 34.0 kg/m2).

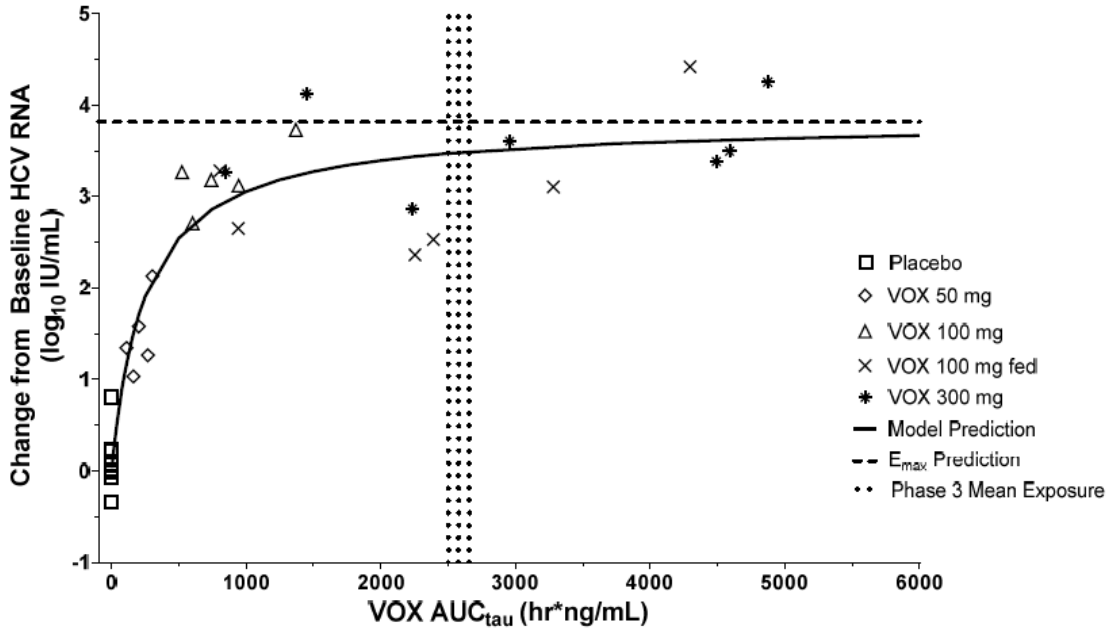
The majority of subjects had the genotype 1a subtype (41.6%), followed by the genotype 3a (31.5%), 1b (10.1%), and 2b (9.0%) subtypes. Genotype 2, 2a/2c, 4, and 4a/4c/4d subtypes were present in ≤ 2.2% of subjects each. Viral load was similar across treatments with a mean HCV RNA of 6.2 $\log_{10}$ IU/mL at baseline (range: 4.0 to 7.6 $\log_{10}$ IU/mL). The majority of subjects had a baseline HCV RNA ≥ 800,000 IU/mL (68.5%) and a baseline alanine aminotransferase (ALT) (U/L) ≤ 1.5 × the upper limit of normal (51.7%).

9.2 Efficacy, PK/PD relationship and Pharmacokinetic analysis

Efficacy:

- Three days of monotherapy with GS-9857 100 mg resulted in ≥ 3 $\log_{10}$ IU/mL maximum decline from baseline in HCV RNA in subjects with genotypes 1 through 4 HCV infection, consistent with pangenotypic *in vitro* replicon data.
- The presence of NS3 RAVs at baseline had no impact on response to 3 days of monotherapy with GS-9857. GS-9857 demonstrated an improved resistance profile to 1st generation PIs; the majority of subjects did not have NS3 RAVs during the dosing or post baseline period.
- The majority of the emergent NS3 RAVs did not persist through Week 12, suggesting reduced fitness of these mutants relative to the wild-type virus.

PK/PD relationship analysis:
Exposure-response relationships between GS-9857 plasma exposure parameters and the change from baseline in HCV RNA were explored. Similar median antiviral responses (> 3.0 $\log_{10}$ reduction) were observed in subjects with non-genotype 3 HCV infection (genotypes 1a, 1b, 2, and 4) following GS-9857 doses of 50, 100, or 300 mg. As such, no GS-9857 exposure-response relationships were observed for anti-HCV activity in subjects with genotype 1a, 1b, 2 or 4 HCV-infection administered GS-9857 doses of 50, 100 or 300 mg fasted. In subjects with genotype 3 HCV infection, an exposure-response relationship was identified and adequately described by a simple E_{max} model utilizing GS-9857 AUC_{0-24} on Day 3 of treatment, as shown below.



"Phase 3 Mean Exposure" marker encompasses mean ± 90% CI

The E_{max} model indicated that exposures achieved after administration of GS-9857 100 mg in combination with SOF/VEL FDC (400/100 mg) with food (Phase 2 and 3 studies and Cohort 10 of this study) were predicted to provide > 90% of maximal antiviral response in subjects with genotype 1a, 1b, 2, 3, or 4 HCV infection.

Pharmacokinetic analysis:

Dose linearity: **Table 1** presents statistical comparisons of the dose-normalized primary PK parameters for GS-9857 after administration of single- or multiple-doses of GS-9857 50, 100, or 300 mg in a fasted state from Cohorts 1 through 5. In general, results of the ANOVA and power model analyses are in agreement indicating dose proportional increases in GS-9857 exposure parameters after single- or multiple-dose administration.

Table 1: Dose Linearity Analysis of GS-9857 Pharmacokinetic Parameters following Single- and Multiple-Dose in Cohorts 1-5

	ANOVA Analysis % GLSM Ratio (Dose/100 mg) (90% CI)		Power Model Analysis Slope (90% CI)
GS-9857 PK Parameters	GS-9857 50 mg	GS-9857 300 mg	GS-9857 Across All Doses
Day 1 Single-Dose Administration			
AUC_{0-24}	86.89 (59.31, 127.30)	94.92 (65.36, 137.85)	1.03 (0.80, 1.27)
C_{max}	85.42 (57.89, 126.05)	78.38 (53.59, 114.62)	0.92 (0.68, 1.17)
C_{24}	97.25 (63.15, 149.76)	88.42 (57.98, 134.85)	0.94 (0.67, 1.20)
Day 3 Multiple-Dose Administration			
AUC_{0-24}	62.77 (41.57, 94.78)	100.73 (67.34, 150.68)	1.22 (0.96, 1.48)
C_{max}	60.12 (39.65, 91.15)	93.97 (62.56, 141.13)	1.20 (0.94, 1.47)
C_{24}	85.86 (59.01, 124.94)	69.81 (48.39, 100.71)	0.85 (0.62, 1.09)

ANOVA = analysis of variance; GLSM = geometric least-squares mean
For ANOVA analysis all PK parameters were dose-normalized to 100 mg.

Accumulation: **Table 2** presents the accumulation indices calculated by comparing AUC_{0-24}, C_{max}, and C_{24} estimates following single-dose and multiple-dose administration of GS-9857 on Days 1 and 3, respectively (dose normalized for composite analysis across doses). Consistent with the $t_{1/2}$ of GS-9857, significant accumulation was observed across the dose levels evaluated as well as the composite analysis across doses. The composite analysis of dose normalized exposure GS-9857 parameters revealed accumulation ratios of 2.6, 2.0, and 2.5 for AUC_{0-24}, C_{max} and C_{24}, respectively.

Table 2: GS-9857 Accumulation Analysis

	% GLSM Ratio (Multiple-Dose/ Single-Dose) (90% CI)				
GS-9857 PK Parameter	GS-9857 50 mg fasted (N = 14)	GS-9857 100 mg fasted (N = 29[a])	GS-9857 100 mg fed (N = 6)	GS-9857 300 mg fasted (N = 15)	GS-9857 All Doses (N = 64[b])
AUC_{0-24}	197.85 (129.90, 301.34)	273.89 (197.02, 380.75)	294.26 (144.38, 599.72)	290.65 (182.58, 462.69)	260.89 (193.86, 351.10)
C_{max}	141.55 (91.06, 220.03)	201.13 (144.52, 279.92)	253.89 (125.30, 514.45)	241.15 (150.52, 386.34)	198.87 (147.99, 267.25)
C_{24}	243.82 (147.15, 404.01)	276.16 (196.60, 387.91)	236.68 (100.20, 559.07)	218.02 (146.23, 325.05)	250.06 (189.84, 329.39)

GLSM = geometric least-squares mean
a Day 3 parameters (N = 29 for all), Day1 parameters (N = 30 for AUC_{0-24} and C_{max} and N = 28 for C_{24})
b Day 3 parameters (N = 64 for all), Day1 parameters (N = 65 for AUC_{0-24} and C_{max} and N = 63 for C_{24})

PK across genotypes: Table 3 presents PK parameters of GS-9857 after a single dose or 3 daily doses of GS-9857 in subjects with genotype 1a, 1b, 2, 3, or 4 HCV infection. The plasma PK of GS-9857 was generally similar across the GS-9857 doses administered (50, 100, or 300 mg) between subjects with genotype 1a, 1b, 2, 3, or 4 HCV infection. GS-9857 exposure (AUC_{0-24} and C_{max}) were modestly increased when 100 mg GS-9857 was administered in a fed state compared to a fasted state.

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Table 3: Plasma GS-9857 Pharmacokinetic Parameters Following Single-Dose and Multiple-Dose Administration in Subjects with Genotype 1 to 4 HCV Infection

PK Parameter Mean (%CV)[a]	50 mg GS-9857 fasted			100 mg GS-9857 fasted						100 mg GS-9857 fed	300 mg GS-9857 fasted		
	GT 1a (N = 8)	GT 3 (N = 6)	All GTs (N = 14)	GT 1a (N = 8)	GT 1b (N = 6)	GT 2 (N = 6)	GT 3 (N = 6)	GT 4 (N = 4)	All GTs (N = 30)	GT 3 (N = 6)	GT 1a (N = 8)	GT3 (N=7)	All GTs (N = 15)
Day 1 Single-Dose Administration													
AUC_{0-24} (ng•h/mL)	222.7 (50.5)	130.7 (36.6)	183.3 (54.3)	796.0 (68.6)	411.8 (33.3)	290.0 (70.0)	252.6 (45.7)	570.7 (116.9)	479.2 (88.2)	789.1 (53.8)	1276.6 (70.4)	1170.2 (47.2)	1227.0 (59.8)
C_{max} (ng/mL)	32.6 (67.9)	23.3 (47.1)	28.6 (63.8)	110.5 (61.9)	72.0 (50.5)	47.7 (62.6)	45.1 (52.1)	60.3 (112.1)	70.4 (74.8)	124.8 (51.7)	167.2 (77.6)	144.8 (37.6)	156.7 (63.2)
C_{24} (ng/mL)	5.8 (97.4)	2.1 (53.0)	4.2 (109.4)	13.7 (66.6)	8.2 (51.3)	4.0 (69.3)	2.4[b] (66.4)	10.4 (116.7)	8.2[c] (95.5)	15.3 (66.3)	20.7 (64.4)	15.9 (39.5)	18.5 (57.4)
$t_{1/2}$ (h)	11.87 (8.39, 18.91)	11.87 (9.44, 13.36)	11.87 (8.46, 15.11)	12.85 (9.20, 16.62)	13.31 (6.67, 22.96)	10.04 (8.12, 11.01)	10.93 (8.37, 11.61)	12.11 (9.76, 14.86)	11.18 (8.13, 13.75)	11.45 (11.03, 31.53)	10.66 (8.56, 11.36)	7.56 (6.72, 10.39)	9.80 (6.93, 11.02)
T_{max} (h)	2.50 (1.75, 4.00)	2.25 (1.50, 3.00)	2.50 (1.50, 4.00)	1.25 (1.00, 2.00)	3.50 (2.00, 5.00)	1.75 (1.50, 2.00)	1.50 (1.50, 2.00)	2.48 (1.75, 3.98)	2.00 (1.50, 2.97)	3.50 (2.00, 5.00)	1.75 (1.50, 2.50)	4.00 (1.50, 5.00)	2.00 (1.50, 5.00)
T_{last} (h)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
Day 3 Multiple-Dose Administration													
AUC_{0-24} (ng•h/mL)	473.2 (60.1)	237.7 (42.4)	372.3 (66.9)	1605.3 (64.7)	1058.6 (26.8)	904.1 (99.2)	834.4[b] (40.5)	2644.2 (134.1)	1357.6[c] (108.2)	2327.2 (57.8)	4683.0 (73.4)	3062.7 (53.1)	3926.9 (70.9)
C_{max} (ng/mL)	49.3 (62.5)	27.2 (33.2)	39.8 (65.1)	190.7 (53.1)	117.7 (33.9)	97.7 (98.9)	88.1[b] (48.1)	309.9 (147.9)	155.1[c] (116.9)	323.3 (66.4)	484.1 (85.5)	333.6 (57.6)	413.8 (79.3)
C_{24} (ng/mL)	10.9 (58.9)	5.6 (50.6)	8.6 (66.3)	27.8 (67.9)	17.5 (31.2)	15.0 (67.6)	15.0[b] (34.8)	29.3 (117.2)	21.0[c] (80.0)	35.1 (58.1)	53.5 (67.4)	31.4 (47.8)	43.1 (68.6)
$t_{1/2}$ (h)[a]	41.91 (39.04, 48.56)	37.33 (28.18, 47.63)	39.71 (35.02, 47.63)	30.38 (25.57, 36.41)	35.56 (32.31, 38.67)	41.85 (31.08, 47.22)	37.17[b] (35.98, 44.60)	38.85 (37.77, 40.50)	37.28[c] (31.42, 40.96)	35.44 (33.29, 41.60)	28.69 (23.22, 35.36)	31.20 (28.74, 47.00)	30.33 (26.59, 40.40)

PK Parameter Mean (%CV)[a]	50 mg GS-9857 fasted			100 mg GS-9857 fasted						100 mg GS-9857 fed	300 mg GS-9857 fasted		
	GT 1a (N = 8)	GT 3 (N = 6)	All GTs (N = 14)	GT 1a (N = 8)	GT 1b (N = 6)	GT 2 (N = 6)	GT 3 (N = 6)	GT 4 (N = 4)	All GTs (N = 30)	GT 3 (N = 6)	GT 1a (N = 8)	GT3 (N=7)	All GTs (N = 15)
T_{max} (h)	2.00 (1.25, 2.00)	3.00 (3.00, 3.00)	2.00 (2.00, 3.00)	1.75 (1.01, 3.50)	2.25 (1.50, 4.00)	3.00 (1.50, 5.00)	5.00[b] (4.00, 5.00)	3.00 (2.25, 5.53)	3.00[c] (1.50, 5.00)	3.50 (3.00, 5.00)	3.00 (2.00, 3.51)	3.00 (1.00, 3.00)	3.00 (2.00, 3.02)
T_{last} (h)	168.00 (120.48, 168.02)	120.00 (118.98, 120.00)	120.48 (120.00, 168.00)	156.17 (120.00, 168.00)	168.06 (168.00, 170.15)	168.00 (167.98, 168.00)	168.00[b] (168.00, 168.00)	132.00 (119.99, 155.99)	168.00[c] (144.00, 168.00)	167.99 (166.98, 168.00)	168.00 (143.99, 168.00)	168.00 (166.98, 168.00)	168.00 (166.98, 168.00)

GT = genotype
a All PK parameters were reported as mean (%CV), except for T_{max}, T_{last}, and $t_{1/2}$, which were reported as median (Q1, Q3).
b N = 5
c N=29

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9.3 Safety Analysis
GS-9857 was safe and well tolerated when administered once daily for 3 days to HCV-infected males and females at doses from 50 to 300 mg, and when administered at a dose of 100 mg with SOF/VEL FDC (400/100 mg). All AEs were Grade 1 and Grade 2 in severity, with no subjects experiencing Grade 3 or 4 AEs during the study. There were no reports of AEs leading to permanent study drug discontinuation, AEs leading to modification or interruption of any study drug, or deaths.

10. Sponsor's Conclusions

- GS-9857 exhibited dose-proportional increases in AUC and C_{max} across the dose range of 50 to 300 mg administered fasted to subjects with HCV infection.
- Consistent with its long median $t_{1/2}$ (29 to 42 hours), significant accumulation (2.0- to 2.6-fold) of GS-9857 exposure was observed.
- GS-9857 plasma PK was similar for subjects with genotypes 1a, 1b, 2, 3, or 4 HCV infection.
- GS-9857 exposure achieved after administration of GS-9857 100 mg in combination with SOF/VEL FDC (400/100 mg) with food is predicted to provide > 90% of maximal antiviral response in subjects with genotypes 1a, 1b, 2, 3, or 4 HCV infection.

11. Reviewer's Assessment

The study design was appropriate for addressing the objectives and the sponsor's conclusions are reasonable. The VOX monotherapy efficacy results are consistent with results from Phase 2 and 3 studies for HCV genotype 1 to 4. The VOX 100 mg dose co-administered with SOF/VEL FDC at 400/100 mg provided near maximal viral suppression, aligning with the high SVR12 rate observed in Phase 2 and 3 studies.

4.4.3 GS-US-338-1124 Mass Balance Study for VOX

1. Title

A Phase 1 Study to Evaluate the Pharmacokinetics, Metabolism, and Excretion of GS-9857

2. Information Regarding the Clinical Trial Site and Duration of the Trial

The trial was conducted (b) (4) from April 24, 2015 to June 17, 2015, with the final report date of May 4, 2016.

3. Objectives

Primary objectives:

- To determine the mass balance of voxilaprevir (VOX) using a dose of radiolabeled [^{14}C]VOX

Secondary objective:

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

4. Trial Design

This was a Phase 1, open-label, mass balance study of VOX administered as a single, oral dose of radiolabeled [^{14}C]VOX in healthy male subjects (planned n = 8).

On Day 1, after an overnight fast and completion of moderate-fat breakfast, each subject received a single, 100-mg dose of VOX administered orally as 2 capsules, each containing 50 μCi [^{14}C]VOX (equivalent to 0.78 mg VOX) and 49.22 mg of nonradiolabeled VOX. Serial whole blood and plasma samples, cumulative voided urine, and all feces were collected and pooled at predetermined intervals.

Eligible subjects were nonsmoking (for at least 90 days prior to dosing) males of 18 to 45 years of age (inclusive). In addition, subjects had a body mass index between 19 and 30 kg/m^2, no significant medical history, normal renal function (an estimated creatinine clearance of at least 80 mL/min as determined by the Cockcroft-Gault method using actual body weight), and were in good general health as determined by the investigator at a screening evaluation performed no more than 28 days prior to study drug administration.

5. Excluded Medications and Restrictions

- Any prescription medications and OTC medications including herbal products and antacids with the exception of vitamins, and/or acetaminophen and/or ibuprofen and/or hormonal contraceptive medications. However, the short-term use of topical hydrocortisone cream or A&D ointment to treat minor skin irritation due to ECG leads was allowed.
- Subjects were required to refrain from the consumption of food and beverages containing alcohol products, and from consumption of grapefruit juice, grapefruits, and Seville orange juice 72 hours prior to the first dose of study drug and during the course of the study through discharge.
- Subjects were required to refrain from the use of nicotine or nicotine-containing products from screening through discharge.

6. Rationale for Doses Used in the Trial

VOX: 100 mg is the dose administered with SOF/VEL FDC at 400/100 mg with food in Phase 2 and 3 studies, and it is reasonable for evaluation of ADME in healthy subjects. The 100 mg dose was administered as 2 capsules, each containing 50 µCi [^{14}C]VOX (equivalent to VOX 0.78 mg) and unlabeled VOX (49.22 mg).

7. Drugs Used in the Trial

VOX capsule: unlabeled VOX (49.22 mg) and 50 µCi [^{14}C]VOX (equivalent to VOX 0.78 mg) – Batch Number(s): Lot DY1503A (IP-12775) (Manufactured by (b) (4)

8. Sample Collection, Bioanalysis, Pharmacokinetic Assessments, and Statistical Analysis

Sample Collection

Whole blood and plasma samples were collected for radioactivity analysis at each of the following time points: 0 (pre-dose, ≤ 10 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 the whole blood and plasma discontinuation criteria had been met.

All urine voided was collected and pooled for radioactivity, PK, and metabolite profiling/identification analysis, starting 12 hours pre-dose 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 hour postdose. All stools were collected for radioactivity and metabolite profiling/identification analysis, starting pre-dose (within a 24 hour period prior to Day 1 dose) and continuing over the following collection: 0-24, 24-48, 48-72, 72-96, and 96-120 hours postdose and at 24 hour intervals until subjects met discharge criteria.

Bioanalytical method

- Validation and Bioanalytical Report:

Analyte	Matrix	Validation Report	Bioanalytical Report
GS-9857 (VOX)	Plasma	(b) (4) 8109.123113.1	(b) (4) 8223.083015, (b) (4) P 1267
	Urine	(b) (4) 8207.061515	(b) (4) 10122.083015.1
Metabolites	Plasma, Urine, Feces	NA*	(b) (4) 8322210

* *Not applicable*

- Bioanalytical Method:

Method type: LC/MS/MS;

Analyte	Matrix	Range (ng/mL)	Stability in frozen matrix (days)
GS-9857 (VOX)	Plasma	0.5 to 1000	420 at −20ºC and at −70ºC
	Urine	50 to 50,000	200 at −20ºC and at −70ºC

Validation	▪ Method validated prior to use	☒ Yes ☐ No ☐ NA
	▪ Method validation acceptable	☒ Yes ☐ No ☐ NA
Study Samples Analysis	▪ Samples analyzed within the established stability period	☒ Yes ☐ No
	▪ Quality control samples range acceptable	☒ Yes ☐ No
	▪ Chromatograms provided	☒ Yes ☐ No
	▪ Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	▪ Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	▪ Incurred samples analysis is acceptable	☒ Yes ☐ No
	▪ Overall performance acceptable	☒ Yes ☐ No
Inspection	▪ Will the bioanalytical site be inspected	☐ Yes ☒ No

Pharmacokinetic Assessments
The PK parameters (C_{max}, T_{max}, C_{last}, T_{last}, AUC_{last}, AUC_{inf}, $\%AUC_{exp}$, $t_{1/2}$, CL/F, and V_z/F) were estimated.

Liquid scintillation counting for ^{14}C radioactivity was performed on all whole blood, plasma, urine and stool samples for real-time monitoring of the recovery of the ^{14}C radioactive dose. Accelerator mass spectrometry was also performed on all whole blood and plasma samples for determination of ^{14}C radioactivity. The radioactivity in each sample was expressed by actual quantity, concentration and the percent of the administered radioactive dose recovered.

9. Results

9.1 Subject Demographics and Disposition
A total of 8 subjects were enrolled in this study. All 8 subjects received the study drug on Day 1 and completed the study as planned. All 8 enrolled subjects were included in the PK and Safety Analysis Sets.

In accordance with the entry criteria, all of the enrolled subjects were male. The subjects had a mean (SD) age of 27 (6.0) years, were primarily white (75.0%; 6 of 8 subjects), and had a mean (SD) BMI of 25.6 (2.40) kg/m^2. The mean (SD) CLcr at baseline for subjects enrolled in the study was 125.83 (25.789) mL/min.

9.2 Pharmacokinetic Analysis

The results of this study demonstrate that the total mean recovery of ^{14}C radioactivity following single oral dose administration of [^{14}C]VOX was approximately 94%, with all the radioactivity recovered in the feces and none recovered in the urine. The majority of the radioactivity (approximately 92.9%) was recovered in the feces within 288 hours postdose (**Figure 1**), with only minor increases in recovered radioactivity noted thereafter, consistent with the elimination half-life of VOX at 30 to 40 hours. Following administration of [^{14}C]VOX, systemic exposure was almost exclusively parent drug (91.0%). The whole blood-to-plasma concentration ratio from 1 to 120 hours postdose ranged from 0.500 to 0.798, indicating that total radioactivity was excluded from erythrocytes. Overall, the human data were consistent with the established profile of VOX in nonclinical species.

Voxilaprevir was the major species identified in feces, accounting for a mean of 39.8% of the administered dose and 42.3% the total radioactivity recovered in feces. One known hydrolysis metabolite, M19 (des-[methylcyclopropylsulphonamide]-VOX, 22.1%) was identified and could be further oxidized into M21 (des-[methylcyclopropylsulphonamide]-oxy-VOX-1, 5.4%) or M25 (des-[methylcyclopropylsulphonamide]-oxy-VOX-2, 3.87%), and one known dehydrogenation metabolite, M9 (dehydro-VOX-2, 7.50 %).

The proposed biotransformation pathway for [^{14}C]VOX in humans is shown in **Figure 2**. Collectively, these results indicated that, in humans, VOX accounts for > 90% of systemic plasma exposure and that the extent of VOX metabolism is moderate, with elimination exclusively in the feces (approximately 94% of the dose) predominantly as parent VOX (approximately 40% of the dose).

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Figure 1: Mean (SD) Cumulative Percent of Total ^{14}C Radioactivity Recovery in Urinary, Fecal, and Urinary + Fecal Samples versus Time

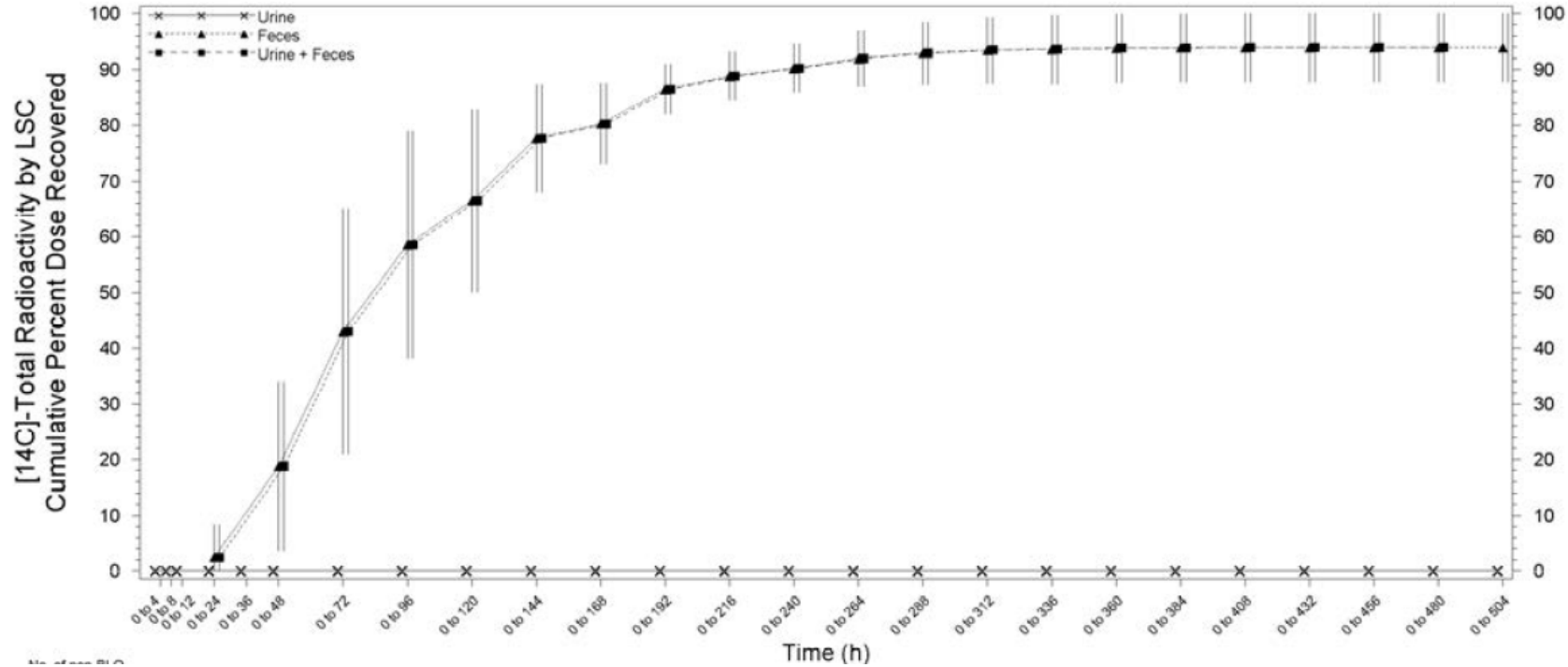
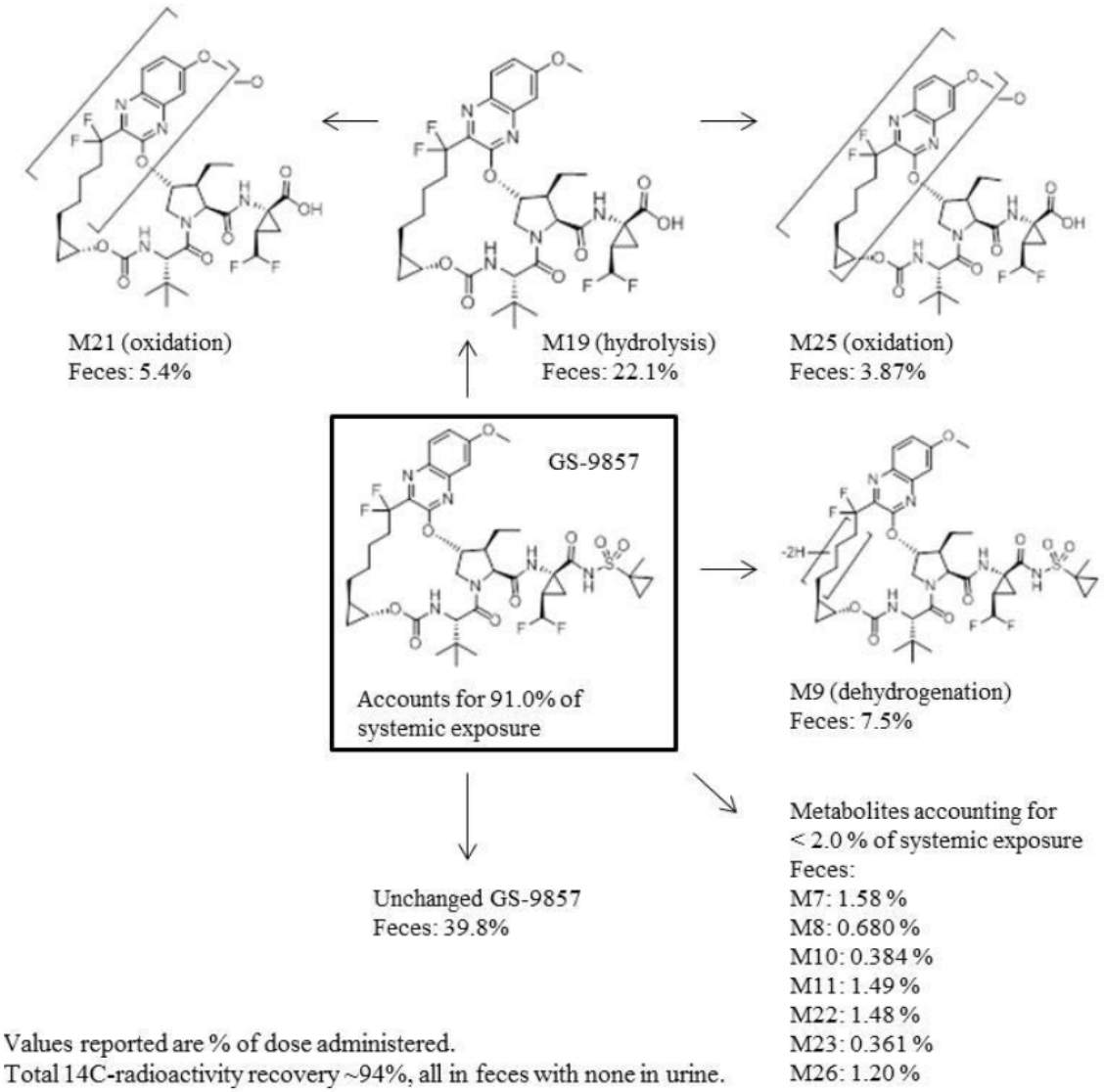


Figure 2: Proposed Major Biotransformation and Excretion Pathways of VOX in Humans



9.3 Safety Analysis

A single dose of [^{14}C]VOX was generally safe and well tolerated. All of the events were Grade 1 in severity and none were considered related to study drug.

10. Sponsor's Conclusions

- The predominant species circulating in plasma was VOX (> 90%).
- Voxilaprevir was exclusively eliminated in the feces, with no recovery in the urine.
- Voxilaprevir was moderately metabolized, with approximately 40% of the dose recovered in feces as parent.

11. Reviewer's Assessment

This mass balance study demonstrated that VOX was the predominant circulating component in the plasma (> 90%), and the major elimination route for VOX was biliary excretion of metabolites (47% of the dose recovered in the feces as metabolites) and potentially parent, with no detectable recovery in the urine. However, it is unclear if 40% of the dose recovered in feces as parent is unabsorbed drug from oral dose or is eliminated from direct biliary excretion after oral absorption.

4.4.4 GS-US-338-1125 Renal Impairment Study (by Jeffrey Froude)

1. Title

A Phase I Open-Label, parallel-Group, Single-Dose Study to Evaluate the Pharmacokinetics of GS-9857 in Subjects with Normal Real Function and Severe Renal Impairment

2. Information Regarding the Clinical Trial Site and Duration of the Trial

The trial was conducted (b) (4) from May 5, 2015 to September 28, 2015 with the final report date of April 25, 2016.

3. Objectives

Primary objectives:

- To evaluate the single-dose pharmacokinetics (PK) of voxilaprevir (VOX, GS-9857) in subjects with severe renal impairment and matched healthy control subjects

Secondary objective:

- To evaluate the safety and tolerability of a single dose of VOX in subjects with severe renal impairment and matched healthy control subjects

4. Trial Design

This Phase 1, single-cohort, open-label, multicenter, single-dose, 2-group study evaluated the PK, safety, and tolerability of VOX in subjects with severe renal impairment and subjects with normal renal function.

Twenty subjects total (10 subjects with severe renal impairment and 10 subjects with normal renal function) were planned and all 20 subjects were included in the safety and PK analysis sets.

Subjects were males and nonpregnant, nonnursing females aged 18 to 79 years (inclusive) with body mass index (BMI) 18 to 36 kg/m^2 (inclusive). Subjects with severe

renal impairment had creatinine clearance (CLcr) < 30 mL/min as determined using the Cockcroft-Gault formula at screening and were not on dialysis or anticipated to require dialysis within 90 days of study entry. Subjects with normal renal function had CLcr ≥ 90 mL/min, and each was matched to an individual subject in the severe renal impairment group for age (± 10 years), sex, and BMI (± 15%).

Reviewer's comment:
Three of the ten subjects in the severe renal impairment group fell into the end-stage renal disease (ESRD) category with a eGFR below 15 mL/min/1.73m^2 (overall group min, max of 8.0 to 22.0) when assessing with the Modification of Diet in Renal Disease (MDRD) equation for chronic kidney disease (CKD). Two of these three remained <15 mL/min/1.73m^2 throughout the study. However, this did not impact the study conclusions.

5. Excluded Medications and Restrictions

Subjects with Normal Renal Function:

- Any prescription medications and OTC medications including herbal products with the exception of vitamins, and/or acetaminophen and/or ibuprofen and/or oral contraceptive medications. However, the short-term use of topical hydrocortisone cream or A&D ointment to treat minor skin irritation due to ECG leads was allowed.

Subjects with Severe Renal Impairment

- Hematologic-stimulating agents (e.g., erythropoiesis-stimulating agents, granulocyte colony stimulating factor, thrombopoietin mimetics)
- Chronic systemic immunosuppressants, including, but not limited to, corticosteroids (prednisone equivalent of > 10 mg/day for > 2 weeks), azathioprine, or monoclonal antibodies (e.g., infliximab)
- Investigational agents or devices for any indication
- Concomitant use of certain medications or herbal/natural supplements (inhibitors or inducers of drug transporters) with study drug(s) that could result in PK interactions resulting in increases or decreases in exposure of study drug.

Drug Class	Agents Disallowed
Antibiotics	Azithromycin, clarithromycin, erythromycin
Acid-Reducing Agents	Proton-pump inhibitors, H2-receptor antagonists, antacids[a]
Anticonvulsants[b]	Phenobarbital, phenytoin, carbamazepine, oxcarbazepine
Antifungals	Itraconazole, ketoconazole
Antimycobacterials[c]	Rifamycins, isoniazid
Cardiac Medications	Amiodarone, dronedarone, felodipine, verapamil, quinidine, ranolazine, bosentan, olmesartan, telmisartan, valsartan
Herbal/Natural Supplements[b]	St. John's wort, echinacea, milk thistle (ie, silymarin), Chinese herb sho-saiko-to (or xiao-shai-hu-tang)
HMG-CoA Reductase Inhibitors[c]	Fluvastatin, lovastatin, pitavastatin , pravastatin, rosuvastatin
Selective Serotonin Reuptake Inhibitors	Fluvoxamine
Other	Modafinil

HMG-CoA = 3-hydroxy-3-methylglutaryl-coenzyme A

a Antacids that directly neutralize stomach pH (ie, Tums®, Maalox®) were permitted but prohibited within 4 hours (before or after) study drug administration.

b May result in a decrease in the concentrations of study drugs.

c Use with study drug may result in an increase in the increase in the concentration of the HMG-CoA reductase inhibitors. The 28-day washout period did not apply to HMG-CoA reductase inhibitors, which could have been taken up to the day before Day 1

Medications for disease conditions excluded from the protocol (e.g., HIV-1 infection, active cancer, transplantation) were also disallowed in the study.

Other Protocol Restrictions:

- Subjects were required to refrain from the consumption of food and beverages containing alcohol products, and from consumption of grapefruit juice, grapefruits, and Seville orange juice 72 hours prior to the first dose of study drug and during the course of the study through discharge.

6. Rationale for Doses Used in the Trial

VOX: 100 mg with moderate-fat food is the proposed VOX dosage in Phase 2 and Phase 3 studies.

7. Drugs Used in the Trial

VOX 100 mg tablets – Batch Number: DY1407B2 (Manufactured by (b) (4)

8. Sample Collection, Bioanalysis, Pharmacokinetic Assessments, and Statistical Analysis

Sample Collection

Plasma samples were collected at the following time points relative to dosing: predose (≤ 5 min) and 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, 96, and 120 hours postdose.

On Day 1, plasma protein binding samples were collected at 3 and 5 hours postdose.

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

Bioanalytical method

- Validation and Bioanalytical Report:

Analyte	Matrix	Validation Report	Bioanalytical Report
GS-9857 (VOX)	Plasma	(b) (4) 8109.123113.1	(b) (4) 8222.112515, (b) (4) 60N-1535
	Urine	(b) (4) 8207.061515	(b) (4) 10168.013016.1

- Bioanalytical Method:

Method type: LC/MS/MS;

Analyte	Matrix	Range (ng/mL)	Stability in frozen matrix (days)
GS-9857 (VOX)	Plasma	0.5 to 1000	420 at −20ºC and at −70ºC
	Urine	50 to 50,000	200 at −20ºC and at −70ºC

Validation	▪ Method validated prior to use	☒ Yes ☐ No ☐ NA
	▪ Method validation acceptable	☒ Yes ☐ No ☐ NA
Study Samples Analysis	▪ Samples analyzed within the established stability period	☒ Yes ☐ No
	▪ Quality control samples range acceptable	☒ Yes ☐ No
	▪ Chromatograms provided	☒ Yes ☐ No
	▪ Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	▪ Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	▪ Incurred samples analysis is acceptable	☒ Yes ☐ No
	▪ Overall performance acceptable	☒ Yes ☐ No
Inspection	▪ Will the bioanalytical site be inspected	☐ Yes ☒ No

Pharmacokinetic Assessments

The following plasma PK parameters were calculated for VOX: AUC_{inf}, AUC_{last}, $\%AUC_{exp}$, C_{max}, C_{last} T_{max}, T_{last}, $t_{1/2}$, λ_z, V_z/F, and CL/F.
Plasma protein binding was determined and unbound PK parameters were estimated.

Statistical Analysis

An analysis of variance (ANOVA) was fit to the natural log-transformed VOX PK parameters (AUC_{last}, AUC_{inf}, and C_{max}). Ninety percent CIs were constructed for the geometric least-squares mean (GLSM) ratios of VOX PK parameters in the severe renal impairment group compared with the normal renal function group. The relationship

between measures of renal function and the VOX primary PK parameters was also explored.

9. Results

9.1 Subject Demographics and Disposition
A total of 20 subjects were enrolled in this study, 10 subjects in the severe renal impairment group and 10 subjects in the normal renal function group. All subjects completed the study. Overall, the mean age was 57 years (range: 23-78). The majority of subjects were male (80.0%, 16 subjects), white (95.0%, 19 subjects), and not Hispanic or Latino (90%, 18 subjects).

9.2 Pharmacokinetic and Statistical Analysis
VOX plasma PK parameters were calculated for subjects with severe renal impairment and subjects with normal renal function matched for age (± 10 years), sex, and BMI (± 15%) following administration of a single dose of VOX 100 mg with a moderate-fat meal (**Table 1**). Statistical comparisons of VOX primary PK parameters (AUC_{inf}, AUC_{last}, and C_{max}) were conducted between groups (**Table 2**). Following a single oral dose of VOX 100 mg with a moderate-fat meal, VOX plasma exposures (AUC_{inf} and AUC_{last}) were approximately 71% to 73% higher and C_{max} was approximately 45% higher in subjects with severe renal impairment compared with subjects with normal renal function. Based on the available VOX safety data, the modest increase in VOX exposure in subjects with severe renal impairment compared with subjects with normal renal function is not considered clinically relevant. No VOX dose adjustment is warranted in subjects with mild, moderate, or severe renal impairment. Nonparametric analysis showed no statistically significant ($p > 0.05$) differences in $t_{1/2}$, CL/F, and V_z/F in subjects with severe renal impairment compared with subjects with normal renal function.

VOX plasma protein binding was similar between subjects with severe renal impairment and subjects with normal renal function. VOX concentrations in the urine were assessed, but undetectable for all samples analyzed. There was no strong relationship between PK exposure (AUC or C_{max}) and renal function.

Table 1: Plasma PK Parameters of VOX in Subjects with Severe Renal Impairment and Normal Renal Function

VOX PK Parameter	Mean (%CV)	
	Severe Renal Impairment (N = 10)	Normal Renal Function (N = 10)
AUC_{last} (ng•h/mL)	910.8 (82.2)	444.9 (55.3)
$\%AUC_{exp}$	13.35 (84.55)	14.88 (30.20)
AUC_{inf} (ng•h/mL)	1033.7 (76.0)	525.8 (57.3)
C_{max} (ng/mL)	68.4 (86.9)	38.4 (60.5)
C_{last} (ng/mL)	1.80 (78.27)	1.19 (54.64)
T_{max} (h)[a]	5.00 (4.00, 5.00)	4.00 (2.00, 4.00)
T_{last} (h)[a]	120.00 (120.00, 120.00)	120.00 (96.00, 120.00)
$t_{1/2}$ (h)[a]	38.70 (32.69, 44.57)	40.83 (36.69, 46.81)
CL/F (L/h)	172.8 (75.8)	256.9 (53.2)
V_z/F (L)	10425.5 (68.2)	15274.4 (47.6)

a Values are displayed as median (Q1, Q3).

Table 2: Statistical Comparisons of PK Parameters of VOX in Subjects with Severe Renal Impairment and Normal Renal Function

VOX PK Parameter	GLSM		%GLSM Ratio (Impaired/Normal)	90% CI
	Severe Renal Impairment (N = 10)	Normal Renal Function (N = 10)		
AUC_{last} (ng•h/mL)	662.75	383.27	172.92	97.93, 305.33
AUC_{inf} (ng•h/mL)	772.13	450.83	171.27	97.65, 300.38
C_{max} (ng/mL)	49.23	33.85	145.43	83.77, 252.48

GLSM = geometric least-squares mean

9.3 Safety Analysis

A single dose of VOX was generally well tolerated in subjects with severe renal impairment and subjects with normal renal function. All AEs were Grade 1 in severity, and there were no Grade 3 or 4 AEs, SAEs, AEs leading to permanent discontinuation of study drug, or deaths reported during the study.

10. Sponsor's Conclusions

- VOX PK exposure was modestly increased in subjects with severe renal impairment compared with subjects with normal renal function; the modest increase was not considered clinically significant based on the safety profile of VOX.

- Dose modification of VOX in subjects with mild, moderate, or severe renal impairment is not warranted.

11. Reviewer's Assessment

The study design is appropriate and the sponsor's conclusions are valid. However, although there is no need for dose adjustment for VOX in subjects with mild, moderate, or severe renal impairment, the dose recommendation for use of SOF/VEL/VOX in subjects with renal impairment is driven by GS-331007 exposures (~5-20 fold increase across severe and ESRD pre- and post-dialysis demonstrated in study P7977-0915). Due to this magnitude of increase in GS-331007 exposure and the lack of safety data to support it, dosage recommendations cannot be made for patients with severe or end stage renal disease.

4.4.5 GS-US-338-1126 Hepatic Impairment Study

1. Title

A Phase 1 Open-Label, Parallel-Group, Single-Dose Study to Evaluate the Pharmacokinetics of GS-9857 in Subjects with Normal Hepatic Function and Moderate or Severe Hepatic Impairment

2. Information Regarding the Clinical Trial Site and Duration of the Trial

The trial was conducted (b) (4) from March 24, 2015 to March 04, 2016, with the final report date of August 26, 2016.

3. Objectives

Primary objectives:
To evaluate the single-dose pharmacokinetics (PK) of voxilaprevir (VOX; GS-9857) in subjects with normal hepatic function, moderate hepatic impairment, or severe hepatic impairment
Secondary objective:
To evaluate the safety and tolerability of VOX single-dose administration in subjects with normal hepatic function, moderate hepatic impairment, or severe hepatic impairment

4. Trial Design

This Phase 1, open-label, parallel-group, staggered-cohort study investigated the single-dose PK, safety, and tolerability of VOX in subjects with normal hepatic function, moderate hepatic impairment, or severe hepatic impairment. Subjects with moderate hepatic impairment had a score of 7 to 9 on the CPT classification, and subjects with severe hepatic impairment had a score of 10 to 15 on the CPT classification. Subjects with moderate hepatic impairment were enrolled in Cohort 1, and subjects with severe hepatic impairment were enrolled in Cohort 2. Subjects with normal hepatic function were enrolled in both cohorts and served as controls for subjects with hepatic impairment. In both cohorts, each subject with hepatic impairment was matched for sex, age (± 10

years), and body mass index (BMI; ± 15%) with a control subject. All subjects received VOX (1 × 100-mg tablet) administered orally in the morning on Day 1 with a moderate-fat-calorie breakfast containing ~600 kcal and 25% to 30% fat.

In Cohort 1, once a subject with moderate hepatic impairment completed the PK assessments, dosing in a control-matched subject with normal hepatic function occurred. Based on review of safety data and available PK data from Cohort 1, dosing of Cohort 2 was initiated. A subject with normal hepatic function in Cohort 1 could have also served as the control match to a subject with severe hepatic impairment in Cohort 2. Otherwise, once a subject with severe hepatic impairment completed PK assessments, dosing in a control-matched subject with normal hepatic function occurred.

Twenty subjects were planned to be enrolled for Cohort 1, including 10 subjects with moderate hepatic impairment and 10 matched controls. Eighteen subjects were planned to be enrolled for Cohort 2, including 9 subjects with severe hepatic impairment and 9 matched controls.

Eligible subjects were male and non-pregnant/non-nursing female subjects who were 18 to 70 years of age (inclusive) and were not actively infected with hepatitis C virus (HCV). Subjects also had creatinine clearance (CLcr) ≥ 50 mL/min (using Cockcroft-Gault method) based on serum creatinine and actual body weight as measured at the screening evaluation.

5. Excluded Medications and Restrictions

Subjects with Normal Hepatic Function:

- Any prescription medications and OTC medications including herbal products with the exception of vitamins, and/or acetaminophen and/or ibuprofen and/or oral contraceptive medications. However, the short-term use of topical hydrocortisone cream or A&D ointment to treat minor skin irritation due to ECG leads was allowed.

Subjects with Hepatic Impairment

- Hematologic-stimulating agents (e.g., erythropoiesis-stimulating agents, granulocyte colony stimulating factor, thrombopoietin mimetics)
- Chronic systemic immunosuppressants, including, but not limited to, corticosteroids (prednisone equivalent of > 10 mg/day for > 2 weeks), azathioprine, or monoclonal antibodies (e.g., infliximab)
- Investigational agents or devices for any indication
- Concomitant use of certain medications or herbal/natural supplements (inhibitors or inducers of drug transporters) with study drug(s) that may have resulted in PK interactions resulting in increases or decreases in exposure of study drug.

Drug Class	Agents Disallowed
Antibiotics	Azithromycin, clarithromycin, erythromycin
Acid-Reducing Agents	Proton-pump inhibitors, H2-receptor antagonists, antacids[a]
Anticonvulsants[b]	Phenobarbital, phenytoin, carbamazepine, oxcarbazepine
Antifungals	Itraconazole, ketoconazole
Antimycobacterials[c]	Rifamycins, isoniazid
Cardiac Medications	Amiodarone, dronedarone, felodipine, verapamil, quinidine, ranolazine, bosentan, olmesartan, telmisartan, valsartan
Herbal/Natural Supplements[b]	St. John's wort, echinacea, milk thistle (ie, silymarin), Chinese herb sho-saiko-to (or xiao-shai-hu-tang)
HMG-CoA Reductase Inhibitors[c]	Fluvastatin, lovastatin, pitavastatin , pravastatin, rosuvastatin
Selective Serotonin Reuptake Inhibitors	Fluvoxamine
Other	Modafinil

HMG-CoA = 3-hydroxy-3-methylglutaryl-coenzyme A

a Antacids that directly neutralize stomach pH (ie, Tums®, Maalox®) were permitted but prohibited within 4 hours (before or after) study drug administration.

b May result in a decrease in the concentrations of study drugs.

c Use with study drug may result in an increase in the increase in the concentration of the HMG-CoA reductase inhibitors. The 28-day washout period did not apply to HMG-CoA reductase inhibitors, which could have been taken up to the day before Day 1

Medications for disease conditions excluded from the protocol (eg, HIV-1 infection, active cancer, transplantation) were also disallowed in the study.

Other Protocol Restrictions:

- Subjects were required to refrain from the consumption of food and beverages containing alcohol products, and from consumption of grapefruit juice, grapefruits, and Seville orange juice 72 hours prior to the first dose of study drug and during the course of the study through discharge.
- Subjects were required to refrain from the use of nicotine or nicotine-containing products from screening through discharge.

6. Rationale for Doses Used in the Trial

VOX: 100 mg with moderate-fat food is the proposed VOX dosage in Phase 2 and Phase 3 studies.

7. Drugs Used in the Trial

VOX 100 mg tablets – Batch Number(s): DY1407B2, DY1502B1 (Manufactured by (b) (4)

8. Sample Collection, Bioanalysis, Pharmacokinetic Assessments, and Statistical Analysis

Sample Collection

Plasma samples were collected relative to study drug administration at the following time points: 0 (pre-dose ≤ 5 min), 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, 96, and 120 hours post-dose. Additional blood samples were collected at the 3 and 5 hours post-doses, for plasma protein binding evaluation.

Bioanalytical method

- Validation and Bioanalytical Report:

Analyte	Matrix	Validation Report	Bioanalytical Report
GS-9857 (VOX)	Plasma	(b) (4) 8109.123113.1	(b) (4) 10073.113015, (b) (4) 60N-1536 (protein binding)

- Bioanalytical Method:

Method type: LC/MS/MS; Matrix: K_2EDTA plasma

Analyte	Range (ng/mL)	Stability in frozen matrix (days)
GS-9857 (VOX)	0.5 to 1000	420 at −20ºC and at −70ºC

Validation	▪ Method validated prior to use	☒ Yes ☐ No ☐ NA
	▪ Method validation acceptable	☒ Yes ☐ No ☐ NA
Study Samples Analysis	▪ Samples analyzed within the established stability period	☒ Yes ☐ No
	▪ Quality control samples range acceptable	☒ Yes ☐ No
	▪ Chromatograms provided	☒ Yes ☐ No
	▪ Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	▪ Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	▪ Incurred samples analysis is acceptable	☒ Yes ☐ No
	▪ Overall performance acceptable	☒ Yes ☐ No
Inspection	▪ Will the bioanalytical site be inspected	☐ Yes ☒ No

Pharmacokinetic Assessments

The following single dose plasma PK parameters of VOX were calculated: AUC_{last}, AUC_{inf}, $\%AUC_{exp}$, C_{max}, C_{last}, T_{max}, T_{last}, λ_z, CL/F, V_z/F, and $t_{1/2}$ as appropriate.

Statistical Analysis

An analysis of variance (ANOVA) appropriate for a parallel design was fit to the natural logarithmic transformation of primary PK parameters (AUC_{last}, AUC_{inf}, and C_{max}) for VOX. The 90% confidence intervals (CIs) were constructed for the geometric least-squares mean (GLSM) ratios of PK parameters for VOX in the moderate or severe hepatic impairment group versus the respective control (normal hepatic function) group

for that cohort.

To evaluate the protein binding of VOX, protein binding plasma samples were collected at 3 and 5 hours postdose and the percentages of bound and unbound VOX at T_{max} and T_{last} were summarized by hepatic function group.

9. Results

9.1 Subject Demographics and Disposition
A total of 33 subjects were enrolled in the study: 10 subjects with moderate hepatic impairment in Cohort 1, 9 subjects with severe hepatic impairment in Cohort 2, and 14 matched control subjects with normal hepatic function. Five subjects with normal hepatic function who were enrolled in Cohort 1 also served as matched controls to subjects with severe hepatic impairment in Cohort 2. The majority of subjects were male (22 of 33 subjects, 66.7%) and white (26 of 33 subjects, 78.8%). Approximately half of the subjects were Hispanic or Latino (15 of 33 subjects, 45.5%). Overall, subjects had a mean (SD) age of 54 (8.0) years (range: 38 to 67 years) and mean (SD) BMI of 27.8 (3.18) kg/m^2.

9.2 Pharmacokinetic and Statistical Analysis
The summary of PK parameters and statistical analysis of VOX in subjects with moderate hepatic impairment, severe hepatic impairment, or normal hepatic function is presented in **Table 1**. Following a single dose of VOX 100 mg with a moderate-fat meal, VOX AUC_{inf} and C_{max} were approximately 299% and 238% higher, respectively, in subjects with moderate hepatic impairment relative to subjects with normal hepatic function. VOX AUC_{inf} and C_{max} were approximately 500% and 614% higher, respectively, in subjects with severe hepatic impairment relative to subjects with normal hepatic function. Nonparametric analysis showed no statistically significant ($p > 0.05$) differences in VOX $t_{1/2}$ in subjects with moderate or severe hepatic impairment compared with subjects with normal hepatic function.

Mean VOX protein binding was determined by measuring the percentage unbound VOX concentration in each subject at 3 and 5 hours postdose. The mean (%CV) percentage unbound VOX values were similar in subjects with moderate hepatic impairment (0.38% [33.92%]) and those with normal hepatic function (0.29% [14.49%]). The mean (%CV) percentage unbound VOX was higher in subjects with severe hepatic impairment (0.71% [39.24%]) relative to subjects with normal hepatic function (0.38% [70.94%]). Overall, the higher unbound VOX concentrations are associated with lower plasma albumin.

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Table 1: Plasma PK Parameters and Statistical Comparisons for VOX in Normal, Moderate and Severe Hepatic Function Groups

PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	Normal Hepatic Function Group (Reference)	Hepatic Impairment Group (Test)	
Moderate Hepatic Impairment (N = 10) vs Matched Normal Hepatic Function (N = 10)			
AUC_{last} (ng•h/mL)	614.5 (72.9)	2801.3 (60.9)	411.62 (214.72, 789.08)
AUC_{inf} (ng•h/mL)	673.0 (70.2)	3021.9 (61.0)	399.24 (210.89, 755.81)
C_{max} (ng/mL)	63.7 (53.6)	304.8 (95.4)	338.41 (168.96, 677.79)
$\%AUC_{exp}$	10.24 (46.77)	7.47 (60.39)	
C_{last} (ng/mL)	1.03 (54.49)	3.89 (79.45)	
T_{max} (h)[a]	4.00 (2.00, 5.00)	5.00 (5.00, 6.00)	
T_{last} (h)[a]	120.00 (120.00, 120.00)	120.00 (120.00, 120.00)	
$t_{1/2}$ (h)[a]	35.95 (34.48, 47.02)	34.45 (30.48, 45.40)	
CL/F (mL/h)	215751.5 (68.3)	71163.0 (107.3)	
Weight-Normalized CL/F (mL/h/kg)	2684.1 (59.2)	823.0 (97.1)	
V_z/F (mL)	12160930.3 (65.1)	3517765.6 (100.4)	
Weight-Normalized V_z/F (mL/kg)	150018.0 (55.3)	42470.0 (105.6)	
Severe Hepatic Impairment (N = 9) vs Matched Normal Hepatic Function (N = 9)			
AUC_{last} (ng•h/mL)	714.6 (65.0)	4816.4 (60.2)	644.15 (364.67, 1137.82)
AUC_{inf} (ng•h/mL)	810.6 (62.5)	5066.1 (59.3)	599.63 (342.36, 1050.22)
C_{max} (ng/mL)	58.8 (57.6)	498.1 (66.7)	713.92 (384.07, 1327.06)
$\%AUC_{exp}$	12.12 (39.96)	5.62 (80.77)	
C_{last} (ng/mL)	1.46 (54.51)	5.58 (74.62)	
T_{max} (h)[a]	4.00 (2.00, 5.00)	4.00 (3.00, 4.00)	
T_{last} (h)[a]	120.00 (120.00, 120.00)	120.00 (120.00, 120.00)	
$t_{1/2}$ (h)[a]	42.93 (35.25, 49.56)	29.61 (26.61, 33.49)	
CL/F (mL/h)	171329.8 (57.4)	31132.2 (75.2)	
Weight-Normalized CL/F (mL/h/kg)	2071.2 (58.8)	384.1 (72.1)	
V_z/F (mL)	10533923.1 (64.7)	1392959.3 (82.8)	
Weight-Normalized V_z/F (mL/kg)	125709.7 (60.3)	16661.6 (69.2)	

GLSM = geometric least-squares mean
a Median (Q1, Q3)
Treatment = VOX (1 × 100-mg tablet)
An ANOVA model with hepatic function as a fixed effect was fitted to the natural-log transformation of PK parameters.

9.3 Safety Analysis

Following a single dose, voxilaprevir was generally well tolerated when administered to subjects with moderate or severe hepatic impairment, as well as subjects with normal hepatic function. All AEs were Grade 1 or 2, and no AEs leading to discontinuation of study drug or study participation, SAEs, deaths were reported during the study.

10. Sponsor's Conclusions

Voxilaprevir overall exposure (AUC_{inf}) was approximately 299% higher in subjects with moderate hepatic impairment, and approximately 500% higher in subjects with severe hepatic impairment, compared with matched control subjects with normal hepatic function.

11. Reviewer's Assessment

The study design is appropriate and the sponsor's conclusions are reasonable. Due to the magnitude of VOX exposure increase in this study, the reviewer agrees with the sponsor's proposal for the following labeling recommendation: SOF/VEL/VOX is not recommended in patients with moderate or severe hepatic impairment.

4.4.6 GS-US-338-1130 DDI, BA, FE, SAD, Steady State PK

1. Title

A Phase 1, Open-label, Study in Healthy Volunteers to Evaluate Pharmacokinetics of a Modified Formulation of GS-9857 and Investigate the Drug-Drug Interaction Between Two Formulations of GS-9857 and HCV Antivirals

2. Information Regarding the Clinical Trial Site and Duration of the Trial

The trial was conducted (b) (4) from June 20, 2014 to July 17, 2015, with the final report date of December 11, 2015.

3. Objectives

Primary objectives:

- To evaluate the pharmacokinetics (PK) of GS-9857 upon coadministration with sofosbuvir (SOF)/velpatasvir (VEL; GS-5816) fixed-dose combination (FDC) (SOF/VEL FDC)
- To evaluate the PK of SOF (and metabolites as applicable) and VEL upon coadministration of SOF/VEL FDC with GS-9857
- To evaluate the single- and multiple-dose PK of a modified formulation of GS-9857
- To evaluate the effect of coadministration of GS-9857 and ledipasvir (LDV)/SOF FDC on the PK of GS-9857, LDV, SOF and its metabolites (not related to this submission)

Secondary objective:

- To evaluate the safety and tolerability of coadministration of GS-9857 with SOF/VEL FDC or LDV/SOF FDC

4. Trial Design

This was a randomized, open-label, 7-cohort, multiple-dose, Phase 1 study in healthy subjects. Subjects were randomized to 1 of 7 cohorts and received treatments as follows:

Cohort 1: Conventional Formulation GS-9857 plus SOF/VEL (n=24)
Treatment A: SOF/VEL FDC (1 × 400/100-mg tablet) once daily, administered orally under fasted conditions for 4 days
Treatment B: GS-9857 200 mg (using 10 and/or 50-mg tablets) once daily, administered orally under fasted conditions for 7 days
Treatment C: GS-9857 200 mg (using 10 and/or 50-mg tablets) + SOF/VEL FDC (1 × 400/100-mg tablet) once daily, administered orally under fasted conditions for 4 days

Days			
1-4	**5-6**	**7-13**	**14-17**
Treatment A: SOF/VEL	Washout	**Treatment B:** GS-9857	**Treatment C:** GS-9857 + SOF/VEL

Cohort 2: Tablet Modified Formulation GS-9857 PK and Food Effect (n=15)
Treatment D: GS-9857 100 mg modified formulation (1 × 100-mg tablet) single dose, administered orally under fasted conditions
Treatment E: GS-9857 100 mg modified formulation (1 × 100-mg tablet) single dose, administered orally after a moderate-fat meal
Treatment F: GS-9857 100 mg conventional formulation (2 × 50-mg tablets) single dose, administered orally under fasted conditions

Days				
1	**2-7**	**8**	**9-14**	**15**
Treatment D: GS-9857 Modified Formulation Fasted	Washout	Treatment E: GS-9857 Modified Formulation Moderate-Fat Meal	Washout	Treatment F: GS-9857 Conventional Formulation Fasted

Cohort 3: Effect of Food on GS-9857 100-mg Tablet Modified Formulation plus SOF/VEL (n=15)
Treatment G: SOF/VEL FDC (1 × 400/100-mg tablet) + GS-9857 100 mg modified formulation (1 × 100-mg tablet) single dose, administered orally under fasted conditions
Treatment H: SOF/VEL FDC (1 × 400/100-mg tablet) + GS-9857 100 mg modified formulation (1 × 100-mg tablet) single dose, administered orally after a moderate-fat meal
Treatment I: SOF/VEL FDC (1 × 400/100-mg tablet) + GS-9857 100 mg modified formulation (1 × 100-mg tablet) single dose, administered orally after a light-fat meal

Days				
1	2-7	8	9-14	15
Treatment G: SOF/VEL + GS-9857 Modified Formulation Fasted	Washout	Treatment H: SOF/VEL + GS-9857 Modified Formulation Moderate-Fat Meal	Washout	Treatment I: SOF/VEL + GS-9857 Modified Formulation Light-Fat Meal

Cohorts 4 through 7 were adaptive cohorts with dosing initiated after review of safety and PK data from previous treatments.

Cohort 4: GS-9857 Modified Formulation Tablet Linearity (n=16)
Treatment J: GS-9857 300 mg modified formulation (3 × 100-mg tablets) single dose, administered orally after a moderate-fat meal
Treatment K: GS-9857 600 mg modified formulation (6 × 100-mg tablets) single dose, administered orally after a moderate-fat meal
Treatment L: GS-9857 900 mg modified formulation (9 × 100-mg tablets) single dose, administered orally after a moderate-fat meal

Days				
1	2-14	15	16-28	29
Treatment J: GS-9857 300 mg Modified Formulation	Washout	Treatment K: GS-9857 600 mg Modified Formulation	Washout	Treatment L: GS-9857 900 mg Modified Formulation

Cohort 5: GS-9857 Modified Formulation Tablet Steady-State Exposure (n=15)
Treatment M: GS-9857 200 mg modified formulation (2 × 100-mg tablets) once daily, administered orally after a moderate-fat meal for 10 days

Cohort 6: GS-9857 Modified Formulation plus LDV/SOF FDC Fed (not related to this submission)

Cohort 7: GS-9857 Modified Formulation plus LDV/SOF FDC Fasted (not related to this submission)

The light-fat breakfast contained approximately 400 kcal and 10% fat, and the moderate-fat breakfast contained approximately 600 kcal and 27% fat was provided

Eligible subjects were healthy males and non-pregnant, non-lactating females from 18 to 45 years of age, inclusive, with a body mass index (BMI) of 19.0 to 30.0 kg/m^2 (inclusive), normal 12-lead electrocardiogram (ECG), normal renal function with an estimated creatinine clearance (CLcr) (calculated using the Cockcroft-Gault equation) ≥ 80 mL/min, and no significant medical history.

5. Excluded Medications and Restrictions

- Any prescription medications and OTC medications including herbal products and antacids with the exception of vitamins, and/or acetaminophen and/or ibuprofen and/or

hormonal contraceptive medications. However, the short-term use of topical hydrocortisone cream or A&D ointment to treat minor skin irritation due to ECG leads was allowed.
- Smokers, use of nicotine or nicotine-containing products within 90 days prior to the first dose of study drug and/or a urine cotinine ≥ 500 ng/mL at screening and clinic admission
- Alcohol or substance abuse as assessed by the investigator
- Had previously participated in an investigational trial involving administration of any investigational compound within 30 days prior to the study dose
- Had taken systemic steroids, immunosuppressant therapies or chemotherapeutic agents within 3 months of study screening or expected to receive these agents during the study (e.g., corticosteroids, immunoglobulins, and other immune- or cytokine-based therapies)

6. Rationale for Doses Used in the Trial

SOF/VEL: FDC at 400/100 mg once daily with or without food is the recommended dose in Epclusa® (sofosbuvir and velpatasvir) US PI.

VOX: 100 mg with SOF/VEL FDC at 400/100 mg is the dose used in most Phase 1 studies and also the dose in Phase 2 and 3 studies.

VOX: 200 mg is the dose proposed for DDI perpetrator studies in healthy subjects to encompass efficacious exposure observed in HCV patients.

7. Drugs Used in the Trial

VOX conventional 10 mg tablets – Lot Number(s): DY1401B1 (Manufactured by (b) (4)
VOX conventional 50 mg tablets – Lot Number(s): DY1403B1 (Manufactured by (b) (4)
VOX modified 100 mg tablets – Lot Number(s): DY1407B2, DY1407B1 (Manufactured by (b) (4)
SOF/VEL FDC 400/100 mg tablets – Lot Number(s): DU1301B1, DU1404B1 (Manufactured by Patheon Inc.)

8. Sample Collection, Bioanalysis, Pharmacokinetic Assessments, and Statistical Analysis

Sample Collection
Intensive PK sampling occurred relative to dosing of study drug at the following time points:
Cohort 1:
- Day 4: Predose (≤ 5 min), 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, 20, and 24 hours postdose
- Day 13: Predose (≤ 5 min), 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 20, and 24

hours postdose

- Day 17: Predose (≤ 5 min), 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 16, 20, 24, 48, 72, and 96 hours postdose
- Additional predose plasma samples were collected on study days 8 through 12, 15 and 16.

Cohorts 2 and 3:

- Days 1, 8, and 15: Predose (< 5 min), 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, and 96 hours postdose

Cohort 4:

- Days 1, 15, and 29: Predose (< 5 min), 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, and 96 hours postdose

Cohort 5:

- Day 1: Predose (< 5 min), 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, and 24 hours postdose
- Day 10: Predose (< 5 min), 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, and 96 hours postdose
- Additional plasma samples were drawn predose on Days 3 through 9 and at 4 hours postdose on Days 2, 3, 4, 5, 6, 7, 8, and 9.

Bioanalytical method

- Validation and Bioanalytical Report:

Analyte	Matrix	Validation Report	Bioanalytical Report
GS-7977 (SOF), GS-331007	Plasma	(b) (4) 60-1323 Amendment 2	(b) (4) 60-1440B Amendment 1
GS-5816 (VEL)	Plasma	(b) (4) 60-1393 Amendment 1	(b) (4) 60-1440A
GS-9857 (VOX)	Plasma	(b) (4) 8109.123113.1	(b) (4) 8221.031515

- Bioanalytical Method:

Method type: LC/MS/MS; Matrix: K_2EDTA plasma

Analyte	Range (ng/mL)	Stability in frozen matrix (days)
GS-7977 (SOF)	5 to 2500	174 at −20°C and 813 at −70°C
GS-331007	10 to 5000	174 at −20°C and 813 at −70°C
GS-5816 (VEL)	1 to 1000	161 at −20°C and 570 at −70°C
GS-9857 (VOX)	0.5 to 1000	420 at −20ºC and at −70ºC

Validation	▪ Method validated prior to use	☒ Yes ☐ No ☐ NA
	▪ Method validation acceptable	☒ Yes ☐ No ☐ NA
Study Samples Analysis	▪ Samples analyzed within the established stability period	☒ Yes ☐ No
	▪ Quality control samples range acceptable	☒ Yes ☐ No
	▪ Chromatograms provided	☒ Yes ☐ No
	▪ Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	▪ Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	▪ Incurred samples analysis is acceptable	☒ Yes ☐ No
	▪ Overall performance acceptable	☒ Yes ☐ No
Inspection	▪ Will the bioanalytical site be inspected	☐ Yes ☒ No

Pharmacokinetic Assessments
The following multiple-dose plasma PK parameters of GS-9857, VEL, and SOF and its metabolites GS-566500 and GS-331007 were calculated: AUC_{tau}, C_{max}, C_{last}, T_{max}, T_{last}, λ_z, CL/F, and $t_{1/2}$, as appropriate. C_{tau} was calculated for GS-9857 and VEL.

The following single-dose plasma PK parameters of GS-9857, VEL, SOF, GS-566500, and GS-331007 were calculated: AUC_{last}, AUC_{inf}, % AUC_{exp}, C_{max}, C_{last}, T_{max}, T_{last}, λ_z, CL/F, and $t_{1/2}$, as appropriate.

Statistical Analysis
For Cohorts 1, 2, and 3 an analysis of variance (ANOVA) was performed for the natural logarithms of PK parameters AUC_{tau}, C_{max}, and C_{tau} for GS-9857 and VEL and AUC_{tau} and C_{max} for SOF and GS-331007 (as applicable, per specific cohort).

For Cohort 4, dose proportionality information was obtained by comparing PK parameters of GS-9857 across the 4 dose levels (Treatment E and 3 dose levels). The primary method for evaluation of dose proportionality was based on AUC_{inf}, AUC_{last}, and C_{max} using a power model that was fitted using all 4 doses.

For Cohort 5, the time to achieve steady-state plasma concentration of GS-9857 was evaluated by assessing linear trend in trough plasma concentrations (C_{trough}) during multiple-dose administration for 10 days. Plots of individual and mean trough concentrations over time were also examined.

9. Results

9.1 Subject Demographics and Disposition
A total of 133 subjects were enrolled in the study. All 133 enrolled subjects were included in the safety analysis set. Of these subjects, 1 subject (0.8%) prematurely

discontinued study drug due to withdrawn consent. Subject 3648-4008 (Cohort 4, treatment sequence JKL) withdrew consent after completing Day 1 administration of GS-9857 300 mg and Day 15 administration of GS-9857 600 mg with a moderate-fat meal. One additional subject (0.8%) prematurely discontinued from the study due to being lost to follow-up.

Overall, the majority of subjects were male (72.9%, 97 subjects), white (66.2%, 88 subjects) and Hispanic or Latino (71.4%, 95 subjects). Subjects had a mean (SD) age of 33 (7.0) years (range: 19 to 45 years), a mean (SD) BMI of 26.3 (2.47) kg/m^2, and a mean (SD) CLcr of 120.47 (20.339) mL/min.

9.2 Pharmacokinetic and Statistical Analysis
In Cohort 1 (DDI, fasted), coadministration of GS-9857 (200 mg, 10 or 50 mg conventional formulation) with SOF/VEL (400/100 mg) FDC under fasted conditions resulted in 63%, 72%, and 41% reduction (**Table 1**) in GS-9857 AUC_{tau}, C_{max}, and C_{tau}, respectively, whereas no significant change in exposure parameters for SOF, GS-331007, and VEL were observed.

Table 1: Plasma PK Parameters and Statistical Comparisons for VOX in Cohort 1

GS-9857 PK Parameter Mean (%CV)[a]	SOF/VEL + GS-9857 (200 mg, Conventional Formulation) Fasted (n = 24)	GS-9857 (200 mg, Conventional Formulation) Fasted (n = 24)	% GLSM Ratio (90% CI)
AUC_{tau} (ng•hr/mL)	413.0 (61.3)	1061.8 (49.9)	36.84 (30.45, 44.58)
C_{max} (ng/mL)	45.6 (73.4)	145.5 (54.1)	28.19 (21.79, 36.46)
C_{tau} (ng/mL)	6.6 (53.6)	10.9 (43.9)	59.41 (51.21, 68.93)
T_{max} (hr)	5.00 (3.50, 5.00)	5.00 (3.00, 5.00)	—
$t_{1/2}$ (hr)	14.31 (9.87, 19.51)	11.10 (8.38, 15.11)	—

GLSM = geometric least-squares mean
a T_{max} and $t_{1/2}$ are reported as Median (Q1, Q3)

In Cohort 2 (BA, fasted; FE), GS-9857 PK parameters were similar between the modified formulation (100 mg tablet) and the conventional formulation (2 x 50 mg tablets) under fasted conditions. Moderate-fat meal increased AUC_{inf} and C_{max} of GS-9857 by approximately 50% when administered as the modified formulation (100 mg tablet), compared to fasted conditions (**Table 2**).

Table 2: Plasma PK Parameters and Statistical Comparisons for VOX in Cohort 2

GS-9857 PK Parameter	GS-9857[b] Fed, Moderate Fat Day 8 (n = 15)		GS-9857[b] Fasted Day 1 (n = 15)		GS-9857 100 mg, Conventional Formulation Fasted Day 15 (n = 15)
	Mean (%CV)[a]	% GLSM Ratio (90% CI) to Modified Formulation Fasted (Day 8/Day 1)	Mean (%CV)[a]	% GLSM Ratio (90% CI) to Conventional Formulation Fasted (Day 1/Day 15)	Mean (%CV)[a]
AUC_{inf} (ng•hr/mL)	495.0 (43.3)	149.45 (122.24, 182.70)	369.7 (64.5)	108.58 (83.51, 141.16)	328.0 (68.1)
AUC_{last} (ng•hr/mL)	421.7 (45.9)	155.31 (122.00, 197.72)	314.2 (71.6)	109.50 (81.17, 147.72)	274.5 (74.0)
C_{max} (ng/mL)	44.2 (44.2)	153.45 (122.14, 192.78)	32.7 (75.7)	143.34 (106.47, 192.97)	21.7 (57.5)
T_{max} (hr)	3.00 (2.00, 4.00)	—	2.00 (1.50, 4.00)	—	4.00 (2.00, 4.00)
$t_{1/2}$ (hr)	41.89 (36.90, 48.00)	—	37.08 (32.73, 44.49)	—	40.74 (33.69, 52.22)

GLSM = geometric least-squares mean
a T_{max} and $t_{1/2}$ are reported as Median (Q1, Q3)
b 100 mg, modified formulation

In Cohort 3 (FE), light- and moderate-fat meal effect on GS-9875 (100 mg modified formulation) + SOF/VEL FDC (400/100 mg) was evaluated and the overall food effect is summarized in **Table 3**. The PK parameters and statistical comparison of VOX, VEL, SOF, GS-331007 are presented in **Table 4 to 6**, respectively.

GS-9857 AUC_{inf} and C_{max} increased 185% and 259%, when given after a moderate-fat meal, and increased 112% and 147%, when given after a light-fat meal, compared to fasted conditions. The effect of food on exposures of VEL and SOF from SOF/VEL plus GS-9857 was greater than from SOF/VEL. This is likely a result of a combination of food effect on SOF and VEL absorption, and a greater degree of intestinal P-gp and BCRP inhibition by GS-9857 when administered with food, according to the sponsor.

Table 3: Summary of Food Effect in Cohort 3

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	GS-US-338-1130 (SOF/VEL+VOX Fed Moderate-Fat Meal vs Fasted)			
	SOF	**GS-331007**	**VEL**	**VOX**
AUC_{inf}	↑144%	↔	↑129%	↑185%
C_{max}	↑76%	↔	↑146%	↑259%
	GS-US-338-1130 (SOF/VEL+VOX Fed Light Meal vs Fasted)			
	SOF	**GS-331007**	**VEL**	**VOX**
AUC_{inf}	↑118%	↔	↑166%	↑112%
C_{max}	↑73%	↔	↑187%	↑147%

Table 4: Plasma PK Parameters and Statistical Comparisons for VOX in Cohort 3

GS-9857 PK Parameter	**SOF/VEL + GS-9857[b] Fed, Moderate Fat Day 8 (n = 15)**		**SOF/VEL + GS-9857[b] Fed, Light Fat Day 15 (n = 15)**		**SOF/ VEL + GS-9857[b] Fasted Day 1 (n = 15)**
	Mean (%CV)[a]	**% GLSM Ratio (90% CI) to Combination Fasted (Day 8/Day 1)**	**Mean (%CV)[a]**	**% GLSM Ratio (90% CI) to Combination Fasted (Day 15/Day 1)**	**Mean (%CV)[a]**
AUC_{inf} (ng•hr/mL)	626.2 (61.2)	284.55 (209.40, 386.69)	480.7 (70.4)	211.70 (158.98, 281.91)	293.4 (99.1)
AUC_{last} (ng•hr/mL)	563.3 (57.8)	323.18 (229.13, 455.83)	405.2 (57.4)	230.67 (166.53, 319.50)	242.6 (100.6)
C_{max} (ng/mL)	64.3 (47.8)	359.43 (251.41, 513.87)	43.4 (41.1)	247.46 (189.74, 322.73)	22.5 (94.1)
T_{max} (hr)	3.00 (3.00, 5.00)	—	4.00 (3.00, 5.00)	—	2.00 (1.00, 5.00)
$t_{1/2}$ (hr)	33.76 (30.09, 36.58)	—	32.81 (27.11, 37.52)	—	33.81 (29.38, 42.64)

GLSM = geometric least-squares mean
a T_{max} and $t_{1/2}$ are reported as Median (Q1, Q3)
b 100 mg, modified formulation of GS-9857

Table 5: Plasma PK Parameters and Statistical Comparisons for VEL in Cohort 3

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VEL PK Parameter Mean (%CV)	SOF/VEL + GS-9857[b] Fed, Moderate Fat Day 8 (n = 15)		SOF/VEL + GS-9857[b] Fed, Light Fat Day 15 (n = 15)		SOF/VEL + GS-9857[b] Fasted Day 1 (n = 15)
	Mean (%CV)[a]	% GLSM Ratio (90% CI) to Fasted (Day 8/Day 1)	Mean (%CV)[a]	% GLSM Ratio (90% CI) to Fasted (Day 15/Day 1)	Mean (%CV)[a]
AUC_{inf} (ng•hr/mL)	5735.8 (56.2)	228.94 (133.72, 391.94)	6212.7 (37.6)	266.04 (161.48, 438.30)	3498.6 (78.6)
AUC_{last} (ng•hr/mL)	5657.5 (56.0)	242.34 (130.87, 448.75)	6097.0 (37.1)	280.63 (157.76, 499.20)	3438.6 (78.9)
C_{max} (ng/mL)	647.1 (39.7)	246.40 (129.66, 468.24)	729.4 (29.9)	287.31 (160.93, 512.95)	389.9 (68.0)
T_{max} (hr)	4.00 (3.00, 5.00)	—	4.00 (3.00, 4.00)	—	3.00 (2.00, 3.00)
$t_{1/2}$ (hr)	17.12 (15.36, 18.53)	—	18.59 (17.57, 19.61)	—	17.79 (15.39, 19.55)

a T_{max} and $t_{1/2}$ are reported as Median (Q1, Q3)
b 100 mg, modified formulation of GS-9857

Table 6: Plasma PK Parameters and Statistical Comparisons for SOF and GS-331007 in Cohort 3

PK Parameter Mean (%CV)	SOF/VEL + GS-9857[b] Fed, Moderate Fat Day 8 (n = 15)		SOF/VEL + GS-9857[b] Fed, Light Fat Day 15 (n = 15)		SOF/VEL + GS-9857[b] Fasted Day 1 (n = 15)
	Mean (%CV)[a]	% GLSM Ratio (90% CI) to Fasted Day 8/Day 1	Mean (%CV)[a]	% GLSM Ratio (90% CI) to Fasted Day 15/Day 1	Mean (%CV)[a]
SOF					
AUC_{inf} (ng•hr/mL)	2692.1 (22.3)	243.93 (168.75, 352.61)	2418.7 (23.6)	217.68 (150.75, 314.33)	1357.1 (53.0)
AUC_{last} (ng•hr/mL)	2684.0 (22.3)	245.56 (169.23, 356.34)	2409.6 (23.8)	218.91 (150.95, 317.46)	1347.9 (53.3)
C_{max} (ng/mL)	1704.5 (35.7)	176.41 (122.69, 253.66)	1653.5 (35.0)	173.08 (121.08, 247.41)	1083.8 (47.5)
T_{max} (hr)	2.00 (1.50, 3.00)	—	2.00 (1.00, 3.00)	—	0.50 (0.50, 1.50)
$t_{1/2}$ (hr)	0.49 (0.45, 0.51)	—	0.48 (0.41, 0.53)	—	0.46 (0.40, 0.52)
CL/F (mL/h)	155433.0 (21.7)	—	176240.2 (29.4)	—	672246.0 (186.0)

GS-331007					
AUC_{inf} (ng•hr/mL)	13077.2 (18.3)	113.36 (107.53, 119.50)	12993.1 (17.4)	112.80 (106.23, 119.78)	11653.3 (23.9)
AUC_{last} (ng•hr/mL)	11829.8 (18.7)	110.97 (103.20, 119.33)	11865.1 (17.1)	111.60 (103.09, 120.81)	10855.9 (26.3)
C_{max} (ng/mL)	664.2 (24.0)	76.36 (70.31, 82.93)	711.2 (26.1)	81.28 (72.89, 90.63)	876.6 (27.1)
T_{max} (hr)	4.00 (3.00, 5.00)	—	4.00 (3.00, 4.00)	—	3.00 (2.00, 4.00)
$t_{1/2}$ (hr)	29.37 (27.63, 32.72)	—	29.04 (26.32, 30.89)	—	25.85 (22.55, 29.07)

GLSM = geometric least-squares mean
a T_{max} and $t_{1/2}$ are reported as Median (Q1, Q3)
b 100 mg, modified formulation of GS-9857

In Cohort 4 (PK linearity, fed), GS-9857 exposure (AUC_{inf} and C_{max}) increased in a greater than dose proportional manner when single doses of GS-9857 greater than 100 mg (300, 600, or 900 mg) were administered with a moderate-fat meal (**Table 7**).

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Table 7: GS-9857 Plasma Pharmacokinetic Parameters and Statistical Analyses (Cohort 2 and Cohort 4)

GS-9857 PK Parameters	ANOVA Analysis							Power Model Analysis Slope (90% CI)
	GS-9857 100 mg Fed, Moderate Fat Cohort 2, Day 8 (n = 15)	GS-9857 300 mg Fed, Moderate Fat Cohort 4, Day 1 (n = 16)		GS-9857 600 mg Fed, Moderate Fat Cohort 4, Day 15 (n = 16)		GS-9857 900 mg Fed, Moderate Fat Cohort 4, Day 29 (n = 15)		
	Mean (%CV)	Mean (%CV)	Dose-Normalized %GLSM[a] Ratio (90% CI) to 100 mg	Mean (%CV)	Dose-Normalized %GLSM[a] Ratio (90% CI) to 100 mg	Mean (%CV)	Dose-Normalized %GLSM[a] Ratio (90% CI) to 100 mg	GS-9857 Across All Doses (n=31)
AUC_{inf} (ng•hr/mL)	495.0 (43.3)	4078.2 (69.3)	251.49 (174.82, 361.79)	17712.4 (70.8)	510.17 (354.63, 733.92)	43528.7 (58.1)	903.32 (624.28, 1307.08)	1.98 (1.83, 2.14)
AUC_{last} (ng•hr/mL)	421.7 (45.9)	3994.9 (70.3)	289.90 (200.16, 419.88)	17604.9 (71.1)	597.53 (412.56, 865.44)	43354.6 (58.3)	1064.65 (730.73, 1551.16)	2.06 (1.90, 2.21)
C_{max} (ng/mL)	44.2 (44.2)	784.4 (86.1)	470.76 (308.48, 718.42)	3711.9 (67.2)	1167.35 (764.94, 1781.46)	6764.0 (57.9)	1645.53 (1071.01, 2528.24)	2.30 (2.12, 2.48)

GLSM = geometric least-squares mean
a GLSM of Dose-Normalized PK Parameters (ie, AUC_{inf} /D, AUC_{last} /D, and C_{max} /D) to 100 mg dose

In Cohort 5 (steady state PK), GS-9857 modified formulation at 200 mg was administered after a moderate-fat meal for 10 days, and the AUC_{tau}, C_{max}, and C_{tau} (**Table 8**) were comparable to preliminary steady-state exposures observed in non-cirrhotic HCV-infected subjects administered SOF/VEL (400/100 mg) FDC plus GS-9857 100 mg with food in on-going Phase 2 studies.

Table 8: Summary of Steady-State GS-9857 Plasma PK Parameters (Cohort 5)

GS-9857 PK Parameter Mean (%CV)[a]	GS-9857[b] Fed, Moderate Fat (n = 15) Day 1	GS-9857[b] Fed, Moderate Fat (n = 15) Day 10
AUC_{tau} (ng•hr/mL)	932.4 (39.6)	2953.0 (66.6)
C_{max} (ng/mL)	153.0 (56.2)	589.2 (75.4)
C_{tau} (ng/mL)	10.3 (29.7)	19.0 (52.9)
T_{max} (hr)	4.00 (4.00, 5.00)	5.00 (4.00, 5.00)
$t_{1/2}$ (hr)	8.62 (7.69, 11.36)	7.43 (6.85, 8.64)

a T_{max} and $t_{1/2}$ are reported as Median (Q1, Q3)
b 100 mg, modified formulation

9.3 Safety Analysis
GS-9857 was generally safe and well tolerated when administered alone and in combination with SOF/VEL, and all related AEs were Grade 1 or Grade 2 in severity. There were no deaths or SAEs reported in this study.

10. Sponsor's Conclusions

1. GS-9857 exposure is significantly reduced when coadministered with SOF/VEL under fasting conditions which is mitigated by administration with food. Therefore, the combination of GS-9857 plus SOF/VEL should be administered with food.
2. Modified and conventional formulations of GS-9857 provide comparable GS-9857 exposure.
3. GS-9857 displayed greater than dose-proportional increases in exposure above the 100 mg dose when administered with food.

11. Reviewer's Assessment

The study design for cohorts 1 to 5 and the sponsor's conclusions are reasonable.

For cohort 1, 200 mg GS-9857 (10x10 mg + 2x50 mg conventional formuation, based on information from the sponsor) was dosed in Treatment B and C. The GS-9857 AUC was decreased by 63% after co-administration with SOF/VEL under fasted conditions, likely via inhibition of the intestinal uptake transporter OATP2B1 by VEL, based on the proposal from the sponsor. However, it is unclear if that is a valid explanation since it is unknown if GS-9857 is a substrate of OATP2B1.

4.4.7 GS-US-367-1176 Relative BA and High-fat Meal FE

1. Title

A Phase 1, Randomized, Open-label, Single Dose Study to Evaluate the Relative Bioavailability and the Effect of Food on Sofosbuvir/GS-5816/GS-9857 Fixed-Dose Combination Tablet in Healthy Subjects

2. Information Regarding the Clinical Trial Site and Duration of the Trial

The trial was conducted (b) (4) from May 26, 2015 to June 24, 2015, with the final report date of December 15, 2015.

3. Objectives

Primary objective:

- To evaluate the relative bioavailability of a fixed-dose combination (FDC) tablet of sofosbuvir (SOF)/velpatasvir (VEL)/GS-9857 (SOF/VEL/GS-9857) relative to existing tablet formulations of SOF/VEL FDC and individual agent GS-9857
- To evaluate the effect of food on the pharmacokinetics (PK) of the SOF/VEL/GS-9857 FDC tablet in healthy subjects

Secondary objective:

- To evaluate the safety and tolerability of the SOF/VEL/GS-9857 FDC tablet following single-dose administration to healthy subjects

4. Trial Design

This Phase 1, open-label, randomized, single-center, 2-cohort crossover study in healthy subjects was designed to evaluate the relative bioavailability, safety, and tolerability of SOF/VEL/GS-9857 relative to the existing tablet formulations of SOF/VEL and the individual agent GS-9857. The study also evaluated the effect of food on SOF/VEL/GS-9857.

Thirty-four subjects were enrolled into each cohort and randomized 1:1 to treatment sequence AB or BA (Cohort 1) or treatment sequence CD or DC (Cohort 2). Each subject received 2 single doses of study drug on Days 1 and 10 separated by a washout period from Days 2 through 9.

Cohort	Treatment Sequence	Day 1	Days 2-9	Day 10
Cohort 1	AB	A	Washout	B
	BA	B	Washout	A
Cohort 2	CD	C	Washout	D
	DC	D	Washout	C

Cohort 1: Relative Bioavailability

- Treatment A: SOF/VEL FDC (1 × 400/100-mg tablet single dose) + GS-9857 (1 × 100-mg tablet single dose) co-administered orally within 5 minutes of completing a moderate-fat meal
- Treatment B: SOF/VEL/GS-9857 FDC (1 × 400/100/100-mg tablet single dose) administered orally within 5 minutes of completing a moderate-fat meal

Cohort 2: Effect of Food

- Treatment C: SOF/VEL/GS-9857 FDC (1 × 400/100/100-mg tablet single dose) administered orally under fasted conditions
- Treatment D: SOF/VEL/GS-9857 FDC (1 × 400/100/100-mg tablet single dose) administered orally within 5 minutes of completing a high-fat/high-calorie meal

In Cohort 1 on Days 1 and 10, a moderate-fat-calorie breakfast containing approximately 600 kcal and 25% to 30% fat was provided. In Cohort 2 Sequence CD Day 10 and Sequence DC Day 1, a high-fat/high-calorie breakfast containing approximately 1000 kcal and 45% to 55% fat was provided. The meal was to be initiated 30 minutes prior to study drug administration and the dose administered within 5 minutes after completing the meal.

In Cohort 2, Sequence CD Day 1 and Sequence DC Day 10, study drug was administered under fasting conditions; after an overnight fast subjects were not allowed any food until 4 hours after study drug administration. Other than the water provided with dosing, water and other fluids were withheld 1 hour before and 2 hours after dose administration.

Eligible subjects were healthy male and non-pregnant, non-lactating female subjects, 18 to 45 years of age (inclusive), with a body mass index (BMI) $\geq$ 19 and $\leq$ 30 kg/m^2, estimated creatinine clearance (CL_{cr}) $\geq$ 80 mL/min (calculated using the Cockcroft-Gault method), and no significant medical history.

5. Excluded Medications and Restrictions

- Any prescription medications and OTC medications including herbal products and antacids with the exception of vitamins, and/or acetaminophen and/or ibuprofen and/or hormonal contraceptive medications. However, the short-term use of topical hydrocortisone cream or A&D ointment to treat minor skin irritation due to ECG leads was allowed.
- Subjects were required to refrain from the consumption of food and beverages containing alcohol products, and from consumption of grapefruit juice, grapefruits, and Seville orange juice 72 hours prior to the first dose of study drug and during the course of the study through discharge.
- Subjects were required to refrain from the use of nicotine or nicotine-containing products from screening through discharge.

6. Rationale for Doses Used in the Trial

SOF/VEL 400/100mg FDC + GS-9857 100mg or SOF/VEL/GS-9857 400/100/100mg FDC is the dose regimen evaluated in different Phase 1 studies and also is the efficacious dose evaluated in Phase 2 and 3 studies. The dose is reasonable assuming the relative BA and food effect are similar in healthy subjects compared to HCV-infected patients.

7. Drugs Used in the Trial

SOF/VEL/GS-9857 400/100/100mg FDC tablets – Lot Number(s): 15GEI001URA (Manufactured by Gilead Sciences)

SOF/VEL 400/100mg FDC tablets – Lot Number(s): DU1404B1 (Manufactured by Gilead Sciences)
GS-9857 100mg tablets – Lot Number(s): DY1407B1 (Manufactured by (b) (4)

8. Sample Collection, Bioanalysis, Pharmacokinetic Assessments, and Statistical Analysis

Sample Collection
Serial blood samples were collected on Day 1 and Day 10 at the following time points: predose (≤ 5 minutes), and 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, 96, and 120 hours postdose.

Bioanalytical method

- Validation and Bioanalytical Report:

Analyte	Matrix	Validation Report	Bioanalytical Report
SOF, GS-566500, GS-331007	Plasma	(b) (4) 60-1323Amendment 4	(b) (4) 60-1527B
VEL	Plasma	(b) (4) 60-1393 Amendment 1	(b) (4) 60-1527A
GS-9857 (VOX)	Plasma	(b) (4) 8109.123113.1	(b) (4) 10031.121515

- Bioanalytical Method:

Method type: LC/MS/MS; Matrix: K_2EDTA plasma

Analyte	Range (ng/mL)	Stability in frozen matrix (days)
SOF	5 to 2500	174 at −20°C and 813 at −70°C
GS-331007	10 to 5000	174 at −20°C and 813 at −70°C
VEL	1 to 1000	161 at −20°C and 570 at −70°C
GS-9857 (VOX)	0.5 to 1000	420 at −20ºC and at −70ºC

Validation	▪ Method validated prior to use	☒ Yes ☐ No ☐ NA
	▪ Method validation acceptable	☒ Yes ☐ No ☐ NA
Study Samples Analysis	▪ Samples analyzed within the established stability period	☒ Yes ☐ No
	▪ Quality control samples range acceptable	☒ Yes ☐ No
	▪ Chromatograms provided	☒ Yes ☐ No
	▪ Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	▪ Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	▪ Incurred samples analysis is acceptable	☒ Yes ☐ No

	▪ Overall performance acceptable	☒ Yes ☐ No
Inspection	▪ Will the bioanalytical site be inspected	☐ Yes ☒ No

Pharmacokinetic Assessments
PK parameters include: AUC_{last}, AUC_{inf}, C_{max}, $\%AUC_{exp}$, T_{max}, T_{last}, C_{last}, λ_z, $t_{1/2}$, and CL/F (parent SOF, VEL, and GS-9857 only).

Statistical Analysis
For Cohort 1, the relative bioavailability of SOF/VEL/GS-9857 was compared with SOF/VEL+GS-9857. The natural log-transformed AUC_{last}, AUC_{inf}, and C_{max} of each analyte were evaluated for each treatment. The 90% CI was constructed for the ratio of geometric least-squares means (GLSM) of PK parameters for each analyte and treatment pair.

For Cohort 2, the effect of food (high-calorie/high-fat) on the PK of SOF/VEL/GS-9857 was estimated using the similar mixed-effects model. The GLSM of fed versus fasted and their 90% CIs were constructed for the primary PK parameters AUC_{last}, AUC_{inf}, and C_{max} of SOF, GS-566500, GS-331007, VEL, and GS-9857.

9. Results

9.1 Subject Demographics and Disposition
All subjects completed the treatments and study as planned.

Overall, the mean age was 30 years (range 18 to 45), 66.2% were male subjects, 47.1% of subjects were white, 45.6% were black or African American, and 86.8% were non-Hispanic or Latino. The mean (SD) BMI was 26.0 (2.59) kg/m^2 and mean (SD) creatinine clearance at baseline was 115.30 (20.983) mL/min. Characteristics were generally similar between sequences within cohorts.

9.2 Pharmacokinetic and Statistical Analysis
Primary PK parameters for SOF, GS-566500, GS-331007, VEL, and GS-9857 are summarized in **Table 1**.

SOF/VEL/GS-9857 FDC and SOF/VEL+GS-9857 resulted in comparable exposures of SOF, GS-331007, VEL, and GS-9857 when administered after a moderate-fat meal.

Administration of SOF/VEL/GS-9857 following a high-fat/high-calorie meal resulted in substantially increased GS-9857 exposure, and modestly increased SOF and VEL exposures compared with exposures achieved under fasted conditions. These data are consistent with the known effect of food on the PK of SOF, GS-331007, VEL, and GS-9857.

The large increase in GS-9857 exposure following administration of SOF/VEL/GS-9857 under fed conditions compared with fasted conditions was expected based upon the combination of a positive food effect and the known drug-drug interaction (significantly

reduced GS-9857 exposure) that occurs between SOF/VEL and GS-9857 administered in the fasted state.

Table 1: Statistical analysis of primary PK parameters for SOF, GS-331007, VEL, and GS-9857

PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test / Reference
	Reference Treatment (N = 34)	Test Treatment (N = 34)	
Cohort 1: SOF/VEL+GS-9857 vs SOF/VEL/GS-9857			
SOF PK: SOF/VEL+GS-9857 (Reference) vs SOF/VEL/GS-9857 (Test)			
AUC_{last} (h•ng/mL)	2548.8 (37.6)	2505.9 (43.5)	97.38 (89.60, 105.84)
AUC_{inf} (h•ng/mL)	2559.8 (37.5)	2517.9 (43.3)	97.43 (89.71, 105.82)
C_{max} (ng/mL)	1579.2 (32.2)	1588.8 (46.6)	95.27 (82.70, 109.74)
GS-331007 PK: SOF/VEL+GS-9857 (Reference) vs SOF/VEL/GS-9857 (Test)			
AUC_{last} (h•ng/mL)	13,271.7 (22.5)	13,563.2 (22.7)	102.13 (99.76, 104.55)
AUC_{inf} (h•ng/mL)	141,12.5 (22.5)	14,400.6 (22.9)	101.93 (99.67, 104.24)
C_{max} (ng/mL)	655.4 (20.8)	661.5 (26.2)	99.56 (94.20, 105.23)
VEL PK: SOF/VEL+GS-9857 (Reference) vs SOF/VEL/GS-9857 (Test)			
AUC_{last} (h•ng/mL)	6243.8 (33.3)	6376.9 (30.8)	104.64 (96.27, 113.75)
AUC_{inf} (h•ng/mL)	6291.6 (33.2)	6421.1 (30.8)	104.45 (96.20, 113.42)
C_{max} (ng/mL)	714.9 (32.4)	735.8 (29.3)	104.90 (96.39, 114.16)
GS-9857 PK: SOF/VEL+GS-9857 (Reference) vs SOF/VEL/GS-9857 (Test)			
AUC_{last} (h•ng/mL)	456.8 (37.9)	485.5 (49.0)	100.22 (90.43, 111.07)
AUC_{inf} (h•ng/mL)	503.5 (35.4)	528.2 (45.2)	100.10 (90.90, 110.23)
C_{max} (ng/mL)	49.8 (53.2)	63.0 (73.3)	112.66 (95.20, 133.31)
Cohort 2: SOF/VEL/GS-9857 Fed vs Fasted			
SOF PK: SOF/VEL/GS-9857, fasted (Reference) vs SOF/VEL/GS-9857, fed (Test)			
AUC_{last} (h•ng/mL)	1568.4 (42.8)	2421.1 (37.3)	163.93 (138.03, 194.69)
AUC_{inf} (h•ng/mL)	1576.3 (42.5)	2439.6 (36.9)	163.51 (139.00, 192.34)
C_{max} (ng/mL)	1369.2 (38.6)	1481.3 (48.8)	109.26 (87.32, 136.72)

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PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test / Reference
	Reference Treatment (N = 34)	Test Treatment (N = 34)	
GS-331007 PK: SOF/VEL/GS-9857, fasted (Reference) vs SOF/VEL/GS-9857, fed (Test)			
AUC_{last} (h•ng/mL)	11,799.3 (20.9)	11,822.1 (18.4)	100.69 (95.49, 106.17)
AUC_{inf} (h•ng/mL)	12,349.1 (19.9)	12,481.3 (17.9)	101.40 (96.43, 106.62)
C_{max} (ng/mL)	864.6 (29.9)	546.8 (17.0)	64.69 (59.51, 70.32)
VEL PK: SOF/VEL/GS-9857, fasted (Reference) vs SOF/VEL/GS-9857, fed (Test)			
AUC_{last} (h•ng/mL)	4616.7 (46.7)	5963.4 (38.9)	141.11 (112.78, 176.56)
AUC_{inf} (h•ng/mL)	4662.5 (46.4)	6011.3 (38.7)	140.36 (112.78, 174.69)
C_{max} (ng/mL)	509.0 (40.7)	658.5 (38.0)	137.35 (110.88, 170.16)
GS-9857 PK: SOF/VEL/GS-9857, fasted (Reference) vs SOF/VEL/GS-9857, fed (Test)			
AUC_{last} (h•ng/mL)	158.6 (117.2)	711.3 (40.6)	642.09 (503.80, 818.34)
AUC_{inf} (h•ng/mL)	193.0 (104.7)	758.9 (39.7)	535.11 (427.91, 669.17)
C_{max} (ng/mL)	16.8 (169.4)	85.0 (41.7)	779.93 (615.80, 987.79)

9.3 Safety Analysis

SOF/VEL/GS-9857 (under both fasted and fed conditions) was generally safe and well tolerated and the safety profile was similar compared with administration of SOF/VEL+GS-9857 (under fed conditions). No deaths, SAEs, Grade 3 or 4 AEs, or discontinuations due to AEs were reported in this study.

10. Sponsor's Conclusions

- SOF/VEL/GS-9857 and SOF/VEL+GS-9857 resulted in equivalent exposures of SOF, GS-331007, VEL, and GS-9857 when administered after a moderate-fat meal.
- Administration of SOF/VEL/GS-9857 after a high-fat/high-calorie meal substantially increased GS-9857 exposure, with changes in exposures of SOF, GS-331007 and VEL consistent with the known effect of food on these analytes.

11. Reviewer's Assessment

- The study design is reasonable and the PK parameters for SOF, GS-331007, VEL, and VOX are comparable to results from other Phase 1 studies in healthy subjects following administration of SOF/VEL/VOX at 400/100/100 mg under fasted conditions or with moderate-fat meal.
- Administration of SOF/VEL/GS-9857 and SOF/VEL+GS-9857 resulted in comparable exposures when administered with moderate-fat meal. A high-fat meal may lead to substantial increases of GS-9857 exposure, which may contribute to the high variability in Phase 2 and 3 studies where the fat content in food was not specified.

4.4.8 GS-US-367-1417 DDI Enzyme/Transporter Inhibition/Induction on VOX

1. Title

A Phase 1 Study to Evaluate Transporter and Cytochrome P450 (CYP)-Mediated Drug-Drug Interactions between GS-9857 and Probe Drugs

2. Information Regarding the Clinical Trial Site and Duration of the Trial

The trial was conducted (b) (4) from February 19, 2015 to April 28, 2015, with the final report date of December 3, 2015.

3. Objectives

- To evaluate the effect of a mixed organic anion transporting polypeptide (OATP)/multidrug resistance-associated protein 2 (MRP2)/ P-glycoprotein (P-gp)/ breast cancer resistance protein (BCRP) inhibitor or selective OATP1B1/1B3 inhibitor on the pharmacokinetics (PK) of GS-9857
- To evaluate the effect of selective inhibitors of cytochrome P450 (CYP) 3A (CYP3A) or CYP2C8 on the PK of GS-9857
- To evaluate the effect of CYP3A/CYP2C8/P-gp induction on the PK of GS-9857
- To evaluate the effect of intestinal uptake transport inhibition on the PK of GS-9857

4. Trial Design

This Phase 1 study was a randomized (Cohort 1 only), open-label, 4-cohort study designed to evaluate transporter and CYP-mediated drug-drug interactions (DDI) between GS-9857 and probe drugs in healthy subjects. Following completion of screening and Day-1 assessments, eligible subjects were enrolled to 1 of 4 cohorts to receive study treatments as described below. In Cohort 1, all study drugs were administered under fasted conditions in the morning. In Cohorts 2 to 4, all study drugs were administered under fed conditions with a moderate-fat meal.

Cohort 1 (OATP/MRP2/P-gp/BCRP Inhibitor Cyclosporine; OATP1B1/1B3 Inhibitor Rifampin):
Subjects were randomized to receive each of the following treatments in 1 of 4 treatment sequences.

- Treatment A: single dose of GS-9857 100 mg (1 × 100-mg tablet)
- Treatment B: single dose of cyclosporine (cyclosporin A [CsA]) 600 mg (6 × 100-mg capsules)
- Treatment C: single dose of CsA 600 mg (6 × 100-mg capsules) + GS-9857 100 mg (1 × 100-mg tablet)
- Treatment D: single dose of rifampin (RIF) 600 mg (2 × 300-mg capsules) + GS-9857 100 mg (1 × 100-mg tablet)

The treatment sequences were as follows:

Sequence	Day 1	Days 2-8	Day 9	Days 10-16	Day 17	Days 18-24	Day 25
ABCD	GS-9857	Washout	CsA	Washout	CsA + GS-9857	Washout	RIF + GS-9857
BDAC	CsA	Washout	RIF + GS-9857	Washout	GS-9857	Washout	CsA + GS-9857
CADB	CsA + GS-9857	Washout	GS-9857	Washout	RIF + GS-9857	Washout	CsA
DCBA	RIF + GS-9857	Washout	CsA + GS-9857	Washout	CsA	Washout	GS-9857

Cohort 2 (CYP3A Inhibitor Voriconazole; CYP2C8 Inhibitor Gemfibrozil):

- Treatment E: single dose of GS-9857 100 mg (1 × 100-mg tablet) on Day 1
- Treatment F: voriconazole (VCZ) 200 mg (1 × 200-mg tablet) twice daily (12 hours apart) on Days 9 to 12, coadministered with a single dose of GS-9857 100 mg (1 × 100-mg tablet) on Day 9. The last dose of VCZ was administered in the evening on Day 12.
- Treatment G: gemfibrozil (GFZ) 600 mg (1 × 600-mg tablet) twice daily (12 hours apart) administered on Days 20 to 23, coadministered with a single dose of GS-9857 100 mg (1 × 100-mg tablet) on Day 20. The last dose of GFZ was administered in the evening on Day 23.

Cohort 3 (CYP3A/CYP2C8/P-gp Inducer Rifampin):

- Treatment H: single dose of GS-9857 100 mg (1 × 100-mg tablet) on Day 1
- Treatment I: RIF 600 mg (2 × 300-mg capsules) once daily administered on Days 9 to 15 in the evening followed by a single dose of GS-9857 100 mg (1 × 100-mg tablet) in the morning on Day 16 (12 hours apart from the evening dose of RIF)

Cohort 4 (Intestinal Uptake Transport Inhibitor Grapefruit Juice):

- Treatment J: single dose of GS-9857 100 mg (1 × 100-mg tablet) on Day 1
- Treatment K: single dose of grapefruit juice (10 ounces [~300 mL]) + GS-9857 100 mg (1 × 100-mg tablet) on Day 9

Eligible subjects were healthy males and non-pregnant, non-lactating females, 18 to 45 years of age (inclusive), with a body mass index (BMI) of 19 to 30 kg/m2 (inclusive), 12-lead electrocardiograms (ECGs) without clinically significant abnormalities, normal renal function, no significant medical history, and in good general health as determined by the investigator at screening.

5. Excluded Medications and Restrictions

- Any prescription medications and OTC medications including herbal products and antacids with the exception of vitamins, and/or acetaminophen and/or ibuprofen and/or hormonal contraceptive medications. However, the short-term use of topical hydrocortisone cream or A&D ointment to treat minor skin irritation due to ECG leads was allowed.
- Subjects were required to refrain from the consumption of food and beverages containing alcohol products, and from consumption of grapefruit juice, grapefruits, and Seville orange juice 72 hours prior to the first dose of study drug and during the course of the study through discharge.
- Subjects were required to refrain from the use of nicotine or nicotine-containing products from screening through discharge.

6. Rationale for Doses Used in the Trial

CsA: 600 mg is around the highest recommended initial dose in Neoral® (cyclosporine) USPI (9 mg/kg/day), and food can decrease CsA exposure. CsA has elimination half-life at 8.4 h with minimal accumulation, and thus a single dose of CsA at 600 mg under fasted condition as a perpetrator drug for transporter inhibition is reasonable.

RIF-Cohort 1 single dose: 600 mg is the highest recommended dose in Rifadin® (rifampin) USPI, and food can decrease RIF exposure. RIF has short elimination half-life at 3.4 h, and thus a single dose of RIF at 600 mg under fasted condition as a perpetrator drug is reasonable for OATP1B1/3 inhibition.

RIF-Cohort 3 multiple doses: rifampin has been documented to effectively induce CYP enzymes and transporter P-gp after 7 days of 600 mg QD administration (Reference: Niemi M et.al, Clin Pharmacokinet. 2003; 42: 819), thus a dosage regimen at 600 mg QD for 7 days is reasonable for CYP3A/CYP2C8/P-gp induction.

VCZ: 200 mg twice daily is the recommended dose in Vfend®(voriconazole) USPI. Food can only decrease VCZ exposure slightly (20 to 30%), and thus a dosage regimen at 200 mg BID for 4 days under fed conditions within VOX PK sampling duration is reasonable for CYP3A inhibition.

GFZ: 600 mg twice daily is the recommended dose in Lopid®(gemfibrozil) USPI. Food has no significant effect on GFZ exposure, and thus a dosage regimen at 600 mg BID for 4 days under fed conditions within VOX PK sampling duration is reasonable for CYP2C8 inhibition.

Grapefruit juice: a single dose at 10 ounces (~300 mL) is reasonable as a perpetrator agent.

VOX: a single dose at 100 mg under fasted or fed conditions is reasonable as a victim drug.

7. Drugs Used in the Trial

VOX 100 mg modified formulation individual-agent tablets – Lot Number(s): DY1407B1 (Manufactured by (b) (4)
RIF 300 mg capsules – Lot Number(s): 3116525 (Manufactured by Sanofi-Aventis US, LLC)
CsA 100 mg capsules – Lot Number(s): F4164 (Manufactured by Novartis Pharmaceuticals Corporation)
VCZ 200 mg tablets – Lot Number(s): E10442431 (Manufactured by Pfizer, Inc)
GFZ 600 mg tablets – Lot Number(s): H79656 (Manufactured by Parke-Davis, Division of Pfizer, Inc)
Grapefruit juice 300 mL – Lot Number(s): C5051 0546 (Manufactured by Everfresh)

8. Sample Collection, Bioanalysis, Pharmacokinetic Assessments, and Statistical Analysis

Sample Collection
Intensive PK sampling occurred relative to dosing of study drug as follows: predose (< 5 min), 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, and 96 hours postdose on Days 1, 9, 17, and 25 (Cohort 1), Days 1, 9, and 20 (Cohort 2), Days 1 and 16 (Cohort 3), and Days 1 and 9 (Cohort 4).

Bioanalytical method

- Validation and Bioanalytical Report:

Analyte	Matrix	Validation Report	Bioanalytical Report
VOX	Plasma	(b) (4) 8109.123113.1	(b) (4) 8892.112515
CsA	Plasma	(b) (4) 10082.063015	(b) (4) 8893.112515

- Bioanalytical Method:

Method type: LC/MS/MS; Matrix: K_2EDTA plasma

Analyte	Range (ng/mL)	Stability in frozen matrix (days)
VOX	0.5 to 1000	420 days at −20ºC and at −70ºC
CsA	5 to 2500	16 days at −20°C and 52 days at −80°C

Validation	▪ Method validated prior to use	☒ Yes ☐ No ☐ NA
	▪ Method validation acceptable	☒ Yes ☐ No ☐ NA
Study Samples Analysis	▪ Samples analyzed within the established stability period	☒ Yes ☐ No
	▪ Quality control samples range acceptable	☒ Yes ☐ No
	▪ Chromatograms provided	☒ Yes ☐ No
	▪ Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No

	▪ Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	▪ Incurred samples analysis is acceptable	☒ Yes ☐ No
	▪ Overall performance acceptable	☒ Yes ☐ No
Inspection	▪ Will the bioanalytical site be inspected	☐ Yes ☒ No

Pharmacokinetic Assessments
The primary PK parameters were AUC_{last}, AUC_{inf}, and C_{max} of GS-9857 and CsA. The following PK parameters were also determined for GS-9857 and CsA: $\%AUC_{exp}$, C_{last}, T_{max}, T_{last}, λ_z, CL/F, V_z/F, and $t_{1/2}$ as appropriate.

Statistical Analysis
The statistical comparisons of the natural log-transformed PK parameters for each analyte and treatment comparison of interest within each cohort were performed. To evaluate the effect of GS-9857 on CsA, the PK parameters (AUC_{last}, AUC_{inf}, C_{max}) of CsA were compared when dosed in combination with GS-9857 (test treatment: C) versus when dosed alone (reference treatment: B). To evaluate the effect of CsA, single-dose RIF, VCZ, GFZ, multiple-dose RIF, and grapefruit juice on GS-9857, the PK parameters (AUC_{last}, AUC_{inf}, C_{max}) of GS-9857 were compared when dosed in combination with probe drugs (test treatment: C, D, F, G, I, or K) versus when dosed alone (reference treatment: A, E, H, or J).

9. Results

9.1 Subject Demographics and Disposition
A total of 98 subjects were enrolled in 4 cohorts, with 26 subjects in Cohort 1, and 24 subjects each in Cohorts 2 to 4. All 98 enrolled subjects also received at least 1 dose of study drug; 93 of 98 subjects (94.9%) received all doses of study drug and 92 of 98 subjects (93.9%) completed the study. A total of 5 subjects prematurelydiscontinued study drug: 4 subjects in Cohort 1 (1 due to an AE [Grade 1 headache], 1 due to pregnancy, and 2 due to investigator's decision), and 1 subject in Cohort 2 (completed study drug but withdrew consent). One subject in Cohort 4 completed study drug, but withdrew consent before completing the study.

The majority of subjects were black or African American (62.2%; 61 subjects) and non-Hispanic or Latino (82.7%; 81 subjects). Most subjects were male (56.1%; 55 subjects). Subjects had a mean (SD) age of 33 (6.8) years (range: 20 to 45 years), a mean (SD) BMI of 25.7 (2.87) kg/m2, and a mean (SD) baseline creatinine clearance of 113.51 (21.015) mL/min.

9.2 Pharmacokinetic and Statistical Analysis
A summary of the PK parameters of GS-9857 and CsA is presented in **Table 1**.

Cyclosporine, an OATP/MRP2/P-gp/BCRP inhibitor, significantly increased GS-9857 AUC_{inf} (9.4-fold) and C_{max} (19-fold) in the fasted state. Single-dose RIF (a hepatic OATP

inhibitor) significantly increased GS-9857 AUC_{inf} (7.9-fold) and C_{max} (11-fold) in the fasted state. Grapefruit juice (intestinal OATP inhibitor) did not affect GS-9857 exposure, suggesting GS-9857 is not subject to intestinal OATP inhibition when administered with grapefruit juice. Coadministration of GS-9857 with CsA did not alter the exposure of CsA (a CYP3A substrate).

Voriconazole, a potent CYP3A inhibitor, increased GS-9857 AUC_{inf} (1.8-fold) with minimal impact on C_{max} (1.1-fold) after a moderate-fat meal. Gemfibrozil, a potent CYP2C8 inhibitor, had no effect on GS-9857 exposure after a moderate-fat meal.

Multiple-dose RIF, a potent inducer of CYP3A/CYP2C8/P-gp, significantly reduced GS-9857 AUC_{inf} (by 73%) and median $t_{1/2}$ (31 hours to 8 hours) with no effect on C_{max} following a moderate-fat meal.

Table 1: Plasma PK Parameters and Statistical Comparisons for VOX and CsA

PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test / Reference
	Reference Treatment	Test Treatment	
GS-9857 PK: GS-9857 (Reference, N = 22) vs CsA+GS-9857 (Test, N = 25)			
AUC_{last} (h*ng/mL)	342.5 (54.7)	3841.0 (51.5)	1101.40 (853.77, 1420.85)
AUC_{inf} (h*ng/mL)	401.9 (50.8)	3911.1 (51.1)	938.64 (736.79, 1195.78)
C_{max} (ng/mL)	42.0 (117.3)	600.0 (38.8)	1902.07 (1412.39, 2561.52)
GS-9857 PK: GS-9857 (Reference, N = 22) vs single-dose RIF+GS-9857 (Test, N = 24)			
AUC_{last} (h*ng/mL)	342.5 (54.7)	3188.3 (52.9)	947.50 (733.51, 1223.92)
AUC_{inf} (h*ng/mL)	401.9 (50.8)	3200.6 (52.7)	790.77 (619.98, 1008.61)
C_{max} (ng/mL)	42.0 (117.3)	366.9 (50.7)	1110.37 (822.83, 1498.40)
GS-9857 PK: GS-9857 (Reference, N = 24) vs Grapefruit Juice+GS-9857 (Test, N = 24)			
AUC_{last} (h*ng/mL)	625.2 (51.3)	614.1 (40.9)	102.32 (94.55, 110.74)
AUC_{inf} (h*ng/mL)	697.6 (50.2)	680.3 (39.7)	101.45 (94.61, 108.78)
C_{max} (ng/mL)	75.7 (57.9)	66.3 (41.8)	95.36 (81.90, 111.04)
GS-9857 PK: GS-9857 (Reference, N = 24) vs VCZ+GS-9857 (Test, N = 24)			
AUC_{last} (h*ng/mL)	494.2 (53.6)	861.7 (57.3)	171.08 (154.25, 189.75)
AUC_{inf} (h*ng/mL)	581.4 (52.8)	1069.3 (53.3)	183.70 (166.41, 202.80)
C_{max} (ng/mL)	57.3 (56.5)	62.2 (44.7)	113.43 (97.99, 131.29)
GS-9857 PK: GS-9857 (Reference, N = 24) vs GFZ+GS-9857 (Test, N = 24)			
AUC_{last} (h*ng/mL)	494.2 (53.6)	607.6 (69.9)	115.51 (104.14, 128.11)
AUC_{inf} (h*ng/mL)	581.4 (52.8)	677.8 (67.9)	111.21 (100.74, 122.77)
C_{max} (ng/mL)	57.3 (56.5)	57.4 (60.4)	97.87 (84.55, 113.29)
GS-9857 PK: GS-9857 (Reference, N = 24) vs following multiple-dose RIF+GS-9857 (Test, N = 24)			
AUC_{last} (h*ng/mL)	550.1 (30.4)	171.1 (59.1)	28.27 (24.26, 32.96)
AUC_{inf} (h*ng/mL)	622.9 (28.8)	182.1 (55.8)	27.01 (23.23, 31.40)
C_{max} (ng/mL)	55.9 (31.2)	56.4 (54.7)	91.39 (76.00, 109.90)
CsA PK: CsA (Reference, N = 24) vs CsA+GS-9857 (Test, N = 25)			
AUC_{last} (h*ng/mL)	13381.6 (25.3)	12859.5 (30.5)	93.97 (84.11, 104.99)
AUC_{inf} (h*ng/mL)	14164.8 (26.4)	13657.3 (31.3)	94.32 (84.27, 105.57)
C_{max} (ng/mL)	1715.4 (17.8)	1642.8 (20.4)	95.48 (88.48, 103.03)

9.3 Safety Analysis
GS-9857 was generally well tolerated when coadministered with probe drugs in healthy subjects. There were no deaths or serious adverse events reported, and all AEs were Grade 1 in severity.

10. Sponsor's Conclusions

- Administration of GS-9857 with hepatic OATP inhibitors is not recommended.
- Administration of GS-9857 with potent or moderate inducers of CYP3A/CYP2C8/P-gp is not recommended.
- GS-9857 may be coadministered with inhibitors of CYP3A or CYP2C8 without dose modification.

11. Reviewer's Assessment

The study design and sponsor's conclusions are reasonable.

The reviewer agrees with the sponsor's proposal for the DDI management strategy, regarding to coadministration with OATP inhibitors, P-gp and/or CYP enzyme inducers, and CYP inhibitors. Please refer to clinical pharmacology review for up-to-date labeling recommendations.

4.4.9 GS-US-367-1657 DDI ARVs

1. Title

Phase 1 Multiple Dose Study to Evaluate the Pharmacokinetic Drug-Drug Interaction Potential between Sofosbuvir/Velpatasvir/GS-9857 Fixed-Dose Combination and HIV Antiretrovirals in Healthy Subjects

2. Information Regarding the Clinical Trial Site and Duration of the Trial

The trial was conducted (b) (4) from January 21, 2016 to April 22, 2016, with the final report date of September 13, 2016.

3. Objectives

Primary objectives:

- To evaluate the pharmacokinetics (PK) of sofosbuvir (SOF), its metabolites GS-566500 and GS-331007, velpatasvir (VEL), and voxilaprevir (VOX; GS-9857) upon administration of SOF/VEL/VOX fixed-dose combination (FDC) with emtricitabine/rilpivirine/tenofovir alafenamide (FTC/RPV/TAF) FDC,

elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (EVG/COBI/FTC/TAF) FDC, or darunavir (DRV)+ritonavir (RTV)+emtricitabine/tenofovir disoproxil fumarate (FTC/TDF) FDC

- To evaluate the PK of COBI, DRV, EVG, FTC, RPV, RTV, TAF, and tenofovir (TFV) upon administration of SOF/VEL/VOX with FTC/RPV/TAF, EVG/COBI/FTC/TAF, or DRV+RTV+FTC/TDF

Secondary objective:

- To evaluate the safety and tolerability of SOF/VEL/VOX and FTC/RPV/TAF, EVG/COBI/FTC/TAF, and DRV+RTV+FTC/TDF administered alone or in combination

4. Trial Design

This Phase 1, randomized, open-label, multiple-dose, single-center, 3-cohort study evaluated the drug-drug interaction (DDI) potential between SOF/VEL/VOX and FTC/RPV/TAF, EVG/COBI/FTC/TAF, or DRV+RTV+FTC/TDF in healthy subjects. For each cohort, following completion of screening and Day-1 assessments, subjects were randomized to 1 of 6 sequences and received study treatments starting on Day 1.

Cohort 1:

Subjects were randomized to 1 of 6 sequences (ABC, BCA, CAB, CBA, ACB, or BAC) and received the following treatments:

- Treatment A: SOF/VEL/VOX FDC (1 × 400/100/100 mg) + VOX (1 × 100 mg) administered orally in the morning with food once daily for 10 days
- Treatment B: SOF/VEL/VOX FDC (1 × 400/100/100 mg) + VOX (1 × 100 mg) +FTC/RPV/TAF FDC (1 × 200/25/25 mg) administered orally in the morning with food once daily for 10 days
- Treatment C: FTC/RPV/TAF FDC (1 × 200/25/25 mg) administered orally in the morning with food once daily for 10 days

Cohort 2:

Subjects were randomized to 1 of 6 sequences (DEF, EFD, FDE, FED, DFE, or EDF) and received the following treatments:

- Treatment D: SOF/VEL/VOX FDC (1 × 400/100/100 mg) + VOX (1 × 100 mg) administered orally in the morning with food once daily for 10 days
- Treatment E: SOF/VEL/VOX FDC (1 × 400/100/100 mg) + VOX (1 × 100 mg) + EVG/COBI/FTC/TAF FDC (1 × 150/150/200/10 mg) administered orally in the morning with food once daily for 10 days
- Treatment F: EVG/COBI/FTC/TAF FDC (1 × 150/150/200/10 mg) administered orally in the morning with food once daily for 10 days

Cohort 3:

Subjects were randomized to 1 of 6 sequences (GHI, HIG, IGH, IHG, GIH, or HGI) and received the following treatments:

- Treatment G: SOF/VEL/VOX FDC (1 × 400/100/100 mg) + VOX (1 × 100 mg) administered orally in the morning with food once daily for 10 days
- Treatment H: SOF/VEL/VOX FDC (1 × 400/100/100 mg) + VOX (1 × 100 mg) + DRV (1 × 800 mg) + RTV (1 × 100 mg) + FTC/TDF FDC (1 × 200/300 mg) administered orally in the morning with food once daily for 10 days
- Treatment I: DRV (1 × 800 mg) + RTV (1 × 100 mg) + FTC/TDF FDC (1 × 200/300 mg) administered orally in the morning with food once daily for 10 days

Eligible subjects were healthy males and non-pregnant, non-lactating females 18 to 45 years of age (inclusive), with body mass index (BMI) 19.0 to 30.0 kg/m^2 (inclusive), normal 12-lead electrocardiogram (ECG) or one without clinically significant abnormalities as assessed by the investigator, normal renal function (creatinine clearance [CLcr] ≥ 80 mL/min as determined by the Cockcroft-Gault method), and no significant medical history.

5. Excluded Medications and Restrictions

- Any prescription medications and OTC medications including herbal products and antacids with the exception of vitamins, and/or acetaminophen and/or ibuprofen and/or hormonal contraceptive medications. However, the short-term use of topical hydrocortisone cream or A&D ointment to treat minor skin irritation due to ECG leads was allowed.
- Subjects were required to refrain from the consumption of food and beverages containing alcohol products, and from consumption of grapefruit juice, grapefruits, and Seville orange juice 72 hours prior to the first dose of study drug and during the course of the study through discharge.
- Subjects were required to refrain from the use of nicotine or nicotine-containing products from screening through discharge.

6. Rationale for Doses Used in the Trial

FTC/RPV/TAF: FDC at 200/25/25 mg once daily with a meal is the recommended dose in the Odefsey® (emtricitabine, rilpivirine, and tenofovir alafenamide) USPI. The elimination half-life values for FTC/RPV/TAF are 10/50/0.51 hours, respectively, and thus the duration of 9 days is sufficient for reaching steady state when co-administered with SOF/VEL/VOX+VOX in Treatment B.

EVG/COBI/FTC/TAF: FDC at 150/150/200/10 mg once daily with food is the recommended dose in the Genvoya® (elvitegravir, cobicistat, emtricitabine, and tenofovir

alafenamide) USPI. The elimination half-life values for EVG/COBI/FTC/TAF are 12.9/3.5/10/0.51 hours, and thus the duration of 9 days is sufficient for reaching steady state when co-administered with SOF/VEL/VOX+VOX in Treatment E.

DRV+RTV+FTC/TDF: DRV at 800 mg + RTV 100 mg once daily with food is the recommended dose in the Prezista® (darunavir) USPI; FTC/TDF FDC at 200/300 mg once daily with or without food is the recommended dose in the Truvada® (emtricitabine/tenofovir disoproxil fumarate) USPI. The elimination half-life values for DRV/RTV/FTC/TDF are 15/3/10/17 hours, respectively, and thus the duration of 9 days is sufficient for reaching steady state when co-administered with SOF/VEL/VOX+VOX in Treatment H.

SOF/VEL/VOX+VOX: 400/100/100+100 mg has been found to provide similar (for SOF and its metabolite GS-331007) or higher exposure (for VEL and VOX) compared to clinical efficacious exposure under fed conditions. The elimination half-life values for SOF/VEL/VOX are 0.5/17/30 hours, respectively, and thus the duration of 9 days is sufficient for reaching steady state in Treatments A, D, and G, and also enough for washout in Treatments C, F and I.

7. Drugs Used in the Trial

SOF/VEL/VOX FDC 400/100/100 mg tablets – Batch Number(s): ER1501B2 (Manufactured by Gilead Sciences)
VOX 100 mg modified formulation individual-agent tablets – Batch Number(s): DY1502B1 (Manufactured by (b) (4)
FTC/RPV/TAF FDC 200/25/25 mg tablets – Batch Number(s): EF1506B1 (Manufactured by (b) (4)
EVG/COBI/FTC/TAF FDC 150/150/200/10 mg tablets – Batch Number(s): CP1406B1 (Manufactured by Patheon)
DRV 800 mg tablets – Batch Number(s): 15GG397 (Manufactured by Janssen Ortho LLC)
RTV 100 mg tablets – Batch Number(s): 1041437 (Manufactured by Abbvie)
FTC/TDF 200/300 mg FDC tablets – Batch Number(s): 004483 (Manufactured by Gilead Sciences)

8. Sample Collection, Bioanalysis, Pharmacokinetic Assessments, and Statistical Analysis

Sample Collection
Serial blood samples were collected pre-dose and at pre-specified time points through 24 hours post-dose on Days 10, 20, and 30: pre-dose (≤ 5 min) and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, and 24 hours post-dose.

Bioanalytical method

- Validation and Bioanalytical Reports:

Analyte	Matrix	Validation Report	Bioanalytical Report
GS-7977 (SOF), GS-331007	Plasma	(b) (4) 60-1323 Amendment 5	(b) (4) 60-1605A
GS-5816 (VEL)	Plasma	(b) (4) 60-1393 Amendment 2	(b) (4) 60-1605B
GS-9857 (VOX)	Plasma	(b) (4) 8109.123113.1	(b) (4) 10441.112517
GS-7340 (Tenofovir alafenamide)	Plasma	(b) (4) 60-1578 Amendment 1	(b) (4) 60-1605C
Tenofovir	Plasma	(b) (4) 60-1435 Amendment 3, (b) (4) 42-1410	(b) (4) 60-1605D, 60-1605E
Emtricitabine	Plasma	42-1410	(b) (4) 60-1605E
Rilpivirine	Plasma	42-1408	60-1605F
Darunavir	Plasma	42-0902 Amendment 4	60-1605H
Ritonavir	Plasma	(b) (4) 42-0830 Amendment 5	60-1605I
GS-9137 (Elvitegravir), GS-9350(Cobicistat)	Plasma	(b) (4) 60-1343 Amendment 2	(b) (4) 60-1605G

- Bioanalytical Method:

Method type: LC/MS/MS; Matrix: K_2EDTA plasma

Analyte	Range (ng/mL)	Stability in frozen matrix (days)
GS-7977 (SOF)	5 to 2500	174 at −20°C and 813 at −70°C
GS-331007	10 to 5000	174 at −20°C and 813 at −70°C
GS-5816 (VEL)	1 to 1000	1302 at −20°C and 1315 at −70°C
GS-9857 (VOX)	0.5 to 1000	420 at −20ºC and at −70ºC
GS-7340 (Tenofovir alafenamide)	1 to 1000	520 at −70°C
Tenofovir	0.3 to 300, 5 to 3000	464 at −20°C and at −70°C, 190 at −20°C and 340 at −70°C
Emtricitabine	5 to 3000	190 at −20°C and 340 at −70°C
Rilpivirine	1 to 500	783 at −20°C and −70°C
Darunavir	20 to 10,000	1635 at −20°C and −70°C
Ritonavir	5 to 2500	721 at −70°C

GS-9137 (Elvitegravir)	20 to 10,000	585 at −70ºC
GS-9350(Cobicistat)	5 to 2500	121 at −10 to −30°C, 1297 at −60 to −80°C

Validation	▪ Method validated prior to use	☒ Yes ☐ No ☐ NA
	▪ Method validation acceptable	☒ Yes ☐ No ☐ NA
Study Samples Analysis	▪ Samples analyzed within the established stability period	☒ Yes ☐ No
	▪ Quality control samples range acceptable	☒ Yes ☐ No
	▪ Chromatograms provided	☒ Yes ☐ No
	▪ Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	▪ Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	▪ Incurred samples analysis is acceptable	☒ Yes ☐ No
	▪ Overall performance acceptable	☒ Yes ☐ No
Inspection	▪ Will the bioanalytical site be inspected	☐ Yes ☒ No

Pharmacokinetic Assessments
The following multiple-dose plasma PK parameters for VOX, VEL, SOF and its metabolites GS-331007, FTC, RPV, EVG, COBI, DRV, RTV, TAF, and TFV were calculated: AUC_{last}, AUC_{tau}, C_{max}, C_{last}, C_{tau}, T_{max}, T_{last}, λ_z, CL_{ss}/F, V_z/F, and $t_{1/2}$, as appropriate.

Statistical Analysis
An analysis of variance was fitted to the natural logarithmic transformation of primary PK parameters (AUC_{tau}, AUC_{last}, C_{max}, and C_{tau}, as applicable) for each analyte within each cohort. A 2-sided 90% confidence interval (CI) for the ratio of geometric least-squares means (GLSMs) of test versus reference treatments was calculated for each of the primary PK parameters by analyte.

9. Results

9.1 Subject Demographics and Disposition
A total of 90 subjects were randomized in Cohorts 1, 2, and 3 (30 subjects per cohort). A total of 86 of 90 subjects (95.6%) completed the study. 4 subjects (4.4%) prematurely discontinued from the study due to withdrawal of consent.
Overall, the mean age was 34 years (range: 19 to 45). The majority of subjects were male (56 subjects, 62.2%), white (66 subjects, 73.3%), and Hispanic or Latino (73 subjects, 81.1%). The majority of subjects were black or African American (62.2%; 61 subjects) and non-Hispanic or Latino (82.7%; 81 subjects).

9.2 Pharmacokinetic and Statistical Analysis
An overall summary of DDI results from Cohorts 1 to 3 is presented in **Table 1**. The PK parameters and statistical comparison of VOX, VEL, SOF, GS-331007, FTC, RPV, EVG, COBI, DRV, RTV, TAF, and TFV are presented in **Table 2 to 13**, respectively.

Effects of ARVs on the exposure of SOF, VEL, and VOX

SOF/VEL/VOX+VOX and FTC/RPV/TAF
No alteration in the exposure (AUC_{tau}, C_{max}, or C_{tau} [as applicable]) of VOX, VEL, SOF, or GS-331007 was observed following administration of SOF/VEL/VOX+VOX with FTC/RPV/TAF.

SOF/VEL/VOX+VOX and EVG/COBI/FTC/TAF
EVG/COBI/FTC/TAF caused a 171%, 92%, and 350% higher voxilaprevir AUC_{tau}, C_{max}, and C_{tau}, respectively, compared to administration of SOF/VEL/VOX+VOX alone. No clinically relevant exposure parameters for sofosbuvir or velpatasvir were altered as compared with SOF/VEL/VOX+VOX alone. Although GS-331007 AUC_{tau} was 43% higher, this increase is unlikely to be clinically significant.

SOF/VEL/VOX+VOX and DRV+RTV+FTC/TDF
Voxilaprevir AUC_{tau}, C_{max}, and C_{tau} were 143%, 72%, and 300% higher, respectively, following administration of SOF/VEL/VOX+VOX with DRV+RTV+FTC/TDF compared with SOF/VEL/VOX+VOX alone. There were no changes in VEL, SOF, or GS-331007 exposure (AUC_{tau}, C_{max}, or C_{tau} [VEL only]) following administration of SOF/VEL/VOX+VOX with DRV+RTV+FTC/TDF compared with SOF/VEL/VOX+VOX alone, except for SOF C_{max} (30% lower).

Effect of SOF/VEL/VOX+VOX on the exposure of ARVs

FTC/RPV/TAF and SOF/VEL/VOX+VOX
Administration of SOF/VEL/VOX+VOX with FTC/RPV/TAF did not alter the exposures of FTC or RPV compared with FTC/RPV/TAF alone. However, mean TAF and TFV exposures were between 32% and 89% higher as compared with FTC/RPV/TAF alone.

EVG/COBI/FTC/TAF and SOF/VEL/VOX+VOX
Following administration of SOF/VEL/VOX+VOX, no significant change in exposures was observed for elvitegravir, TAF, tenofovir, or emtricitabine as compared with administration of EVG/COBI/FTC/TAF alone. Cobicistat AUC_{tau} and C_{tau} were 50% and 250% higher, respectively.

DRV+RTV+FTC/TDF and SOF/VEL/VOX+VOX
Following administration of SOF/VEL/VOX+VOX, no clinically significant change in exposures was observed for ritonavir and emtricitabine. However, darunavir C_{tau} was 34% lower, and exposures of TFV (from TDF) were higher (AUC_{tau}: 39%, C_{max}: 48%, C_{tau}: 47%) following administration of SOF/VEL/VOX+VOX with DRV+RTV+FTC/TDF compared with DRV+RTV+FTC/TDF alone. Despite the lower

trough concentrations, DRV C_{tau} remained > 30-fold higher than the protein-adjusted DRV half-maximal effective concentration value for HIV virus with no DRV resistance-associated mutations (55 ng/mL), according to the sponsor.

Table 1: Summary of DDI Results in Cohorts 1 to 3 for VOX, VEL, SOF, GS-566500, GS-331007, FTC, RPV, EVG, COBI, DRV, RTV, TAF, and TFV.

SOF/VEL/VOX+VOX + ARVs / SOF/VEL/VOX+VOX					
Cohort 1: FTC/RPV/TAF	**VOX**	**VEL**	**SOF**	**GS-566500**	**GS-331007**
AUC_{tau}	↔	↔	↔	↔	↔
C_{max}	↔	↔	↔	↔	↔
C_{tau}	↔	↔	NA	NA	NA
Cohort 2: EVG/COBI/FTC/TAF	**VOX**	**VEL**	**SOF**	**GS-566500**	**GS-331007**
AUC_{tau}	↑171%	↔	↔	↔	↑43%
C_{max}	↑92%	↔	↑27%	↔	↔
C_{tau}	↑350%	↑46%	NA	NA	NA
Cohort 3: DRV+RTV+FTC/TDF	**VOX**	**VEL**	**SOF**	**GS-566500**	**GS-331007**
AUC_{tau}	↑143%	↔	↔	↔	↔
C_{max}	↑72%	↔	↓30%	↔	↔
C_{tau}	↑300%	↔	NA	NA	NA

SOF/VEL/VOX+VOX + ARVs / ARVs				
Cohort 1: FTC/RPV/TAF	**FTC**	**RPV**	**TAF**	**TFV**
AUC_{tau}[a]	↔	↔	↑52%	↑79%
C_{max}	↔	↔	↑32%	↑64%
C_{tau}	↔	↔	NA	↑89%

Cohort 2: EVG/COBI/FTC/TAF	**EVG**	**COBI**	**FTC**	**TAF**	**TFV**
AUC_{tau}[a]	↔	↑50%	↔	↔	↔
C_{max}	↔	↔	↔	↓21%	↔
C_{tau}	↑32%	↑250%	↔	NA	↔

Cohort 3: DRV+RTV+FTC/TDF	**DRV**	**RTV**	**FTC**	**TFV**
AUC_{tau}	↔	↑45%	↔	↑39%
C_{max}	↔	↑60%	↔	↑48%
C_{tau}	↓34%	↔	↔	↑47%

NA = not applicable

a AUC_{last} is presented for TAF.

The 90% CIs of the %GLSM ratios for test versus reference treatments were within (↔), extended above (↑), or extended below (↓) the predefined lack of PK alteration boundaries of 70% to 143%. Percentages indicate the increase or decrease in the geometric mean for the respective PK parameter for test treatment relative to reference treatment.

Table 2: Plasma PK Parameters and Statistical Comparisons for VOX.

VOX PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	SOF/VEL/VOX+VOX (Reference) (N=30)	SOF/VEL/VOX+VOX + FTC/RPV/TAF (Test) (N=30)	
AUC_{tau} (h*ng/mL)	5322.4 (69.4)	4957.2 (62.4)	93.93 (83.85, 105.21)
C_{max} (ng/mL)	1086.6 (63.8)	1044.4 (58.8)	96.47 (83.60, 111.31)
C_{tau} (ng/mL)	26.5 (59.7)	27.9 (69.7)	101.78 (92.44, 112.07)
T_{max} (h)[a]	5.00 (4.00, 5.00)	5.00 (4.00, 5.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a]	6.91 (5.95, 7.94)	7.10 (6.35, 7.91)	—
CL_{ss}/F (mL/h)	57138.9 (70.0)	61898.9 (77.0)	—

VOX PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	SOF/VEL/VOX+VOX (Reference) (N=28)	SOF/VEL/VOX+VOX + EVG/COBI/FTC/TAF (Test) (N=29)	
AUC_{tau} (h*ng/mL)	5837.5 (88.3)	14139.8 (60.7)	271.09 (230.33, 319.06)
C_{max} (ng/mL)	1226.3 (76.0)	2057.3 (49.0)	191.87 (162.92, 225.98)
C_{tau} (ng/mL)	30.2 (85.7)	134.5 (77.8)	450.11 (368.24, 550.19)
T_{max} (h)[a]	5.00 (4.00, 5.00)	5.00 (5.00, 5.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a]	6.74 (5.94, 7.66)	6.68 (6.31, 7.64)	—
CL_{ss}/F (mL/h)	53800.4 (61.2)	19413.9 (55.6)	—

VOX PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	SOF/VEL/VOX+VOX (Reference) (N=29)	SOF/VEL/VOX+VOX + DRV+RTV+FTC/TDF (Test) (N=29)	
AUC_{tau} (h*ng/mL)	3887.8 (67.3)	10101.1 (84.3)	243.19 (215.14, 274.90)
C_{max} (ng/mL)	833.4 (83.8)	1476.7 (86.9)	172.38 (151.23, 196.50)
C_{tau} (ng/mL)	21.1 (47.2)	102.7 (106.6)	399.59 (343.75, 464.50)
T_{max} (h)[a]	5.00 (4.00, 5.00)	5.00 (4.00, 5.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a]	6.94 (6.04, 7.93)	7.01 (6.53, 7.91)	—
CL_{ss}/F (mL/h)	71598.0 (53.1)	30717.3 (55.1)	—

a Values are presented as median (Q1, Q3).

Table 3: Plasma PK Parameters and Statistical Comparisons for VEL.

VEL PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	SOF/VEL/VOX+VOX (Reference) (N=30)	SOF/VEL/VOX+VOX + FTC/RPV/TAF (Test) (N=30)	
AUC_{tau} (h*ng/mL)	8334.8 (33.7)	8448.2 (36.6)	100.58 (94.21, 107.38)
C_{max} (ng/mL)	974.4 (26.3)	1065.4 (41.1)	105.43 (96.21, 115.53)
C_{tau} (ng/mL)	144.5 (47.3)	148.1 (53.6)	101.45 (94.59, 108.81)
T_{max} (h)[a]	4.00 (3.00, 4.00)	4.00 (3.00, 5.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a]	11.49 (10.82, 13.61)	13.09 (11.31, 15.81)	—
CL_{ss}/F (mL/h)	13493.6 (37.9)	13474.8 (37.2)	—

VEL PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	SOF/VEL/VOX+VOX (Reference) (N=28)	SOF/VEL/VOX+VOX + EVG/COBI/FTC/TAF (Test) (N=29)	
AUC_{tau} (h*ng/mL)	8863.9 (47.6)	10074.8 (40.2)	116.31 (106.26, 127.30)
C_{max} (ng/mL)	1030.4 (33.8)	980.3 (31.3)	96.02 (88.57, 104.08)
C_{tau} (ng/mL)	151.7 (71.0)	205.8 (49.9)	146.00 (130.15, 163.79)
T_{max} (h)[a]	4.00 (4.00, 4.50)	4.00 (4.00, 5.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a]	11.71 (10.03, 14.23)	14.05 (11.21, 15.34)	—
CL_{ss}/F (mL/h)	13297.2 (36.7)	11672.3 (44.0)	—

VEL PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	SOF/VEL/VOX+VOX (Reference) (N=29)	SOF/VEL/VOX+VOX + DRV+RTV+FTC/TDF (Test) (N=29)	
AUC_{tau} (h*ng/mL)	6836.1 (28.6)	6458.8 (29.6)	94.64 (87.89, 101.90)
C_{max} (ng/mL)	845.5 (22.1)	660.7 (22.0)	78.21 (72.55, 84.30)
C_{tau} (ng/mL)	111.3 (42.5)	129.2 (43.7)	115.95 (106.71, 125.99)
T_{max} (h)[a]	4.00 (3.00, 4.00)	4.00 (4.00, 5.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a]	10.98 (9.92, 12.80)	13.28 (10.57, 15.23)	—
CL_{ss}/F (mL/h)	16025.7 (34.0)	16874.8 (30.1)	—

a Values are presented as median (Q1, Q3).

Table 4: Plasma PK Parameters and Statistical Comparisons for SOF.

	Mean (% CV)		
	SOF/VEL/VOX+VOX (Reference)	SOF/VEL/VOX+VOX + FTC/RPV/TAF (Test)	%GLSM Ratio (90% CI) Test/Reference
SOF PK Parameter	**(N=30)**	**(N=30)**	—
AUC_{tau} (h*ng/mL)	3256.7 (30.6)	3352.2 (35.7)	101.16 (96.74, 105.79)
C_{max} (ng/mL)	1755.8 (33.6)	1747.8 (41.2)	95.46 (86.41, 105.46)
T_{max} (h)[a]	1.50 (1.50, 2.00)	2.00 (1.50, 3.00)	—
T_{last} (h)[a]	6.00 (5.00, 6.00)	6.00 (6.00, 6.00)	—
$t_{1/2}$ (h)[a]	0.47 (0.43, 0.54)	0.49 (0.46, 0.63)	—
CL_{ss}/F (mL/h)	133324.5 (28.3)	134613.6 (37.1)	—

	Mean (% CV)		
	SOF/VEL/VOX+VOX (Reference)	SOF/VEL/VOX+VOX + EVG/COBI/FTC/TAF (Test)	%GLSM Ratio (90% CI) Test/Reference
SOF PK Parameter	**(N=28)**	**(N=29)**	—
AUC_{tau} (h*ng/mL)	3248.6 (39.0)	3924.7 (35.3)	121.58 (112.36, 131.57)
C_{max} (ng/mL)	1528.8 (41.7)	1916.5 (40.2)	127.28 (109.38, 148.10)
T_{max} (h)[a]	2.00 (1.75, 3.00)	2.00 (1.50, 3.00)	—
T_{last} (h)[a]	6.00 (6.00, 8.00)	6.00 (6.00, 8.00)	—
$t_{1/2}$ (h)[a]	0.54 (0.47, 0.63)	0.58 (0.54, 0.63)	—
CL_{ss}/F (mL/h)	139098.6 (32.9)	115777.6 (43.2)	—

	Mean (% CV)		
	SOF/VEL/VOX+VOX (Reference)	SOF/VEL/VOX+VOX + DRV+RTV+FTC/TDF (Test)	%GLSM Ratio (90% CI) Test/Reference
SOF PK Parameter	**(N=29)**	**(N=29)**	—
AUC_{tau} (h*ng/mL)	2660.2 (24.7)	2058.9 (24.8)	77.58 (72.77, 82.70)
C_{max} (ng/mL)	1447.9 (40.8)	981.8 (30.9)	69.85 (62.26, 78.36)
T_{max} (h)[a]	2.00 (1.50, 2.00)	2.00 (1.50, 2.00)	—
T_{last} (h)[a]	6.00 (6.00, 8.00)	6.00 (6.00, 8.00)	—
$t_{1/2}$ (h)[a]	0.52 (0.48, 0.63)	0.59 (0.52, 0.75)	—
CL_{ss}/F (mL/h)	158342.7 (22.0)	205965.2 (24.9)	—

a Values are presented as median (Q1, Q3).

Table 5: Plasma PK Parameters and Statistical Comparisons for GS-331007.

	Mean (% CV)		
	SOF/VEL/VOX+VOX (Reference)	SOF/VEL/VOX+VOX + FTC/RPV/TAF (Test)	%GLSM Ratio (90% CI) Test/Reference
GS-331007 PK Parameter	**(N=30)**	**(N=30)**	—
AUC_{tau} (h*ng/mL)	10356.7 (18.3)	10776.1 (20.7)	103.54 (101.40, 105.73)
C_{max} (ng/mL)	838.5 (16.7)	854.5 (18.4)	101.63 (97.70, 105.71)
T_{max} (h)[a]	4.00 (3.00, 4.00)	4.00 (3.00, 4.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a]	27.75 (22.98, 32.37)	30.57 (24.40, 35.83)	—

	Mean (% CV)		
	SOF/VEL/VOX+VOX (Reference)	SOF/VEL/VOX+VOX + EVG/COBI/FTC/TAF (Test)	%GLSM Ratio (90% CI) Test/Reference
GS-331007 PK Parameter	**(N=28)**	**(N=29)**	—
AUC_{tau} (h*ng/mL)	11229.9 (19.5)	16142.0 (24.7)	143.23 (139.28, 147.29)
C_{max} (ng/mL)	876.4 (16.2)	1124.5 (18.5)	128.30 (124.68, 132.02)
T_{max} (h)[a]	4.00 (4.00, 4.50)	4.00 (4.00, 5.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a]	27.26 (24.06, 30.82)	28.61 (26.16, 36.61)	—

	Mean (% CV)		
	SOF/VEL/VOX+VOX (Reference)	SOF/VEL/VOX+VOX + DRV+RTV+FTC/TDF (Test)	%GLSM Ratio (90% CI) Test/Reference
GS-331007 PK Parameter	**(N=29)**	**(N=29)**	—
AUC_{tau} (h*ng/mL)	10828.6 (19.1)	12542.0 (20.8)	115.43 (111.75, 119.24)
C_{max} (ng/mL)	882.2 (18.5)	937.7 (23.3)	105.58 (101.40, 109.94)
T_{max} (h)[a]	4.00 (3.00, 5.00)	4.00 (4.00, 5.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a]	31.54 (27.09, 43.31)	24.64 (22.16, 32.91)	—

a Values are presented as median (Q1, Q3).

Table 6: Plasma PK Parameters and Statistical Comparisons for FTC.

FTC PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	FTC/RPV/TAF (Reference) (N=30)	SOF/VEL/VOX+VOX + FTC/RPV/TAF (Test) (N=30)	
AUC_{tau} (h*ng/mL)	10368.0 (18.9)	9648.2 (19.8)	92.86 (89.92, 95.89)
C_{max} (ng/mL)	1940.7 (22.6)	1709.3 (25.8)	87.84 (83.03, 92.94)
C_{tau} (ng/mL)	66.8 (22.4)	72.5 (27.9)	107.30 (101.41, 113.53)
T_{max} (h)[a]	2.00 (2.00, 3.00)	3.00 (2.00, 3.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a]	8.23 (7.74, 9.47)	8.81 (8.00, 9.70)	—

FTC PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	EVG/COBI/FTC/TAF (Reference) (N=28)	SOF/VEL/VOX+VOX + EVG/COBI/FTC/TAF (Test) (N=29)	
AUC_{tau} (h*ng/mL)	12469.6 (19.6)	12020.8 (20.4)	96.40 (94.02, 98.85)
C_{max} (ng/mL)	2053.6 (21.0)	1791.7 (16.5)	87.45 (83.76, 91.30)
C_{tau} (ng/mL)	90.1 (27.5)	104.6 (32.7)	114.36 (109.21, 119.75)
T_{max} (h)[a]	3.00 (2.00, 3.00)	3.00 (2.00, 3.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a]	7.74 (7.46, 8.48)	8.24 (7.64, 9.05)	—

FTC PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	DRV+RTV+FTC/TDF (Reference) (N=28)	SOF/VEL/VOX+VOX + DRV+RTV+FTC/TDF (Test) (N=29)	
AUC_{tau} (h*ng/mL)	9766.3 (17.2)	9807.4 (20.8)	99.27 (96.05, 102.61)
C_{max} (ng/mL)	1705.4 (23.2)	1524.1 (28.5)	87.58 (81.68, 93.91)
C_{tau} (ng/mL)	77.3 (32.4)	91.8 (26.6)	120.18 (114.78, 125.83)
T_{max} (h)[a]	3.00 (2.00, 4.00)	3.00 (3.00, 4.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a]	8.31 (7.84, 8.79)	8.83 (8.15, 9.44)	—

a Values are presented as median (Q1, Q3).

Table 7: Plasma PK Parameters and Statistical Comparisons for RPV.

RPV PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	FTC/RPV/TAF (Reference) (N=30)	SOF/VEL/VOX+VOX + FTC/RPV/TAF (Test) (N=30)	
AUC_{tau} (h*ng/mL)	3387.8 (30.5)	2780.1 (37.7)	80.37 (76.37, 84.58)
C_{max} (ng/mL)	246.0 (27.8)	195.3 (31.5)	78.72 (74.07, 83.66)
C_{tau} (ng/mL)	127.4 (37.4)	107.6 (47.9)	81.96 (77.29, 86.91)
T_{max} (h)[a]	4.00 (4.00, 4.00)	4.00 (4.00, 4.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a,b]	41.15 (28.63, 44.72)	49.98 (29.95, 62.11)	—

a Values are presented as median (Q1, Q3).
b N = 13 for reference treatment and N = 10 for test treatment.

Table 8: Plasma PK Parameters and Statistical Comparisons for EVG.

EVG PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	EVG/COBI/FTC/TAF (Reference) (N=28)	SOF/VEL/VOX+VOX + EVG/COBI/FTC/TAF (Test) (N=29)	
AUC_{tau} (h*ng/mL)	27459.0 (24.6)	25930.3 (30.7)	93.95 (88.08, 100.21)
C_{max} (ng/mL)	2381.8 (23.7)	1896.2 (28.8)	79.47 (74.69, 84.57)
C_{tau} (ng/mL)	536.9 (44.5)	715.5 (43.4)	132.10 (116.95, 149.23)
T_{max} (h)[a]	4.00 (4.00, 5.00)	5.00 (4.00, 5.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a,b]	10.53 (8.71, 12.22)	13.42 (12.33, 16.60)	—

a Values are presented as median (Q1, Q3).
b N = 28 for reference and test treatments.

Table 9: Plasma PK Parameters and Statistical Comparisons for COBI.

COBI PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	EVG/COBI/FTC/TAF (Reference) (N=28)	SOF/VEL/VOX+VOX + EVG/COBI/FTC/TAF (Test) (N=29)	
AUC_{tau} (h*ng/mL)	12419.5 (32.7)	18844.7 (33.3)	150.42 (143.62, 157.55)
C_{max} (ng/mL)	1657.1 (27.5)	2039.0 (24.0)	123.15 (118.21, 128.29)
C_{tau} (ng/mL)	34.2 (68.5)	136.2 (91.1)	350.17 (301.01, 407.36)
T_{max} (h)[a]	3.00 (3.00, 4.00)	4.00 (3.00, 5.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a]	3.36 (2.92, 3.78)	4.27 (3.87, 5.57)	—

a Values are presented as median (Q1, Q3).

Table 10: Plasma PK Parameters and Statistical Comparisons for DRV.

DRV PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	DRV+RTV+FTC/TDF (Reference) (N=28)	SOF/VEL/VOX+VOX + DRV+RTV+FTC/TDF (Test) (N=29)	
AUC_{tau} (h*ng/mL)	86623.4 (27.8)	74667.0 (32.3)	85.54 (80.70, 90.67)
C_{max} (ng/mL)	7877.9 (24.0)	7011.7 (25.3)	89.33 (85.28, 93.58)
C_{tau} (ng/mL)	2429.3 (36.0)	1723.2 (59.1)	65.54 (57.72, 74.41)
T_{max} (h)[a]	4.00 (3.00, 4.00)	4.00 (4.00, 5.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a,b]	17.17 (15.95, 21.74)	14.32 (10.41, 19.07)	—

a Values are presented as median (Q1, Q3).
b N = 12 for reference treatment and N = 19 for test treatment.

Table 11: Plasma PK Parameters and Statistical Comparisons for RTV.

RTV PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	DRV+RTV+FTC/TDF (Reference) (N=28)	SOF/VEL/VOX+VOX + DRV+RTV+FTC/TDF (Test) (N=29)	
AUC_{tau} (h*ng/mL)	4002.7 (36.6)	5729.9 (33.6)	145.31 (134.77, 156.67)
C_{max} (ng/mL)	595.0 (35.7)	944.3 (36.6)	160.43 (147.20, 174.84)
C_{tau} (ng/mL)	37.8 (50.1)	30.9 (56.0)	80.03 (71.65, 89.39)
T_{max} (h)[a]	4.50 (4.00, 5.00)	4.00 (4.00, 5.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a]	6.33 (5.89, 7.66)	4.90 (4.72, 5.50)	—

a Values are presented as median (Q1, Q3).

Table 12: Plasma PK Parameters and Statistical Comparisons for TAF.

TAF PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	FTC/RPV/TAF (Reference) (N=30)	SOF/VEL/VOX+VOX + FTC/RPV/TAF (Test) (N=30)	
AUC_{last} (h*ng/mL)	286.7 (38.0)	437.3 (36.8)	151.75 (143.40, 160.60)
C_{max} (ng/mL)	212.5 (54.1)	276.9 (43.5)	131.57 (117.07, 147.85)
T_{max} (h)[a]	1.50 (1.00, 2.00)	1.50 (1.00, 2.00)	—
T_{last} (h)[a]	5.00 (4.00, 5.00)	6.00 (5.00, 6.00)	—
$t_{1/2}$(h)[a]	0.38 (0.34, 0.44)	0.46 (0.40, 0.49)	—

TAF PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	EVG/COBI/FTC/TAF (Reference) (N=28)	SOF/VEL/VOX+VOX + EVG/COBI/FTC/TAF (Test) (N=29)	
AUC_{last} (h*ng/mL)	231.6 (32.6)	220.1 (34.1)	92.78 (84.92, 101.36)
C_{max} (ng/mL)	181.6 (43.7)	145.8 (44.3)	79.05 (67.61, 92.41)
T_{max} (h)[a]	1.50 (1.00, 2.00)	1.50 (1.00, 2.00)	—
T_{last} (h)[a]	4.00 (4.00, 5.00)	5.00 (4.00, 6.00)	—
$t_{1/2}$(h)[a]	0.40 (0.37, 0.48)	0.50 (0.41, 0.54)	—

a Values are presented as median (Q1, Q3).

Table 13: Plasma PK Parameters and Statistical Comparisons for TFV.

TFV PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	FTC/RPV/TAF (Reference) (N=30)	SOF/VEL/VOX+VOX + FTC/RPV/TAF (Test) (N=30)	
AUC_{tau} (h*ng/mL)	273.1 (20.5)	487.9 (20.2)	178.95 (172.75, 185.37)
C_{max} (ng/mL)	16.6 (19.6)	27.1 (16.7)	163.85 (157.66, 170.28)
C_{tau} (ng/mL)	9.2 (22.8)	17.3 (23.1)	188.74 (181.24, 196.57)
T_{max} (h)[a]	3.00 (3.00, 4.00)	3.00 (3.00, 4.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a]	34.04 (30.63, 38.84)	36.19 (32.45, 43.73)	—

TFV PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	EVG/COBI/FTC/TAF (Reference) (N=28)	SOF/VEL/VOX+VOX + EVG/COBI/FTC/TAF (Test) (N=29)	
AUC_{tau} (h*ng/mL)	320.5 (19.0)	385.0 (18.7)	120.00 (117.22, 122.85)
C_{max} (ng/mL)	19.3 (18.7)	21.2 (17.8)	109.20 (105.22, 113.34)
C_{tau} (ng/mL)	11.0 (23.2)	13.3 (21.7)	121.48 (117.54, 125.55)
T_{max} (h)[a]	3.00 (3.00, 3.00)	3.00 (2.00, 4.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a]	37.16 (32.62, 50.97)	37.30 (32.66, 48.48)	—

TFV PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	DRV+RTV+FTC/TDF (Reference) (N=28)	SOF/VEL/VOX+VOX + DRV+RTV+FTC/TDF (Test) (N=29)	
AUC_{tau} (h*ng/mL)	3337.7 (15.8)	4658.9 (20.2)	138.58 (131.60, 145.92)
C_{max} (ng/mL)	314.8 (19.7)	471.4 (26.6)	148.00 (135.80, 161.29)
C_{tau} (ng/mL)	61.1 (18.3)	90.1 (19.7)	147.02 (138.27, 156.33)
T_{max} (h)[a]	3.00 (2.00, 3.00)	3.00 (3.00, 3.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a]	10.01 (9.12, 10.66)	11.18 (10.28, 11.94)	—

a Values are presented as median (Q1, Q3).

9.3 Safety Analysis

Once-daily dosing of FTC/RPV/TAF, EVG/COBI/FTC/TAF, DRV+RTV+FTC/TDF, or SOF/VEL/VOX+VOX, alone or in combination, for 10 days was generally safe and well tolerated. No Grade 3 or 4 AEs, SAEs, AEs leading to premature study drug discontinuation, or deaths were reported.

10. Sponsor's Conclusions

- No PK interactions warranting dose adjustment were observed following administration of SOF/VEL/VOX+VOX with FTC, RPV, TAF, or TDF.
- Antiretroviral regimens including PK boosters (COBI and RTV) modestly increased VOX exposure (AUC_{tau}: 143% to 171%). No clinically relevant changes in the PK of SOF or its metabolites, VEL, or ARVs were observed.

11. Reviewer's Assessment

The study design and the sponsor's conclusions are reasonable.

The reviewer agrees with the sponsor's proposal for the DDI management strategy, regarding to coadministration with FTC/RPV/TAF, EVG/COBI/FTC/TAF, DRV+RTV+FTC/TDF. Please refer to clinical pharmacology review for up-to-date labeling recommendations.

4.4.10 GS-US-367-1726 DDI with H2-Receptor Antagonist or PPI

1. Title

A Phase 1 Study to Evaluate the Pharmacokinetics of Sofosbuvir/Velpatasvir/GS-9857 Fixed-Dose Combination upon Administration with a Representative H_2-Receptor Antagonist or Proton Pump Inhibitor

2. Information Regarding the Clinical Trial Site and Duration of the Trial

The trial was conducted (b) (4) from October 5, 2015 to March 3, 2016, with the final report date of August 24, 2016.

3. Objectives

Primary objective:

- To evaluate the pharmacokinetics (PK) of sofosbuvir (SOF)/velpatasvir (VEL)/voxilaprevir (VOX; GS-9857) fixed-dose combination (FDC) upon co-administration with a representative H_2-receptor antagonist (H_2RA) or proton pump inhibitor (PPI)

Secondary objective:

- To evaluate the safety of administration of SOF/VEL/VOX FDC with an H_2RA or PPI

4. Trial Design

This was a Phase 1, randomized, open-label, single-center, 5-cohort, single- and multiple-dose study in healthy subjects that evaluated the PK of SOF/VEL/VOX upon coadministration with a representative H_2RA (famotidine) or PPI (omeprazole). The study was planned to include up to 5 cohorts, with Cohorts 3, 4, and 5 being adaptive and subject to results of Cohort 2. Cohorts 1 and 2 were conducted in parallel. Based on available safety and PK data from Cohort 2, and at the discretion of the sponsor and investigator, Cohort 3 was initiated. Cohorts 4 and 5 were not conducted, as Cohort 2 results did not support the initiation of these cohorts.

Eligible subjects were males and non-pregnant, non-lactating females, 18 to 45 years of age (inclusive), with a body mass index (BMI) of 19 to 30 kg/m2 (inclusive), 12-lead electrocardiogram (ECG) without clinically significant abnormalities, an estimated creatinine clearance (CLcr) (calculated using the Cockcroft-Gault equation) ≥ 80 mL/min, and no significant medical history.

Cohort 1

Subjects were randomized to 1 of 6 treatment sequences (6 subjects per sequence) to receive the following 3 study treatments with 6-day washout intervals in between, as follows:

- **Reference (Treatment A):** single dose of SOF/VEL/VOX FDC (400/100/100 mg) administered in the morning with food
- **Simultaneous H_2RA Administration (Treatment B):** single dose of SOF/VEL/VOX FDC (400/100/100 mg) administered simultaneously with single dose of famotidine 40 mg in the morning with food
- **12-Hour H_2RA Stagger (Treatment C)**: single dose of famotidine 40 mg administered in the evening with a standardized meal, 12 hours before single dose of SOF/VEL/VOX FDC (400/100/100 mg) administered in the morning with food

Cohort 2
Subjects were randomized to 1 of 2 treatment sequences (17 subjects per sequence) to receive the following 2 study treatments with 6-day washout intervals in between, as follows:

- **Reference (Treatment D):** single dose of SOF/VEL/VOX FDC (400/100/100 mg) administered in the morning with food
- **2-Hour PPI Stagger (Treatment E):** omeprazole 20 mg administered once daily in the morning for 6 days under fasted conditions followed by a single dose of SOF/VEL/VOX FDC (400/100/100 mg) administered with food 2 hours after omeprazole administration on the sixth day of omeprazole dosing

Cohort 3 (Adaptive)
Subjects were randomized to 1 of 2 treatment sequences (17 subjects per sequence) to receive the following 2 study treatments with 6-day washout intervals in between, as follows:

- **Reference (Treatment F):** single dose of SOF/VEL/VOX FDC (400/100/100 mg) administered in the morning with food
- **4-Hour PPI Stagger (Treatment G):** omeprazole 20 mg administered once daily 1 hour before lunch for 6 days; on the sixth day, a single dose of SOF/VEL/VOX FDC (400/100/100 mg) was administered with food 4 hours before the omeprazole dose

Cohorts 4 and 5
These two cohorts for administration of 40 mg omeprazole at 2 h before or 4 h after dosing of SOF/VEL/VOX FDC (400/100/100 mg) with food were not conducted.

5. Excluded Medications and Restrictions

- Any prescription medications and OTC medications including herbal products and antacids with the exception of vitamins, and/or acetaminophen and/or ibuprofen and/or hormonal contraceptive medications. However, the short-term use of topical

hydrocortisone cream or A&D ointment to treat minor skin irritation due to ECG leads was allowed.

- Subjects were required to refrain from the consumption of food and beverages containing alcohol products, and from consumption of grapefruit juice, grapefruits, and Seville orange juice 72 hours prior to the first dose of study drug and during the course of the study through discharge.
- Subjects were required to refrain from the use of nicotine or nicotine-containing products from screening through discharge.

6. Rationale for Doses Used in the Trial

Famotidine: 40 mg is the highest recommended dose in the Pepcid® (famotidine) USPI, dosed in the evening for basal and nocturnal gastric secretion suppression and dosed in the morning for food-stimulated acid secretion inhibition. Famotidine has a short elimination half-life at 2.5 to 3.5 hours and food has no clinically significant effect on its exposure, and thus a single dose of famotidine at 40 mg with food is reasonable.

Omeprazole: 20 mg and 40 mg are the recommended doses in the Prilosec® (omeprazole) USPI, dosed before a meal. The inhibitory effect of omeprazole on acid secretion increases with repeated once-daily dosing, reaching a plateau after four days. Dosing omeprazole at 20 mg or 40 mg before a meal for 5 days before co-administration on the sixth day for drug interaction is reasonable.

SOF/VEL/VOX FDC: the dose regimen of 400/100/100 mg once daily with food is the regimen evaluated in Phase 2 and 3 studies. Although VOX exposure is 3 to 4-fold higher in HCV-infected patients compared to healthy subjects, it is reasonable to use this dose since SOF/VEL/VOX FDC is the victim drug in this DDI study.

7. Drugs Used in the Trial

SOF/VEL/VOX 400/100/100mg FDC tablets – Batch Number(s): ER1501B2 (Manufactured by Gilead Sciences)
Famotidine 40 mg tablets – Batch Number(s): M974C40048 (Manufactured by Marathon Pharmaceuticals, LLC)
Omeprazole 20 mg delayed-release capsules – Batch Number(s): EC4546 (Manufactured by Lek Pharmaceuticals for Sandoz Inc)

8. Sample Collection, Bioanalysis, Pharmacokinetic Assessments, and Statistical Analysis

Sample Collection
Serial blood samples were collected relative to the dosing of SOF/VEL/VOX at the following time points: predose (≤ 5 minutes) and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, 96, and 120 hours postdose.

Bioanalytical method

- Validation and Bioanalytical Report:

Analyte	Matrix	Validation Report	Bioanalytical Report
GS-7977 (SOF), GS-331007	Plasma	(b) (4) 60-1323 Amendment 5	(b) (4) 60-1593A
GS-5816 (VEL)	Plasma	(b) (4) 60-1393 Amendment 2	(b) (4) 60-1593B
GS-9857 (VOX)	Plasma	(b) (4) 8109.123113.1	(b) (4) 10359.112515

- Bioanalytical Method:

Method type: LC/MS/MS; Matrix: K_2EDTA plasma

Analyte	Range (ng/mL)	Stability in frozen samples (days)
GS-7977 (SOF)	5 to 2500	174 at −20°C and 813 at −70°C
GS-331007	10 to 5000	174 at −20°C and 813 at −70°C
GS-5816 (VEL)	1 to 1000	1302 at −20°C and 1315 at −70°C
GS-9857 (VOX)	0.5 to 1000	420 at −20ºC and at −70ºC

Validation	▪ Method validated prior to use	☒ Yes ☐ No ☐ NA
	▪ Method validation acceptable	☒ Yes ☐ No ☐ NA
Study Samples Analysis	▪ Samples analyzed within the established stability period	☒ Yes ☐ No
	▪ Quality control samples range acceptable	☒ Yes ☐ No
	▪ Chromatograms provided	☒ Yes ☐ No
	▪ Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	▪ Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	▪ Incurred samples analysis is acceptable	☒ Yes ☐ No
	▪ Overall performance acceptable	☒ Yes ☐ No
Inspection	▪ Will the bioanalytical site be inspected	☐ Yes ☒ No

Pharmacokinetic Assessments

PK parameters include: $\%AUC_{exp}$, AUC_{last}, AUC_{inf}, C_{max}, T_{max}, T_{last}, C_{last}, λ_z, CL/F, and $t_{1/2}$.

Statistical Analysis

To evaluate the PK of SOF/VEL/VOX upon co-administration with a representative H_2RA or PPI, a parametric (normal theory) mixed-effects analysis of variance (ANOVA) model was fitted to the natural log-transformed values of the PK parameters (AUC_{last}, AUC_{inf}, and C_{max}) for SOF, GS-331007, VEL, and VOX. For the primary PK parameters

(AUC_{last}, AUC_{inf}, and C_{max}), the 90% CIs were determined for the ratios of geometric least squares means (GLSMs) for each analyte and treatment pair of interest.

9. Results

9.1 Subject Demographics and Disposition
Of the 147 subjects screened, 104 subjects were randomized (36 subjects in Cohort 1 and 34 subjects each in Cohorts 2 and 3). All 104 randomized subjects received study drug and were included in the Safety Analysis Set. Overall, 1 of 104 subjects (1.0%) prematurely discontinued study drug and from the study due to an AE. All other subjects completed study drug and the study.

The majority of subjects were white (64.4%, 67 subjects) and Hispanic or Latino (73.1%, 76 subjects), with more males than females (65.4%, 68 male subjects). At baseline, subjects had a mean (SD) age of 33 (6.7) years (range: 20 to 45), BMI of 25.7 (2.49) kg/m2, and CLcr of 122.22 (20.643) mL/min.

9.2 Pharmacokinetic and Statistical Analysis
Table 1 summarizes the changes in PK parameters of SOF, GS-331007, VEL, and VOX following single-dose administration of SOF/VEL/VOX alone and either simultaneously with or 12 hours after administration of a single dose of famotidine 40 mg (Cohort 1), 2 hours after administration of omeprazole 20 mg once daily (Cohort 2), or 4 hours before administration of omeprazole 20 mg once daily (Cohort 3).

No alteration in the AUC (AUC_{inf} or AUC_{last}) or C_{max} of SOF, GS-331007, VEL, or VOX was observed following administration of SOF/VEL/VOX with famotidine 40 mg, simultaneously or staggered by 12 hours, relative to SOF/VEL/VOX alone.

Sofosbuvir AUC and C_{max} were 27% and 23% lower, respectively, with no alteration in GS-331007 exposure; velpatasvir AUC and C_{max} were 54% and 57% lower, respectively; VOX exposure (AUC) was unchanged, while C_{max} was lower (24%), following the administration of SOF/VEL/VOX 2 hours after administration of omeprazole 20 mg, relative to SOF/VEL/VOX alone.

No alteration in SOF, GS-331007, or VOX AUC or C_{max} was observed following administration of SOF/VEL/VOX 4 hours before the administration of omeprazole 20 mg, relative to SOF/VEL/VOX alone, and velpatasvir AUC and C_{max} were both approximately 51% lower (**Table 2**).

Decreases in VEL exposure in the presence of omeprazole were not uniform across the study population. Those with highest exposure at baseline exhibited the largest decreases in VEL exposure, while those with lower exposure at baseline were minimally affected, according to the sponsor (**Figure 1**).

Reviewer's analysis:

There was a weak trend suggesting the largest declines of VEL exposure occurred in subjects with highest exposures, while less of a decrease occurred in subjects with lower exposures. However, the interaction needs to be assessed by evaluating the AUC_{test} to $AUC_{reference}$ ratios, not by the absolute difference between the AUC_{test} and $AUC_{reference}$. There was no trend suggesting that subjects with lower $AUC_{reference}$ had a lower degree of interaction when assessing AUC_{test} to $AUC_{reference}$ ratios (**Figures 2**).

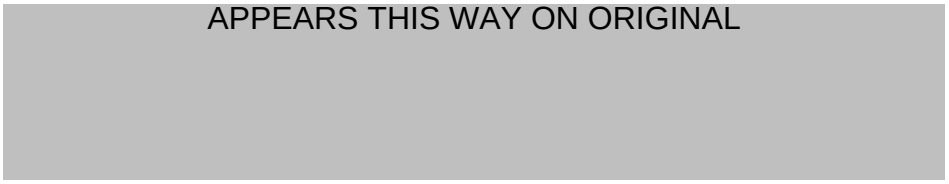


Table 1: AUC and C_{max} change of SOF, GS-331007, VEL, and VOX after co-administration with H_2RA (famotidine) or PPI (omeprazole).

PK Parameter		SOF/VEL/VOX + Simultaneous Famotidine 40 mg (Cohort 1)	SOF/VEL/VOX 12 Hours After Famotidine 40 mg (Cohort 1)	SOF/VEL/VOX 2 Hours After OME 20 mg (Cohort 2)	SOF/VEL/VOX 4 Hours Before OME 20 mg (Cohort 3)
SOF	AUC_{last}	↔	↔	↓ 27%	↔
	AUC_{inf}	↔	↔	↓ 27%	↔
	C_{max}	↔	↔	↓ 23%	↔
GS-566500	AUC_{last}	↔	↔	↔	↔
	AUC_{inf}	↔	↔	↔	↔
	C_{max}	↔	↔	↔	↔
GS-331007	AUC_{last}	↔	↔	↔	↔
	AUC_{inf}	↔	↔	↔	↔
	C_{max}	↔	↔	↔	↔
VEL	AUC_{last}	↔	↔	↓ 54%	↓ 51%
	AUC_{inf}	↔	↔	↓ 54%	↓ 51%
	C_{max}	↔	↔	↓ 57%	↓ 51%
VOX	AUC_{last}	↔	↔	↔	↔
	AUC_{inf}	↔	↔	↔	↔
	C_{max}	↔	↔	↓ 24%	↔

GLSM = geometric least squares mean; OME = omeprazole
The lower bound of the 90% CIs of the %GLSM ratios were within (↔) or extended below (↓) the predetermined equivalence boundary of 70%.

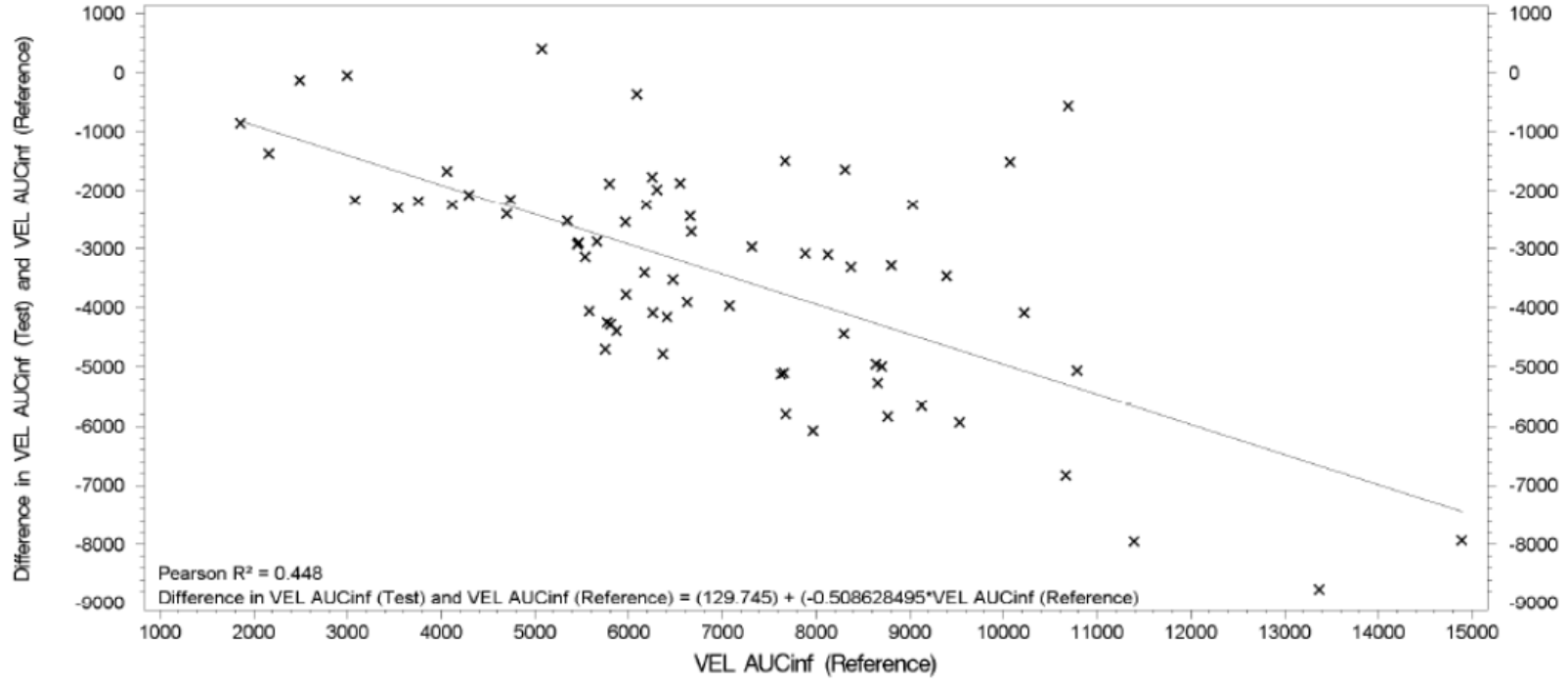
Table 2: VEL Plasma Pharmacokinetic Parameters Following Single-Dose Administration of SOF/VEL/VOX Alone or with Famotidine 40 mg (Co-administered Simultaneously or 12-Hour Stagger) or Omeprazole 20 mg (2- or 4-Hour Stagger) (VEL PK Analysis Set)

	Cohort 1			Cohort 2		Cohort 3	
VEL PK Parameter	SOF/VEL/VOX Alone (N = 35)	SOF/VEL/VOX + Simultaneous Famotidine 40 mg (N = 35)	SOF/VEL/VOX 12 Hours After Famotidine 40 mg (N = 36)	SOF/VEL/VOX Alone (N = 34)	SOF/VEL/VOX 2 Hours After OME 20 mg (N = 34)	SOF/VEL/VOX Alone (N = 34)	SOF/VEL/VOX 4 Hours Before OME 20 mg (N = 34)
AUCexp (%)	0.79 (59.21)	0.95 (55.43)	1.02 (57.48)	0.72 (44.56)	1.27 (45.71)	0.76 (66.56)	1.53 (75.19)
AUC_{last} (ng•h/mL)	6816.4 (41.9)	5984.6 (40.1)	5805.3 (47.5)	7140.3 (30.0)	3495.8 (48.6)	6605.2 (42.3)	3487.8 (58.7)
AUC_{inf} (ng•h/mL)	6870.7 (42.0)	6042.8 (40.4)	5865.9 (47.9)	7191.7 (30.0)	3535.2 (48.3)	6652.6 (42.2)	3527.1 (58.2)
C_{max} (ng/mL)	774.9 (37.2)	684.5 (28.5)	672.6 (34.8)	850.4 (25.6)	394.6 (51.5)	748.6 (30.8)	390.0 (47.9)
C_{last} (ng/mL)	2.09 (72.38)	2.18 (66.79)	2.20 (81.02)	2.00 (44.28)	1.65 (31.15)	1.91 (53.29)	1.85 (43.54)
T_{max} (h)	4.00 (3.07, 5.00)	4.00 (3.00, 4.00)	4.00 (3.03, 4.00)	4.00 (3.00, 4.00)	4.00 (3.00, 5.00)	4.00 (3.00, 5.00)	3.92 (3.00, 3.95)
T_{last} (h)	120.00 (96.00, 120.00)	120.00 (96.00, 120.00)	120.00 (96.00, 120.00)	120.00 (120.00, 120.00)	96.00 (72.00, 120.00)	96.00 (96.00, 120.00)	96.00 (72.00, 120.00)
$t_{1/2}$ (h)	17.33 (14.45, 18.24)	16.98 (14.95, 18.68)	17.93 (14.78, 19.27)	17.80 (15.89, 19.46)	16.02 (13.13, 19.74)	15.88 (13.58, 18.39)	14.78 (12.45, 16.84)
CL/F (mL/h)	18,532.9 (67.0)	19,761.8 (48.7)	21,434.6 (57.7)	15,407.8 (36.0)	34,817.7 (45.3)	18,485.4 (56.5)	41,180.6 (70.4)

OME = omeprazole
All PK parameters are presented as mean (%CV) except for T_{max}, T_{last}, and $t_{1/2}$, which are presented as median (Q1, Q3).

Sponsor's analysis

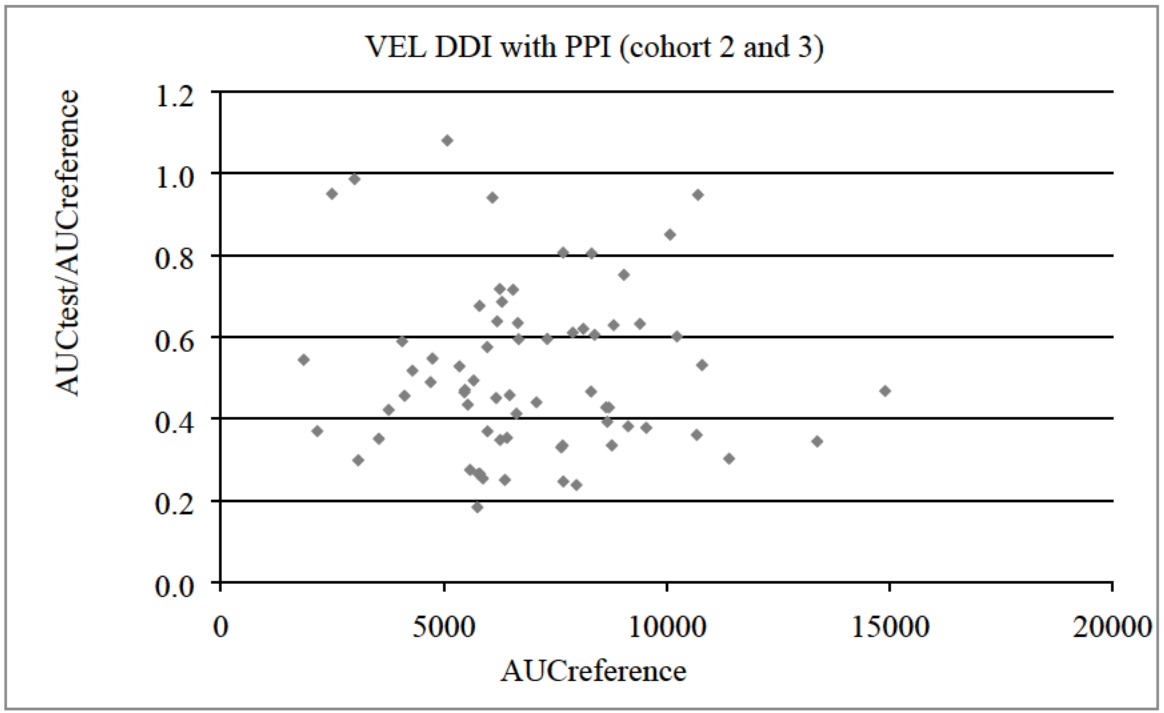
Figure 1: Scatterplot of difference in AUC_{inf} between test and reference treatment versus AUC_{inf} of reference treatment (Analysis Set: VEL PK)



AUC_{inf} from Cohorts 2 and 3 are presented together.
Cohort 2: reference = SOF/VEL/VOX alone; test = SOF/VEL/VOX 2 hours after omeprazole 20 mg
Cohort 3: reference = SOF/VEL/VOX alone; test = SOF/VEL/VOX 4 hours before omeprazole 40 mg

Reviewer's analysis:

Figure 2: Scatterplot of AUC_{inf} ratio between test and reference treatments versus AUC_{inf} of reference treatment (Analysis Set: VEL PK)



9.3 Safety Analysis
Administration of SOF/VEL/VOX with famotidine or omeprazole was generally well tolerated in healthy adult subjects. No Grade 3 or 4 AEs, serious adverse events (SAEs), or deaths were reported.

10. Sponsor's Conclusions

- Famotidine 40 mg did not impact SOF/VEL/VOX PK.

- Administration of SOF/VEL/VOX with omeprazole 20 mg resulted in ~50% lower VEL exposure. Changes in the PK of SOF and VOX were minor and likely secondary to effects on VEL.

11. Reviewer's Assessment

- The study design is reasonable and the PK parameters for SOF, GS-331007, VEL, and VOX are comparable to results from other Phase 1 studies in healthy subjects following administration of SOF/VEL/VOX at 400/100/100 mg with moderate-fat meal.

- The sponsor's conclusion that famotidine 40 mg did not impact SOF/VEL/VOX PK is valid, and the reviewer agrees with the sponsor's proposal that H_2-receptor antagonists may be administered simultaneously with or staggered from SOF/VEL/VOX at a dose that does not exceed doses comparable with famotidine 40 mg twice daily.

- Administration of SOF/VEL/VOX with omeprazole 20 mg resulted in ~ 50% lower VEL exposure, even for subjects with lower exposure at reference. However, given that VEL exposures are already 2-fold higher (in the presence of VOX) as compared with VEL exposures resulting from SOF/VEL administration, the impact of 50% lower VEL exposures in the context of SOF/VEL/VOX dosing is likely very limited, given the high efficacy rates associated with SOF/VEL/VOX. Thus, it is acceptable to allow co-administration of omeprazole with SOF/VEL/VOX at doses that do not exceed 20 mg.

4.4.11 GS-US-367-1727 DDI with Transporter Substrates and ARVs

1. Title

A Phase 1 Study to Evaluate Drug-Drug Interactions between Sofosbuvir/Velpatasvir/GS-9857 Fixed-Dose Combination and Drug Transporter Probe Substrates and Inhibitors

2. Information Regarding the Clinical Trial Site and Duration of the Trial

The trial was conducted (b) (4) from October 21, 2015 to December 12, 2015, with the final report date of August 8, 2016.

3. Objectives

Primary objective:

- To evaluate the effect of sofosbuvir (SOF)/velpatasvir (VEL)/voxilaprevir (VOX; GS-9857) fixed-dose combination (FDC) + VOX 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 antiretrovirals (ARVs) that inhibit OATP (HIV protease inhibitors [PIs] ritonavir [RTV]-boosted darunavir [DRV] [DRV/r], RTV-boosted atazanavir [ATV] [ATV/r], and HIV integrase strand transfer inhibitor [INSTI] cobicistat [COBI]-boosted elvitegravir [EVG] [EVG/c]) on SOF/VEL/VOX FDC pharmacokinetics (PK)

Secondary objective:

- To evaluate the safety and tolerability of SOF/VEL/VOX FDC + VOX and SOF/VEL/VOX FDC when administered with transporter probe drugs and ARVs, respectively

4. Trial Design

This Phase 1 study was an open-label, multiple-dose, single-center study designed to evaluate drug-drug interactions (DDIs) between SOF/VEL/VOX+VOX and phenotypic probes, as well as the DDIs between SOF/VEL/VOX and ARVs. Following screening procedures and baseline assessments, eligible subjects were assigned to 1 of 3 cohorts:

Cohort 1 (OATP substrate pravastatin [PRA] and OATP/BCRP substrate rosuvastatin [ROSU]): Subjects received Treatments A and B with food and a washout period between each treatment.

- Treatment A: Single dose of PRA 40 mg followed by a washout period and a single dose of ROSU 10 mg
- Treatment B: SOF/VEL/VOX FDC (400/100/100 mg) + VOX (100 mg) administered once daily for 15 days with a single dose of PRA 40 mg administered on the eighth day and a single dose of ROSU 10 mg administered on the 12th day

Cohort 2 (P-gp substrate dabigatran etexilate [DAB]): Subjects received Treatments C and D with food and a washout period between each treatment.

- Treatment C: Single dose of DAB 75 mg
- Treatment D: SOF/VEL/VOX FDC (400/100/100 mg) + VOX (100 mg) administered once daily for 11 days with a single dose of DAB 75 mg administered on the eighth day

Cohort 3 (ARVs): Subjects received Treatments E, F, G, and H with food and a washout period between each treatment.

- Treatment E: Single dose of SOF/VEL/VOX FDC (400/100/100 mg)
- Treatment F: SOF/VEL/VOX FDC (400/100/100 mg)+DRV (800 mg)+RTV (100 mg)

- Treatment G: SOF/VEL/VOX FDC (400/100/100 mg)+ATV (300 mg)+RTV (100 mg)
- Treatment H: SOF/VEL/VOX FDC (400/100/100 mg)+EVG (150 mg)+COBI (150 mg)

Eligible subjects were healthy males and non-pregnant, non-lactating females, 18 to 45 years of age (inclusive), with body mass index (BMI) 19 to 30 kg/m2 (inclusive), electrocardiogram (ECG) without clinically significant abnormalities, normal renal function, no significant medical history, and good general health.

5. Excluded Medications and Restrictions

- Any prescription medications and OTC medications including herbal products and antacids with the exception of vitamins, and/or acetaminophen and/or ibuprofen and/or hormonal contraceptive medications. However, the short-term use of topical hydrocortisone cream or A&D ointment to treat minor skin irritation due to ECG leads was allowed.
- Subjects were required to refrain from the consumption of food and beverages containing alcohol products, and from consumption of grapefruit juice, grapefruits, and Seville orange juice 72 hours prior to the first dose of study drug and during the course of the study through discharge.
- Subjects were required to refrain from the use of nicotine or nicotine-containing products from screening through discharge.

6. Rationale for Doses Used in the Trial

PRA: 40 mg is the recommended starting dose in Pravachol® (pravastatin sodium) USPI for adults and adolescents. Food has no effect on its lipid-lowering effects, and thus a single dose of PRA at 40 mg with food as a victim drug is reasonable.

ROSU: 10 mg is lower than the recommended starting dose at 20 mg in Crestor® (rosuvastatin calcium) USPI. This dose was likely chosen due to the expected significant DDI. Food has no effect on ROSU exposure, and thus a single dose of ROSU at 10 mg with food as a victim drug is reasonable.

DAB: 75 mg is lower than the recommended dose at 150 mg twice daily in Pradaxa®(dabigatran etexilate mesylate) USPI. This dose was likely chosen due to the safety concern associated with higher exposure of DAB. Food has no effect on its exposure, and thus a single dose of DAB at 75 mg with food as a victim drug is reasonable.

SOF/VEL/VOX+VOX: In healthy volunteers, a dose of SOF/VEL/VOX+VOX

400/100/100+100 mg has been found to provide similar (for SOF and its metabolite GS-331007) or higher exposure (for VEL and VOX) compared to clinical efficacious exposures in HCV patients under fed conditions. When tested as perpetrator drugs, SOF/VEL/VOX+VOX has been dosed once daily for 7 days before co-administration due to the long half-life of VOX at 30 to 40 hours. When tested as victim drugs, a single dose of SOF/VEL/VOX at 400/100/100 mg with food is reasonable.

DRV+RTV: 800+100 mg once daily with food is the recommended dose in the Prezista® (darunavir) USPI. DRV has a half-life of about 15 hours when co-administered with RTV and RTV also has a short half-life of 3 to 5 hours, and thus significant accumulation is not anticipated. A single dose of DRV/RTV as perpetrator drugs is reasonable.

ATV+RTV: 300+100 mg once daily with food is the recommended dose in the Reyataz® (atazanavir) USPI. ATV has a half-life of about 7 hours and RTV also has a short half-life of 3 to 5 hours, and thus significant accumulation is not anticipated. A single dose of ATV/RTV as perpetrator drugs is reasonable.

EVG+COBI: 150+150 mg once daily with food is the recommended dose as two components of EVG/COBI/FTC/TAF in the GENVOYA® (elvitegravir, cobicistat, emtricitabine, and tenofovir alafenamide) USPI. EVG has a half-life at 12.9 hours and COBI has a short half-life at 3.5 hours, and thus significant accumulation is not anticipated. Furthermore, the choice of EVG/COBI as perpetrator drugs in this exploratory study before the full evaluation of all four components of Genvoya is reasonable, as the other two components of Genvoya are not expected to cause a drug interaction with SOF/VEL/VOX.

7. Drugs Used in the Trial

SOF/VEL/VOX 400/100/100 mg FDC tablets – Batch Number(s): ER1501B2 (Manufactured by Gilead Sciences)
VOX 100 mg tablet – Batch Number(s): DY1502B1 (Manufactured by (b) (4)
PRA 40 mg tablets – Batch Number(s): 4G81472C (Manufactured by Bristol-Myers Squibb Company)
ROSU 10 mg tablets – Batch Number(s): FL0087 (Manufactured by AstraZeneca Pharmaceuticals LP)
DAB 75 mg tablets – Batch Number(s): 408451 (Manufactured by Boehringer Ingelheim Pharmaceuticals, Inc.)
DRV 800 mg tablets – Batch Number(s): 15GG397 (Manufactured by Janssen Therapeutics)
ATV 300 mg capsules – Batch Number(s): 5A88555A (Manufactured by Bristol-Myers Squibb Company)
RTV 100 mg tablets – Batch Number(s): 1041437 (Manufactured by Abbvie Inc.)
EVG 150 mg tablets – Batch Number(s): MFSDA (Manufactured by Patheon Inc.)
COBI 150 mg tablets – Batch Number(s): SHWK (Manufactured by Gilead Sciences, Inc.)

8. Sample Collection, Bioanalysis, Pharmacokinetic Assessments, and Statistical

Analysis

Sample Collection
Plasma concentrations of VOX, VEL, SOF and its metabolites GS-331007, PRA, ROSU, DAB [total (unconjugated + DAB glucuronide) and free (unconjugated)], DRV, ATV, RTV, EVG, and COBI were determined as appropriate and PK parameters evaluated.

Serial blood samples were collected following dosing at the following time points:
Cohort 1:

- Days 1, 5, 18, and 22: predose (< 5 min) and 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, and 96 hours postdose

Cohort 2:

- Days 1 and 13: predose (< 5 min) and 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, and 96 hours postdose

Cohort 3:

- Days 1, 9, 17, and 25: predose (< 5 min) and 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, and 96 hours postdose

Bioanalytical method

- Validation and Bioanalytical Report:

Analyte	Matrix	Validation Report	Bioanalytical Report
SOF (GS-7977), GS-331007	Plasma	(b) (4) 60-1323 Amendment 4	(b) (4) 60-1598A
VEL	Plasma	(b) (4) 60-1393 Amendment 1	(b) (4) 60-1598B
VOX	Plasma	(b) (4) 8109.123113.1	(b) (4) 10360.112515
DAB (free)	Plasma	(b) (4) P1266 Addendum 2	(b) (4)
DAB (total)	Plasma	P1270 Addendum 1	(b) (4)
PRA	Plasma	42-1328	(b) (4) 60-1598C
ROSU	Plasma	42-1043 Amendment 2	60-1598D
DRV	Plasma	42-0902 Amendment 4	60-1598E
ATV, RTV	Plasma	42-0830 Amendment 4	60-1598F
EVG (GS-9137), COBI (GS-9350)	Plasma	60-1343 Amendment 2	60-1598G

- Bioanalytical Method:

Method type: LC/MS/MS; Matrix: K_2EDTA plasma

Analyte	Range (ng/mL)	Stability in frozen matrix (days)
SOF	5 to 2500	174 at −20°C and 813 at −70°C
GS-331007	10 to 5000	174 at −20°C and 813 at −70°C
VEL	1 to 1000	161 at −20°C and 570 at −70°C
VOX	0.5 to 1000	420 at −20ºC and at −70ºC

PRA	0.2 - 100	95 at −20°C and −70°C
ROSU	0.05 - 50	175 at −20°C and at −70°C
DAB (free)	0.5 - 250	288 at −20°C and −70°C
DAB (total)	1.0 - 500	273 at −20°C and −70°C
DRV	20 - 10,000	1635 at −20°C and −70°C
ATV	10 - 5000	721 at −70°C
RTV	5 - 2500	721 at −70°C
EVG	20 - 10,000	585 at −70ºC
COBI	5 - 2500	121 at −10°C to −30°C and 1297 at −60ºC to −80°C

Validation	▪ Method validated prior to use	☒ Yes ☐ No ☐ NA
	▪ Method validation acceptable	☒ Yes ☐ No ☐ NA
Study Samples Analysis	▪ Samples analyzed within the established stability period	☒ Yes ☐ No
	▪ Quality control samples range acceptable	☒ Yes ☐ No
	▪ Chromatograms provided	☒ Yes ☐ No
	▪ Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	▪ Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	▪ Incurred samples analysis is acceptable	☒ Yes ☐ No
	▪ Overall performance acceptable	☒ Yes ☐ No
Inspection	▪ Will the bioanalytical site be inspected	☐ Yes ☒ No

Reviewer note: the method validation and bioanalysis data for dabigatran (free and total) were reviewed and found to be acceptable by the secondary reviewer, Dr. Shirley Seo.

Pharmacokinetic Assessments
PK parameters include: AUC_{last}, AUC_{0-24}, AUC_{inf}, $\%AUC_{exp}$, C_{max}, C_{last}, T_{max}, T_{last}, λ_z, CL/F, and $t_{1/2}$.

Statistical Analysis
An analysis of variance (ANOVA) was fitted to the natural logarithmic transformation of PK parameters AUC_{last}, AUC_{inf}, and C_{max} for each analyte of interest. The geometric least-squares mean (GLSM) ratios and associated two-sided 90% confidence intervals (CIs) were calculated for the PK parameters.

9. Results

9.1 Subject Demographics and Disposition
A total of 71 subjects were enrolled, each into Cohort 1, 2, or 3 as described below. All 71 subjects who enrolled also received at least 1 dose of study drug. All subjects completed the study except for 1 subject who withdrew consent during study drug dosing.

The majority of subjects were white (60.6%, 43 subjects) or black or African-American (33.8%, 24 subjects) and Hispanic or Latino (67.6%, 48 subjects), with more males than females (64.8% male, 46 subjects). Subjects had a mean (SD) age of 35 (7.4) years (range: 19 to 45 years) and a mean (SD) BMI of 26.1 (2.59) kg/m2.

9.2 Pharmacokinetic and Statistical Analysis

Cohort 1 and 2: Effect of SOF/VEL/VOX+VOX on OATP, BCRP, and P-gp Substrates Using Phenotypic Probes

The summary of results for the PK parameters and statistical analysis of PRA, ROSU, and DAB are presented in **Table 1**, **Table 2**, and **Table 3**, respectively. In brief:

- Pravastatin mean AUC_{inf} and mean C_{max} were 116% and 89% higher, respectively, following co-administration with SOF/VEL/VOX+VOX relative to PRA administration alone.
- Rosuvastatin mean AUC_{inf} and mean C_{max} were 639% and 1788% higher, respectively, following co-administration with SOF/VEL/VOX+VOX, relative to ROSU administration alone.
- Co-administration of DAB with SOF/VEL/VOX+VOX resulted in higher total (free + conjugated) DAB exposure (mean AUC_{inf} 161% increase, mean C_{max} 187% increase) and free DAB exposure (mean AUC_{inf} 122% increase, mean C_{max} 134% increase), relative to DAB administration alone.

In summary, the combination SOF/VEL/VOX significantly inhibits BCRP and to a lesser extent, OATP and P-gp.

Cohort 3: Effect of ARVs on SOF/VEL/VOX

The drug interaction potential between SOF/VEL/VOX and the ARVs DRV/r, ATV/r, or EVG/c was evaluated. The summary of results for the PK parameters and statistical analysis of VOX, VEL, SOF, and SOF primary metabolite GS-331007 are presented in **Table 4**, **Table 5**, and **Table 6**, respectively. In brief:

- VOX mean AUC_{inf} and mean C_{max} were 57% and 25% higher, respectively, following co-administration of single doses of SOF/VEL/VOX with DRV/r, relative to SOF/VEL/VOX alone. The PK parameters of VEL, SOF, and GS-331007, were not

altered by DRV/r.

- VOX mean AUC_{inf} and mean C_{max} were 331% and 342% higher, respectively, following co-administration of single doses of ATV/r and SOF/VEL/VOX, relative to SOF/VEL/VOX alone. Velpatasvir mean AUC_{inf} and mean C_{max} were 93% and 29% higher, respectively, and SOF mean AUC_{inf} and mean C_{max} were 40% and 29% higher, respectively, with no change in GS-331007 exposure, following co-administration of single doses of ATV/r and SOF/VEL/VOX.
- Increases in the mean AUC_{inf} of VOX (36%), VEL (30%), SOF (31%) and GS-331007 (35%), without any changes in mean Cmax, were observed following co-administration of single doses of SOF/VEL/VOX and EVG/c, relative to SOF/VEL/VOX alone.

The summary of results for the PK parameters of the component ARV agents following administration of SOF/VEL/VOX in combination with DRV/r, ATV/r, or EVG/c are presented in **Table 7**. All ARVs were co-administered with SOF/VEL/VOX, and not administered alone; therefore, there are no reference groups for statistical comparison. However, DRV, ATV, RTV, EVG, and COBI mean AUCs were consistent with previous exposure data.

APPEARS THIS WAY ON ORIGINAL

Table 1: Pravastatin Plasma PK Parameters and Statistical Comparison Following Administration of PRA Alone or Co-administered with SOF/VEL/VOX+VOX

Pravastatin PK Parameter	Mean[a] (%CV)		% GLSM Ratio (90% CI) Test/Reference
	PRA (Reference) (N = 20)	PRA + SOF/VEL/VOX + VOX (Test) (N = 19)	
AUC_{exp} (%)	1.98 (42.03)	1.03 (108.96)	—
AUC_{last} (ng•h/mL)	95.2 (41.9)	204.8 (36.5)	217.94 (180.71, 262.84)
AUC_{inf} (ng•h/mL)	97.0 (41.7)	206.9 (36.4)	215.89 (179.27, 259.99)
C_{max} (ng/mL)	36.2 (44.6)	70.5 (51.2)	189.18 (153.24, 233.55)
T_{max} (h)[b]	1.50 (1.50, 2.00)	2.00 (2.00, 3.00)	—
C_{last} (ng/mL)	0.37 (33.35)	0.56 (43.92)	—
T_{last} (h)[b]	12.00 (12.00, 24.00)	12.00 (12.00, 12.00)	—
$t_{1/2}$ (h)[b]	2.50 (2.00, 6.50)	1.92 (1.37, 2.46)	—
CL/F (mL/h)	522856.2 (61.6)	218763.2 (36.3)	—

GLSM = geometric least-squares mean; PRA = pravastatin
a Means are presented as unadjusted arithmetic means.
b median (Q1, Q3)

Table 2: Rosuvastatin Plasma PK Parameters and Statistical Comparison Following Administration of ROSU Alone or Co-administered with SOF/VEL/VOX+VOX

Rosuvastatin PK Parameter	Mean[a] (%CV)		% GLSM Ratio (90% CI) Test/Reference
	ROSU (Reference) (N = 20)	ROSU + SOF/VEL/VOX + VOX (Test) (N = 19)	
AUC_{exp} (%)	10.50 (50.68)	1.10 (43.04)	—
AUC_{last} (ng•h/mL)	27.8 (28.4)	223.9 (21.1)	816.70 (733.13, 909.80)
AUC_{inf} (ng•h/mL)	30.8 (25.7)	226.4 (21.2)	738.84 (667.52, 817.77)
C_{max} (ng/mL)	2.7 (31.1)	48.5 (22.1)	1888.23 (1623.37, 2196.29)
T_{max} (h)[b]	5.00 (4.00, 5.00)	4.00 (3.00, 5.00)	—
C_{last} (ng/mL)	0.10 (80.81)	0.10 (62.17)	—
T_{last} (h)[b]	72.00 (48.00, 72.00)	72.00 (72.00, 72.00)	—
$t_{1/2}$ (h)[b]	23.54 (17.31, 29.22)	17.90 (15.41, 19.77)	—
CL/F (mL/h)	349584.1 (31.4)	46116.2 (21.7)	—

GLSM = geometric least-squares mean; ROSU = rosuvastatin
a Means are presented as unadjusted arithmetic means.
b median (Q1, Q3)

Table 3: Total and Free Dabigatran Plasma PK Parameters and Statistical Comparisons Following Administration of DAB Alone or Co-administered with SOF/VEL/VOX+VOX

PK Parameter	Mean[a] (%CV)		% GLSM Ratio (90% CI) Test/Reference
	DAB (Reference) (N = 36)	DAB + SOF/VEL/VOX +VOX (Test) (N= 36)	
Total DAB			
AUC_{exp} (%)	4.63 (65.47)	1.74 (39.75)	—
AUC_{last} (ng•h/mL)	643.1 (29.8)	1709.7 (25.6)	268.72 (247.01, 292.33)
AUC_{inf} (ng•h/mL)	670.9 (27.7)	1738.4 (25.2)	260.66 (240.55, 282.45)
C_{max} (ng/mL)	84.0 (31.5)	237.2 (24.6)	286.67 (260.72, 315.21)
T_{max} (h)[b]	4.00 (3.00, 4.00)	4.00 (4.00, 5.00)	—
C_{last} (ng/mL)	2.25 (69.14)	2.03 (36.30)	—
T_{last} (h)[b]	48.00 (36.00, 48.00)	48.00 (48.00, 72.00)	—
$t_{1/2}$ (h)[b]	9.58 (8.37, 10.42)	9.14 (8.56, 10.93)	—
CL/F (mL/h)	120138.8 (27.1)	45832.8 (24.9)	—
Free DAB			
AUC_{exp} (%)	2.46 (38.03)	1.26 (52.94)	—
AUC_{last} (ng•h/mL)	591.2 (27.9)	1320.1 (25.7)	224.36 (206.39, 243.91)
AUC_{inf} (ng•h/mL)	605.2 (27.4)	1335.7 (25.3)	221.64 (204.15, 240.62)
C_{max} (ng/mL)	76.3 (31.6)	176.1 (25.2)	234.35 (212.43, 258.55)
T_{max} (h)[b]	4.00 (3.00, 4.00)	4.00 (4.00, 5.00)	—
C_{last} (ng/mL)	0.93 (30.64)	1.10 (44.42)	—
T_{last} (h)[b]	48.00 (48.00, 48.00)	72.00 (48.00, 72.00)	—
$t_{1/2}$ (h)[b]	9.87 (9.14, 11.18)	9.52 (7.99, 10.90)	—
CL/F (mL/h)	132926.8 (26.6)	59804.2 (26.3)	—

DAB = dabigatran etexilate; GLSM = geometric least-squares mean
a Means are presented as unadjusted arithmetic means.
b median (Q1, Q3)

Table 4: Voxilaprevir Plasma PK Parameters and Statistical Comparison Following Administration of SOF/VEL/VOX Alone or Co-administered with ARVs (DRV/r, ATV/r, or EVG/c)

	SOF/VEL/VOX (Reference) (N = 15)	SOF/VEL/VOX + DRV/r (Test) (N = 15)		SOF/VEL/VOX +ATV/r (Test) (N = 15)		SOF/VEL/VOX + EVG/c (Test) (N = 15)	
VOX PK Parameter	Mean[a] (%CV)	Mean[a] (%CV)	%GLSM Ratio (90% CI) (Test/Reference)	Mean[a] (%CV)	%GLSM Ratio (90% CI) (Test/Reference)	Mean[a] (%CV)	%GLSM Ratio (90% CI) (Test/Reference)
AUC_{exp} (%)	11.08 (70.59)	8.48 (67.35)	—	3.71 (47.64)	—	8.08 (47.91)	—
AUC_{last} (ng•h/mL)	557.8 (56.1)	987.4 (70.5)	161.71 (131.53, 198.82)	2811.1 (72.8)	468.28 (407.08, 538.67)	804.4 (58.8)	141.30 (122.69, 162.72)
AUC_{inf} (ng•h/mL)	620.3 (52.5)	1060.7 (67.8)	156.82 (130.52, 188.42)	2902.0 (71.6)	430.85 (376.28, 493.32)	865.6 (56.5)	136.27 (120.48, 154.12)
C_{max} (ng/mL)	68.7 (44.8)	99.8 (74.2)	125.32 (98.48, 159.46)	348.4 (80.9)	442.04 (365.12, 535.16)	66.4 (37.5)	99.29 (84.17, 117.11)
T_{max} (h)[b]	3.00 (2.00, 5.00)	4.00 (3.00, 5.00)	—	4.00 (4.00, 5.00)	—	4.00 (3.00, 5.00)	—
C_{last} (ng/mL)	1.18 (48.98)	1.64 (59.28)	—	2.22 (56.61)	—	1.52 (49.75)	—
T_{last} (h)[b]	96.00 (96.00, 96.00)	96.00 (96.00, 96.00)	—	96.00 (96.00, 96.00)	—	96.00 (96.00, 96.00)	—
$t_{1/2}$ (h)[b]	29.23 (26.02, 34.54)	30.00 (24.66, 34.43)	—	29.09 (24.84, 30.48)	—	29.21 (23.82, 31.83)	—
CL/F (mL/h)	211574.9 (69.0)	140482.9 (58.2)	—	51800.0 (69.2)	—	156682.9 (62.6)	—

ATV/r = ritonavir-boosted atazanavir; DRV/r = ritonavir-boosted darunavir; EVG/c = cobicistat-boosted elvitegravir; GLSM = geometric least-squares mean

a Means are presented as unadjusted arithmetic means.

b median (Q1, Q3)

Table 5: Velpatasvir Plasma PK Parameters and Statistical Comparisons Following Administration of SOF/VEL/VOX Alone or Co-administered with ARVs (DRV/r, ATV/r, or EVG/c)

VEL PK Parameter	SOF/VEL/ VOX (Reference) (N = 15)	SOF/VEL/VOX + DRV/r (Test) (N = 15)		SOF/VEL/VOX +ATV/r (Test) (N = 15)		SOF/VEL/VOX+ EVG/c (Test) (N = 15)	
	Mean[a] (%CV)	Mean[a] (%CV)	GLSM % Ratio (90% CI) (Test/ Reference)	Mean (%CV)	GLSM % Ratio (90% CI) (Test/ Reference)	Mean (%CV)	GLSM % Ratio (90% CI) (Test/ Reference)
AUC_{exp} (%)	1.20 (58.88)	1.34 (69.68)	—	1.37 (85.79)	—	1.31 (87.23)	—
AUC_{last} (ng•h/mL)	5927.1 (42.4)	6746.8 (43.3)	115.60 (93.57, 142.82)	10736.9 (32.0)	192.45 (157.08, 235.77)	7667.0 (47.9)	130.09 (107.27, 157.77)
AUC_{inf} (ng•h/mL)	6004.5 (42.8)	6851.9 (43.9)	115.76 (93.87, 142.76)	10907.5 (32.5)	192.79 (157.63, 235.79)	7794.0 (48.6)	130.24 (107.47, 157.84)
C_{max} (ng/mL)	688.9 (32.1)	615.5 (28.7)	91.16 (76.76, 108.25)	849.5 (18.7)	129.20 (107.29, 155.60)	719.1 (29.5)	106.22 (87.28, 129.27)
T_{max} (h)[b]	3.00 (3.00, 4.00)	3.00 (3.00, 4.00)	—	4.00 (3.00, 5.00)	—	4.00 (4.00, 5.00)	—
C_{last} (ng/mL)	3.25 (79.13)	4.23 (89.01)	—	6.76 (85.86)	—	4.96 (101.13)	—
T_{last} (h)[b]	96.00 (72.00, 96.00)	96.00 (96.00, 96.00)	—	96.00 (96.00, 96.00)	—	96.00 (96.00, 96.00)	—
$t_{1/2}$ (h)[b]	14.19 (13.11, 16.93)	14.50 (13.08, 17.80)	—	14.17 (12.49, 17.89)	—	14.32 (13.20, 17.87)	—
CL/F (mL/h)	21475.7 (67.9)	17673.5 (49.3)	—	10052.2 (30.1)	—	15711.9 (45.6)	—

ATV/r = ritonavir-boosted atazanavir; DRV/r = ritonavir-boosted darunavir; EVG/c = cobicistat-boosted elvitegravir; GLSM = geometric least-squares mean

a Means are presented as unadjusted arithmetic means.

b median (Q1, Q3)

Table 6: Sofosbuvir and GS-331007 Plasma PK Parameters and Statistical Comparisons Following Administration of SOF/VEL/VOX Alone or Co-administered with ARVs (DRV/r, ATV/r, or EVG/c)

	SOF/VEL/VOX (Reference) (N = 15)	SOF/VEL/VOX + DRV/r (Test) (N = 15)		SOF/VEL/VOX +ATV/r (Test) (N = 15)		SOF/VEL/VOX + EVG/c (Test) (N = 15)	
PK Parameter	Mean[a] (%CV)	Mean[a] (%CV)	GLSM % Ratio (90% CI) (Test/Reference)	Mean[a] (%CV)	GLSM % Ratio (90% CI) (Test/Reference)	Mean[a] (%CV)	GLSM % Ratio (90% CI) (Test/Reference)
SOF							
AUC_{exp} (%)	0.42 (70.86)	0.42 (102.85)	—	0.39 (82.20)	—	0.42 (91.45)	—
AUC_{last} (ng•h/mL)	2646.7 (26.5)	2624.7 (23.0)	100.32 (89.75, 112.14)	3732.3 (29.7)	140.37 (125.11, 157.50)	3509.2 (31.6)	131.35 (120.47, 143.20)
AUC_{inf} (ng•h/mL)	2657.1 (26.4)	2635.3 (22.9)	100.32 (89.77, 112.11)	3745.3 (29.5)	140.33 (125.13, 157.38)	3523.3 (31.5)	131.35 (120.51, 143.17)
C_{max} (ng/mL)	1715.7 (46.9)	1764.0 (26.7)	107.84 (92.11, 126.25)	2235.1 (43.4)	128.63 (108.51, 152.47)	1914.0 (31.1)	115.06 (96.51, 137.18)
T_{max} (h)[b]	1.50 (1.00, 2.00)	2.00 (1.50, 2.00)	—	2.00 (1.00, 3.00)	—	2.00 (1.50, 3.00)	—
C_{last} (ng/mL)	14.74 (72.42)	12.48 (74.72)	—	16.57 (69.72)	—	18.92 (91.85)	—
T_{last} (h)[b]	6.00 (5.00, 6.00)	6.00 (5.00, 6.00)	—	6.00 (6.00, 8.00)	—	6.00 (6.00, 8.00)	—
$t_{1/2}$ (h)[b]	0.47 (0.44, 0.56)	0.55 (0.48, 0.64)	—	0.56 (0.46, 0.65)	—	0.52 (0.48, 0.56)	—
CL/F (mL/h)	161454.6 (28.6)	158592.3 (20.8)	—	115183.3 (27.9)	—	123625.3 (29.4)	—

PK Parameter	SOF/VEL/VOX (Reference) (N = 15)	SOF/VEL/VOX + DRV/r (Test) (N = 15)		SOF/VEL/VOX +ATV/r (Test) (N = 15)		SOF/VEL/VOX + EVG/c (Test) (N = 15)	
	Mean[a] (%CV)	Mean[a] (%CV)	GLSM % Ratio (90% CI) (Test/Reference)	Mean[a] (%CV)	GLSM % Ratio (90% CI) (Test/Reference)	Mean[a] (%CV)	GLSM % Ratio (90% CI) (Test/Reference)
GS-331007							
AUC_{exp} (%)	8.32 (22.93)	10.61 (16.52)	—	9.83 (24.98)	—	10.47 (29.28)	—
AUC_{last} (ng•h/mL)	11449.1 (22.0)	13379.6 (20.9)	117.30 (110.78, 124.19)	14116.3 (22.7)	123.33 (114.19, 133.19)	15205.9 (24.6)	132.23 (124.09, 140.90)
AUC_{inf} (ng•h/mL)	12511.2 (23.1)	14963.1 (20.6)	120.30 (113.44, 127.57)	15651.2 (22.2)	125.41 (116.06, 135.51)	16972.2 (24.2)	135.45 (126.82, 144.67)
C_{max} (ng/mL)	578.6 (19.7)	603.2 (16.3)	104.90 (98.94, 111.23)	610.0 (20.5)	105.30 (98.60, 112.47)	658.3 (21.6)	113.40 (106.99, 120.21)
T_{max} (h)[b]	3.00 (3.00, 5.00)	4.00 (3.00, 4.00)	—	4.00 (3.00, 5.00)	—	4.00 (4.00, 5.00)	—
C_{last} (ng/mL)	25.85 (35.07)	35.75 (22.50)	—	35.24 (25.57)	—	40.72 (29.85)	—
T_{last} (h)[b]	96.00 (96.00, 96.00)	96.00 (96.00, 96.00)	—	96.00 (96.00, 96.00)	—	96.00 (96.00, 96.00)	—
$t_{1/2}$ (h)[b]	28.65 (26.62, 29.79)	30.75 (28.89, 31.96)	—	29.66 (27.81, 31.54)	—	29.00 (27.43, 32.44)	—

ATV/r = ritonavir-boosted atazanavir; DRV/r = ritonavir-boosted darunavir; EVG/c = cobicistat-boosted elvitegravir; GLSM = geometric least-squares mean

a Means are presented as unadjusted arithmetic means.

b median (Q1, Q3)

Table 7: Plasma PK Parameters of ARV Agents (DRV, RTV, ATV, EVG, and COBI) Following Co-administration with SOF/VEL/VOX

	SOF/VEL/VOX + DRV/r (N = 15)		SOF/VEL/VOX + ATV/r (N = 15)		SOF/VEL/VOX + EVG/c (N = 15)	
PK Parameter	**DRV Mean (%CV)**	**RTV Mean (%CV)**	**ATV Mean (%CV)**	**RTV Mean (%CV)**	**EVG Mean (%CV)**	**COBI Mean (%CV)**
AUC_{exp} (%)	3.09 (146.38)	3.38 (48.75)	0.77 (111.89)	2.00 (59.65)	23.96 (72.48)	1.91 (113.07)
AUC_{last} (ng•h/mL)	73327.2 (32.0)	3550.2 (47.6)	42896.2 (22.4)	7444.3 (32.6)	17578.7 (39.4)	7252.9 (46.3)
AUC_{inf} (ng•h/mL)	75376.6 (30.1)	3679.2 (47.7)	43246.7 (22.7)	7610.3 (33.1)	26123.0 (66.6)	7462.0 (49.2)
C_{max} (ng/mL)	6414.0 (22.4)	575.1 (48.4)	4065.3 (19.2)	1287.9 (23.9)	1427.4 (39.5)	1046.2 (22.2)
T_{max} (h)[a]	4.00 (3.00, 4.00)	4.00 (4.00, 5.00)	4.00 (3.00, 5.00)	4.00 (4.00, 5.00)	4.00 (4.00, 5.00)	4.00 (3.00, 4.00)
C_{last} (ng/mL)	177.85 (142.56)	18.25 (63.47)	27.11 (85.05)	25.43 (73.56)	348.86 (81.51)	32.32 (129.46)
T_{last} (h)[a]	48.00 (48.00, 72.00)	24.00 (24.00, 24.00)	48.00 (48.00, 72.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
$t_{1/2}$ (h)[a]	7.74 (6.54, 8.89)	4.54 (4.30, 5.42)	7.25 (5.69, 8.68)	4.17 (3.78, 4.86)	11.14 (6.43, 13.64)	3.22 (2.83, 4.04)
CL/F (mL/h)	11677.7 (35.7)	34398.8 (52.8)	7287.6 (23.2)	14616.9 (33.8)	7723.7 (48.0)	24150.6 (40.1)

ATV = atazanavir; ATV/r = ritonavir-boosted atazanavir; COBI = cobicistat; DRV = darunavir; DRV/r = ritonavir-boosted darunavir; RTV = ritonavir; EVG = elvitegravir; EVG/c = cobicistat-boosted elvitegravir

a median (Q1, Q3)

9.3 Safety Analysis
SOF/VEL/VOX±VOX was generally well tolerated when administered alone or in combination with probe drugs or ARVs to healthy males and females. No deaths, SAEs, Grade 3 or 4 AEs, AEs leading to discontinuation of study drug or study participation were reported during the study.

10. Sponsor's Conclusions

- The combination of SOF/VEL/VOX+VOX inhibits BCRP and, to a lesser extent, OATP and P-gp, as assessed by co-administration with probe substrates rosuvastatin, pravastatin, and dabigatran etexilate.

- PK results from single-dose administration of SOF/VEL/VOX and DRV/r or EVG/c support further evaluation of multiple-dose PK. However, given the high exposures of VOX with single-dose administration of ATV/r, multiple-dose administration of SOF/VEL/VOX with ATV/r, an OATP inhibitor, will not be further evaluated.

11. Reviewer's Assessment

- The study design is reasonable for cohorts 1 to 3. The PK parameters for SOF, GS-331007, VEL, and VOX in Cohort 3 as victim drugs following a single dose of SOF/VEL/VOX at 400/100/100 mg with moderate-fat meal are comparable to results from other Phase 1 studies in healthy subjects.

- The sponsor's conclusion that SOF/VEL/VOX+VOX at 400/100/100+100 mg with a moderate-fat meal after multiple doses significantly increased exposure of BCRP/OATP substrate ROSU, and increased exposure of OATP substrate PRA and P-gp substrate DAB in healthy subjects is valid. In addition, ATV/r led to moderate to significant exposure increases of VEL and VOX, respectively, after a single dose in healthy subjects.

- Upon consultation with the metabolism review team in OCP (Dr. Jaya Vaidyanathan and Dr. Sang Chung), the proposed labeling recommendation about pravastatin and rosuvastatin by the sponsor was deemed reasonable and refer to clinical pharmacology review for up-to-date labeling recommendations.

- Upon consultation with the cardio/renal review team in OCP (Dr. Sudharshan Hariharan and Dr. Girish Bende), the dose was decreased by half for dabigatran when coadministration with SOF/VEL/VOX in patients with moderate renal impairment. Please

refer to clinical pharmacology review for up-to-date labeling recommendations.

- Atazanavir is not recommendated for coadminstration and refer to clinical pharmacology review for up-to-date labeling recommendations.

4.4.12 GS-US-367-1909 DDI with Hormonal Contraceptive (by Jenny Zheng)

1. Title

A Phase 1, Open-Label, Drug Interaction Study Evaluating the Effect of Sofosbuvir/Velpatasvir/Voxilaprevir (VOX; GS-9857) Fixed-Dose Combination on the Pharmacokinetics of a Representative Hormonal Contraceptive Medication, Norgestimate/Ethinyl Estradiol

2. Information Regarding the Clinical Trial Site and Duration of the Trial

The trial was conducted by Gilead Sciences, Inc. from October 29, 2015 to March 18, 2016, with the final report date of September 9, 2016.

3. Objectives

Primary objective:

- To determine the effect of sofosbuvir (SOF)/velpatasvir (VEL)/voxilaprevir (VOX; GS-9857) + VOX on the pharmacokinetics (PK) of a representative hormonal contraceptive medication, norgestimate (NGM) 0.180 mg/0.215 mg/0.25 mg/ethinyl estradiol (EE) 0.025 mg (Ortho Tri-Cyclen® Lo)
- To assess the effect of NGM/EE on the PK of SOF/VEL/VOX+VOX

Secondary objective:

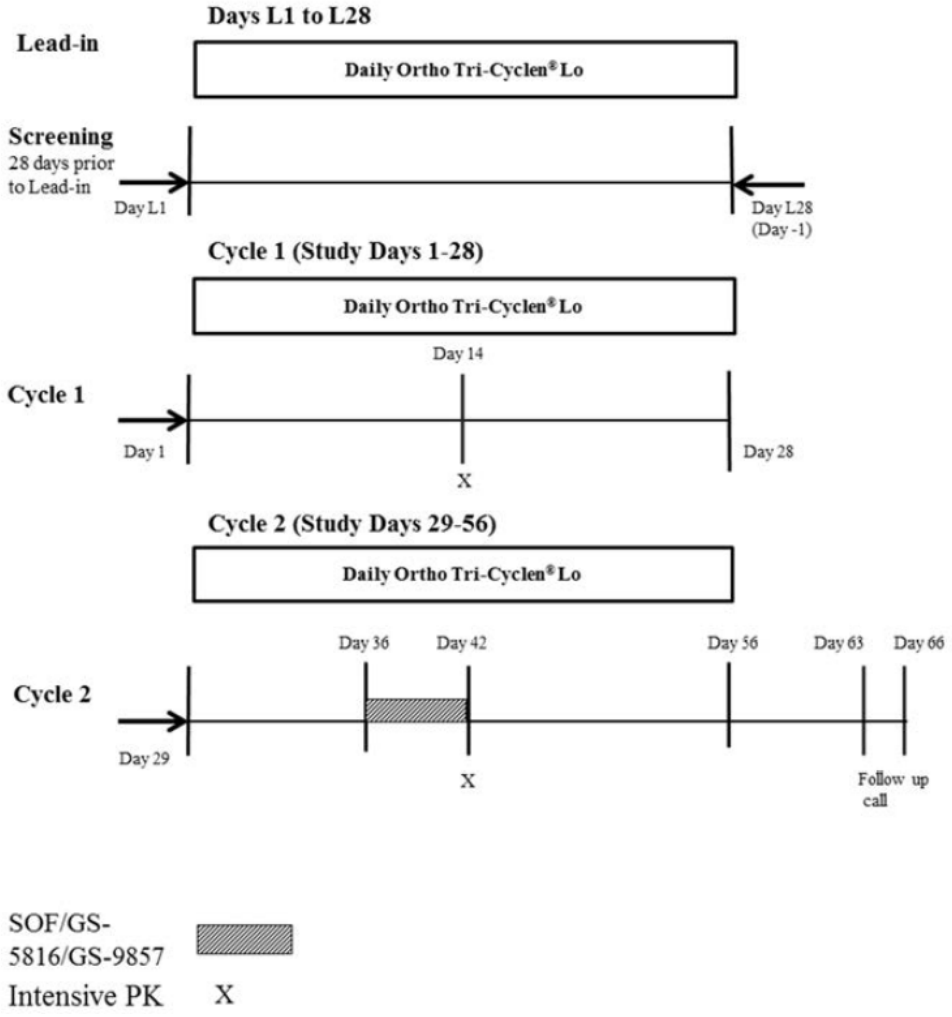
- To evaluate the safety and tolerability of administration of SOF/VEL/VOX+VOX when given with a representative hormonal contraceptive medication, NGM/EE

4. Trial Design

This Phase 1, open-label, single-center, fixed-sequence, multiple-dose study evaluated the PK, safety, and tolerability of SOF/VEL/VOX+VOX when administered with a representative hormonal contraceptive medication, NGM/EE, in healthy females of childbearing potential. Following screening procedures, eligible subjects were either enrolled in a 28-day lead-in period (Part A), during which they completed dosing with NGM/EE prior to initiation of Study Day 1 in Cycle 1 (Part B), or for subjects with a documented history of taking NGM/EE for at least 1 menstrual cycle, directly enrolled into Part B of the study within 28 days of screening.

Figure 1 presents the study design. If enrolled in the lead-in period, subjects were admitted to the study center on Day L –1 (one day before lead-in period) and confined until completion of NGM/EE dosing on Day L 1. Daily dosing with NGM/EE continued for 28 days. For Part B, all subjects returned to the study center on Study Day –1 (Day L28), where they remained until completion of NGM/EE dosing on Study Day 1. Subjects continued daily dosing with NGM/EE through Study Day 56 (2 full 28-day menstrual cycles). Subjects were administered SOF/VEL/VOX+VOX during Cycle 2 on Study Days 36 to 42.

Figure 1. GS-US-367-1909: Study Schema (Source: Sponsor's study report)



All doses of study drug were administered at approximately the same time each day in the morning with 240 milliliter of water within 30 minutes of subjects initiating and 5 minutes of completing a moderate-fat breakfast.

Eligible subjects were premenopausal, non-pregnant females, 18 to 45 years of age (inclusive), with body mass index (BMI) 19 to 30 kg/m^2 (inclusive), electrocardiogram (ECG) without clinically significant abnormalities, normal renal function, no significant medical history, and good general health.

5. Excluded Medications, Restrictions or Exceptions

- Any prescription medications and OTC medications including herbal products and antacids with the exception of vitamins, and/or acetaminophen and/or ibuprofen and/or hormonal contraceptive medications. However, the short-term use of topical hydrocortisone cream or A&D ointment to treat minor skin irritation due to ECG leads was allowed.
- Subjects were required to refrain from the consumption of food and beverages containing alcohol products, and from consumption of grapefruit juice, grapefruits, and Seville orange juice 72 hours prior to the first dose of study drug and during the course of the study through discharge.
- Subjects were required to refrain from the use of nicotine or nicotine-containing products from screening through discharge.

6. Rationale for Doses Used in the Trial

SOF/VEL/VOX+VOX: In healthy volunteers, a dose of SOF/VEL/VOX 400/100/100 mg +VOX 100 mg has been found to provide similar (for SOF and its metabolite GS-331007) or higher exposure (for VEL and VOX) compared to clinical efficacious exposures in HCV patients under fed conditions. When tested as perpetrator drugs, SOF/VEL/VOX+VOX has been dosed once daily for 7 days before co-administration due to the long half-life of VOX at 30 to 40 hours.

NGM/EE: NGM 0.180 mg/0.215 mg/0.25 mg/EE 0.025 mg was administered according to the information in the Ortho Tri-Cyclen® Lo package insert. For each cycle, subjects took one white tablet containing 0.180 mg norgestimate and 0.025 mg ethinyl estradiol once daily for Day 1-7, one light blue tablet containing 0.215 mg norgestimate and 0.025 mg ethinyl estradiol once daily for Day 8-14, one dark blue tablet containing 0.250 mg norgestimate and 0.025 mg ethinyl estradiol once daily for Day 15-21; and one dark green tablet once daily for Day 22-28.

7. Drugs Used in the Trial

The test product batch numbers were ER1501B2 (SOF/VEL/VOX), DY1502B1 (VOX), and 15BM277B (NGM/EE).

8. Sample Collection, Bioanalysis, Pharmacokinetic Assessments, and Statistical Analysis

Sample Collection
Serial blood samples were collected on Day 14 for Cycles 1 and 2 at the following time points relative to study drug dosing: predose and 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.

Bioanalytical method
Concentrations of SOF, GS-566500, GS-331007, VEL, VOX, NGM, norelgestromin (NGMN, also known as 17-desacetyl norgestimate), norgestrel (NG), and EE in plasma samples were determined using fully validated high-performance liquid chromatography-tandem mass spectrometry (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, VEL, NGM, NGMN, NG, and EE were all performed and validated by [redacted] (b) (4) The assays for VOX were performed and validated [redacted] (b) (4) The standard curve and quality control data indicated that the plasma assay methods for SOF, GS-566500, GS-331007, VEL, NGM, NGMN, NG, and EE were precise and accurate.

PK evaluation
Statistical comparisons of NGMN and NG (active metabolites of NGM) and EE primary PK parameters (AUC_{tau}, C_{max}, and C_{tau}) were conducted between test (NGM/EE + SOF/VELVOX+VOX) and reference (NGM/EE) treatments, and the GLSM ratios and corresponding 90% CIs were calculated. The PK parameters for VOX, VEL, SOF, GS-566500, and GS-331007 were descriptively compared with historical data.

9. Results

9.1 Subject Demographics and Disposition

Total of 15 subjects were enrolled. All were female, non-Hispanic or Latino, and the majority were white (14 subjects, 93.3%). The mean age was 24 years (range: 19 to 37); the mean BMI was 23.4 kg/m2 (range: 19.4 to 29.7); and the mean CLcr was 103.95 mL/min (range: 86.17 to 122.28).

9.2 Pharmacokinetic and Statistical Analysis

NGM plasma concentrations were BLQ (< 50 pg/mL) for all subjects at most time points; therefore, NGM PK parameters were, in general, not calculable.

PK of NGMN (norelgestromin)
Table 1 presents NGMN plasma PK parameters and statistical comparisons following administration of NGM/EE alone or in combination with SOF/VEL/VOX+VOX. NGMN mean plasma exposure parameters (AUC_{tau}, C_{max}, and C_{tau}) and median T_{max} were similar following both treatments. The median $t_{1/2}$ following administration of NGM/EE +SOF/VEL/VOX+VOX was modestly prolonged compared with administration of NGM/EE alone, but did not result in significant changes to NGMN exposure parameters.

Table 1: GS-US-367-1909: NGMN Plasma PK Parameters and Statistical Comparisons Following Administration of NGM/EE Alone or With SOF/VEL/VOX+VOX (NGMN PK Analysis Set) (Source: Sponsor's study report)

NGMN PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	NGM/EE (Reference) (N = 15)	NGM/EE + SOF/VEL/VOX+VOX (Test) (N = 15)	
AUC_{tau} (h•pg/mL)	13,757.4 (18.0)	14,690.4 (14.4)	107.36 (103.19, 111.69)
C_{max} (pg/mL)	1080.9 (20.5)	1162.2 (18.0)	107.71 (97.78, 118.65)
C_{tau} (pg/mL)	364.1 (21.1)	413.9 (17.8)	114.19 (107.40, 121.39)
T_{max} (h)[a]	3.00 (2.00, 4.00)	3.00 (1.50, 4.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a]	18.91 (17.08, 23.42)	28.95 (21.90, 36.02)	—

a Values are presented as median (Q1, Q3).

PK of NG (norgestrel)

Table 2 presents NG plasma PK parameters and statistical comparisons following administration of NGM/EE alone or in combination with SOF/VEL/VOX+VOX. NG mean plasma exposure parameters (AUC_{tau}, and C_{max}) and median T_{max} were similar following both treatments. The median $t_{1/2}$ following administration of NGM/EE + SOF/VEL/VOX+VOX was prolonged and the mean C_{tau} was 21% higher compared with administration of NGM/EE alone. The difference is not considered clinically significant.

Table 2: GS-US-367-1909: NG Plasma PK Parameters and Statistical Comparisons Following Administration of NGM/EE Alone or With SOF/VEL/VOX+VOX (NG PK Analysis Set) (Source: Sponsor's study report)

NG PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	NGM/EE (Reference) (N = 15)	NGM/EE + SOF/VEL/VOX+VOX (Test) (N = 15)	
AUC_{tau} (h•ng/mL)	41.1 (26.9)	47.3 (27.9)	115.14 (106.49, 124.50)
C_{max} (ng/mL)	2.0 (26.1)	2.3 (26.3)	115.02 (108.12, 122.37)
C_{tau} (ng/mL)	1.5 (24.5)	1.8 (29.6)	121.62 (110.85, 133.44)
T_{max} (h)[a]	4.00 (2.50, 8.00)	4.00 (2.50, 6.00)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a]	33.76 (30.76, 78.76)	57.42 (49.41, 82.93)	—

a Values are presented as median (Q1, Q3).

PK of EE (ethinyl estradiol)

Table 3 presents EE plasma PK parameters and statistical comparisons following administration of NGM/EE alone or in combination with SOF/VEL/VOX+VOX. The mean plasma exposure parameters (AUC_{tau} and C_{tau}) of EE and the median T_{max} and $t_{1/2}$ were similar following both treatments. The median C_{max} following administration of NGM/EE +

SOF/VEL/VOX+VOX was 21% higher compared with administration of NGM/EE alone. The magnitude of the increase of EE C_{max} is not considered clinically significant.

Table 3: GS-US-367-1909: EE Plasma PK Parameters and Statistical Comparisons Following Administration of NGM/EE Alone or With SOF/VEL/VOX+VOX (EE PK Analysis Set) (Source: Sponsor's study report)

EE PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI) Test/Reference
	NGM/EE (Reference) (N = 15)	NGM/EE + SOF/VEL/VOX+VOX (Test) (N = 15)	
AUC_{tau} (h•pg/mL)	835.2 (44.0)	871.4 (40.8)	105.43 (96.95, 114.66)
C_{max} (pg/mL)	68.4 (45.4)	80.6 (35.3)	121.06 (106.10, 138.12)
C_{tau} (pg/mL)	19.7 (55.0)	18.2 (57.1)	92.86 (82.55, 104.45)
T_{max} (h)[a]	3.00 (1.50, 3.00)	2.00 (1.50, 3.13)	—
T_{last} (h)[a]	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	—
$t_{1/2}$ (h)[a]	13.68 (10.01, 21.06)	10.78 (9.48, 15.67)	—

a Values are presented as median (Q1, Q3).

PK of SOF, GS-566500, GS-331007, VEL and VOX

Table 4 presents SOF, GS-566500, GS-331007, VEL, and VOX plasma PK parameters following administration of NGM/EE with SOF/VEL/VOX+VOX. The mean exposures observed for SOF, GS-566500, GS-331007, VEL, and VOX are generally within the range of observed exposures in healthy volunteers following administration of SOF/VEL/VOX+VOX.

Table 4: GS-US-367-1909: SOF, GS-566500, GS-331007, VEL, and VOX Plasma PK Parameters Following Administration of NGM/EE with SOF/VEL/VOX+VOX (Source: Sponsor's study report)

PK Parameters	Mean (%CV) (NGM/EE + SOF/VEL/VOX+VOX)				
	SOF	GS-566500	GS-331007	VEL	VOX
AUC_{tau} (h•ng/mL)	1997.2 (40.2)	2769.3 (16.1)	12,098.9 (15.9)	8226.3 (25.0)	3857.9 (31.5)
C_{max} (ng/mL)	967.6 (33.3)	491.1 (21.9)	979.4 (12.2)	853.3 (18.9)	512.4 (33.5)
C_{tau} (ng/mL)	-	-	-	154.6 (30.2)	56.1 (105.2)
T_{max} (h)[a]	2.50 (1.00, 3.00)	4.00 (3.00, 4.00)	4.00 (4.00, 6.00)	4.00 (4.00, 6.00)	6.00 (4.00, 6.02)
$t_{1/2}$ (h)[a]	0.68 (0.45, 0.81)	2.70 (2.61, 3.04)	30.06 (24.96, 75.66)	19.75 (15.46, 24.92)	8.51 (7.56, 13.55)
CL_{ss}/F (mL/h)	218,383.1 (24.4)	-	-	12,757.3 (21.0)	56,071.5 (27.2)

[a] Values are presented as median (Q1, Q3).

9.3 Pharmacodynamic Evaluation

Luteinizing hormone and FSH serum concentrations were analyzed on Cycle Day 14 and progesterone serum concentrations were analyzed on Cycle Day 21 in each cycle of the study.

Luteinizing hormone, FSH, and progesterone serum concentrations were similar across both treatments (Table 5). Median concentrations of LH and FSH were lower than those expected for the ovulatory phase (reference ranges: 8.7 to 76.3 mIU/mL and 3.1 to 17.7 mIU/mL, respectively) (b) (4) consistent with decreased LH and FSH serum concentrations caused by oral hormonal contraceptives. Progesterone median values were substantially lower than those expected for the luteal phase (reference range: 2.6 to 21.5 ng/mL) (b) (4) consistent with absence of ovulation.

Table 5: GS-US-367-1909: Summary of LH, FSH, and Progesterone Concentrations Following Administration of NGM/EE Alone or With SOF/VEL/VOX+VOX (Source: Sponsor's study report)

	Median (Q1, Q3)	
PD Analyte	**NGM/EE (N = 15)**	**NGM/EE + SOF/VEL/VOX+VOX (N = 15)**
LH (mIU/mL)	3.30 (0.90, 5.30)	2.80 (0.10, 4.70)
FSH (mIU/mL)	2.90 (1.20, 4.20)	2.40 (0.90, 3.40)
Progesterone (ng/mL)	0.57 (0.41, 0.72)	0.46 (0.41, 0.72)

9.4 Safety Analysis

Overall, 14 of 15 subjects (93.3%) experienced at least 1 AE. All AEs were Grade 1 or 2 in severity; no Grade 3 or 4 AEs, no SAEs, no AEs leading to permanent discontinuation of study drug or study, and no deaths were reported during the study. Overall, 13 of 15 subjects (86.7%) experienced at least 1 laboratory abnormality. Most laboratory abnormalities were Grade 1 in severity, and no Grade 4 laboratory abnormalities were reported. Grade 3 laboratory abnormalities were reported for 2 subjects (13.3%). One subject (08142-1013) had Grade 3 occult blood in urine on Study Day 5 during treatment with NGM/EE alone. The other subject (08142-1008**)** had a Grade 3 elevation in ALT and LDL. Here is the narrative for the patient from the study report:

> *"Subject 08142-1008, a 19-year-old white female, had a Grade 3 elevation in ALT. Screening, baseline, and Study Day 35 laboratory values were within normal limits. On Study Day 43 after completion of dosing with NGM/EE + SOF/VEL/VOX+VOX, ALT, AST, and ALP increased to 91 U/L (Grade 2), 52 U/L, and 164 U/L, respectively. Repeat testing on Study Day 49 indicated that ALT, AST, and ALP were 183 U/L (Grade 3), 64 U/L (Grade 1), and 158 U/L, respectively. On Study Day 50, ALT and AST remained high and ALP was within normal limits. On Study Day 56, ALT, AST, and ALP decreased to 76 U/L (Grade 2), 26 U/L (within normal limits), and 131 U/L (within normal limits), respectively. By Study Day 63, 7 days*

after completion of study drug dosing, ALT, AST, and ALP were within normal limits. Total and direct bilirubin values were normal throughout the study.

This subject also had a Grade 3 elevation in low-density lipoprotein (LDL), which was 200.77 mg/dL (Grade 3) on Study Day 56. Baseline LDL was 166.02 mg/dL (Grade 2), Study Day 43 LDL was 142.86 mg/dL (Grade 1), and Study Day 63 LDL was 181.47 mg/dL (a grade could not be assigned because sampling occurred in a nonfasting state)."

The individual exposures to SOF, GS-331007, VEL, VOX, NGMN, NG and EE for Subject 08142-1008 were compared to other subjects in the study. The exposures for GS-331007, VOX, NGMN, NG and EE for this subject were within the range of other subjects. However, the exposures to SOF and VEL are the highest for Subject 08142-1008 among all the subjects. SOF AUC was 4690 ng*h/mL for Subject 1008, while SOF AUC for other subjects ranged from 1255 to 2296 ng* h/mL with a geometric mean of 1780 ng*h/mL. Likewise, VEL AUC was 13662 ng*h/mL for Subject 1008, while VEL AUC for other subjects ranged from 5739 to 11007 ng*h/mL, with a geometric mean of 7719 ng*h/mL.

No Grade 3 ALT or LDL elevation was observed in the previous reviewed drug-drug interaction study between VEL and NGM/EE (Study GS-US-281-1058) or drug-drug interaction study between SOF and NGM/EE (Study GS-US-334-0146). However, VEL AUC in Study GS-US-281-1058 (range: 2053 -8638 ng.h/mL) or SOF AUC in Study GS-US-334-0146 (range: 266-2436 ng.h/mL) were generally lower than observed in this study. Additional assessments are conducted by the review team to evaluate the risk of ALT elevation for all subjects taking oral contraceptives in 4 Phase 3 trials (GS-US-367-1170, GS-US-1171, GS-US-1172, and GS-US-1173). A total of 13 subjects in Phase 3 took oral contraceptives (OCs). A total of 4 subjects in Phase 3 studies were identified as grade 3 ALT events, of which 2 were administered placebo. None of these 4 subjects took OCs. In addition, 12 subjects experienced Grade 2 ALT events, but none of these subjects took OCs.

Subject 08142-1008 in this study had higher SOF AUC than any subject in Phase 3 studies, while VEL is also higher than other subjects except one in Phase 3 trials (GS-US-367-1172-02760-26211: VEL AUC_{tau}: 14395). Subject 26211 was classified as Grade 2 ALT event. This subject did not take OCs during the study.

Although VEL and VOX are substrates of CYP3A and NGM/EE is a weak CYP3A inhibitor, it is not expected that the effect of NGM/EE on VEL or VOX PK is substantial. This was demonstrated by the similar results from this study (other than Subject 08142-1008) and the historical data for VEL and VOX. It is not clear why Subject 08142-1008 has higher SOF and VEL exposures. It is not clear whether ALT elevation in this patient was due to elevated exposure of SOF and/or VEL or was due to the administration of SOF/VEL/VOX itself as observed in Phase 3 trials. Because none of the subjects who experienced Grade 2 or 3 ALT elevation was associated with OC administration, it is hard to relate this event to either PK or PD drug-drug interactions.

10. Conclusions

- Coadministration of SOF/VEL/VOX+VOX with NGM/EE does not affect the exposures of NGMN, NG and EE.
- The mean exposures of VOX, VEL, SOF, GS-566500, and GS-331007 were within the ranges of exposures observed in historical data.
- No loss of contraceptive efficacy is expected upon coadministration of SOF/VEL/VOX with oral contraceptives.
- Although one subject in this study experienced Grade 3 ALT elevation, it may not be related to a drug interaction between NGM/EE and SOF/VEL/VOX.

4.4.13 In Vitro Study Reports

Part 1: Overall summary

1. Absorption

- AD-230-2074: Bi-Directional Permeability of GS-9857 through Caco-2 Cell Monolayers

Summary: GS-9857 has high forward permeability (P_{app} = 4.36 x 10^{-6} cm/s) through monolayers of caco-2 cells with evidence of efflux transport.

2. Distribution

- AD-338-2005: *In Vitro* Protein Binding Determination of GS-9857 by Equilibrium Dialysis
- AD-338-2013: *In Vitro* Assessment of Blood Distribution of GS-9857

Summary: GS-9857 shows high protein binding across all species tested, with < 1% unbound; GS-9857 is predominantly distributed to plasma with blood to plasma concentration ratio at 0.78 for human.

3. Metabolism and elimination

- AD-338-2018: *In Vitro* Metabolic Stability of GS-9857 in Hepatic Microsomal Fractions and Human Hepatocytes
- AD-338-2006: Cytochrome P450 Metabolic Reaction Phenotyping of GS-9857
- AD-338-2027: Identification of Metabolites in Samples from *In Vitro* Metabolism of ^{3}H-GS-9857 by Mouse, Rat, Dog, Monkey, and Human Hepatic Microsomes

Summary: GS-9857 has low human hepatic metabolic clearance; there is no human specific metabolite(s); GS-9857 is a substrate of CYP2C8, CYP3A4 and CYP1A2.

4. Drug interaction potential

- AD-338-2008: *In Vitro* Assessment of Human Liver Cytochrome P450 Inhibition Potential of GS-9857
- AD-338-2035: *In Vitro* Assessment of Human Hepatic Microsomal Cytochrome P450 Mechanism-Based Inhibition Potential of Voxilaprevir
- AD-338-2009: *In Vitro* Assessment of Human UGT1A1 Inhibition Potential of GS-9857
- AD-338-2034: Microsomal Binding of Voxilaprevir
- AD-338-2007: *In Vitro* Assessment of Induction Potential of GS-9857 in Humans
- AD-338-2026: Evaluation of Induction Potential of GS-9857 in Cultured Human Hepatocytes
- AD-338-2012: Bi-Directional Permeability of GS-9857 through Monolayers of P-glycoprotein and BCRP Over-expressing Cells
- AD-338-2011 (Addendum 1): *In Vitro* Assessment of GS-9857 Uptake in Human Hepatocytes
- AD-338-2037: *In Vitro* Assessment of GS-9857 as Substrate for Human OCT1 Transporter
- AD-338-2010: *In Vitro* Inhibition Assessment of GS-9857 with Human P-gp and BCRP
- AD-230-2075: *In Vitro* Assessment of GS-9857 Inhibition of Human OATP1B1 and OATP1B3
- AD-338-2020: *In Vitro* Assessment of GS-9857 Inhibition of Human MRP2 and BSEP
- AD-338-2036: *In Vitro* Assessment of GS-9857 Inhibition of Human OCT1, OCT2, OAT1, OAT3, and MATE1 Transporters

Summary:
GS-9857 shows no clinical relevant inhibition ($I/K_i < 0.1$ or AUCR < 1.25) or induction potential on CYP and UGT1A1 enzymes, which is confirmed by clinical DDI studies with minimal effect on following drugs: darunavir and rilpivirine (CYP3A4 substrates) and elvitegravir (CYP3A and UGT1A1/3 substrate).

GS-9857 is a substrate of P-gp, BCRP, and hepatic uptake transporters. GS-9857 shows no inhibition on P-gp and BCRP at concentrations up to 10 μM, however, the estimated maximal intestinal VOX concentration at 460 μM far exceeds 10 μM. GS-9857 shows significant inhibition on OATP1B1 (IC_{50} at 0.18 μM), OATP1B3 (IC_{50} at 0.70 μM), BSEP (IC_{50} at 1.5 μM), and shows no inhibition on renal transporters OCT1, OCT2, OAT1, OAT3, and MATE1 at concentrations up to 23 μM.

Note: there was no %recovery data to validate the actual inhibition concentrations in all transporter inhibition assays (GS-9857 has low solubility and > 99% protein binding, and has the potential for non-specific binding or precipitation; 0.5 to 1% BSA was added to the receiver cells in the transporter substrate assays to improve the %recovery with unknown effects on the results).

The following table provides the pharmacokinetic parameters for VOX following oral administration of the SOF/VEL/VOX FDC at 400/100/100 mg to HCV patients under fed conditions, used for the assessment of the potential for drug-drug interactions.

PK parameters	**VOX**
Dose (mg)	100
Total C_{max} (μM)[a]	0.221
Unbound C_{max} (μM)[b]	0.00221[e]
Intestinal (μM)[c]	460
$C_{hep,\ inlet}$ (μM)[d]	7.67
Unbound $C_{hep,\ inlet}$ (μM)[b]	0.0767[e]

[a] Values are the mean C_{max} based on population PK modeling from HCV-infected subjects.
[b] Concentration based on an arbitrary 1% free concentration used to assess DDI potential.
[c] Intestinal concentrations are calculated based on the water volume of 250 mL.
[d] Liver inlet concentration = $C_{max}+(k_a*dose*F_aF_g/Q_h)$; where K_a and F_aF_g have not been determined and are assumed to be 0.1 min^{-1} and 1, respectively. Q_h for human is 1500 mL/min.
[e] Molecular weight of VOX: 869 g/mole.

Based on an estimated unbound hepatic inlet concentration of 0.0767 μM and IC_{50} value at 0.18 and 0.70 μM, the R value is 1.43 and 1.11 for OATP1B1 and OATP1B3, respectively. The R values are greater or close to 1.25, and thus VOX (GS-9857) showed some potential to inhibit the hepatic uptake transporters OATP1B1 and OATP1B3 during first pass. VOX may have the potential to inhibit the intestinal P-gp and BCRP due to the high intestinal concentration (460 μM *vs.* IC_{50} > 10 μM).

Part 2: Individual *in vitro* study review

AD-230-2074: Bi-Directional Permeability of GS-9857 through Caco-2 Cell Monolayers

Introduction

This study assessed the absorption potential of GS-9857 by doing bi-directional permeability studies *in vitro* using a human colon carcinoma cell line (caco-2).

Materials and Methods

Experiments were conducted using HBSS buffer containing 10 mM HEPES, 15 mM glucose. The donor well used HBSS buffer supplemented with 0.1% BSA. Donor buffers had their pH adjusted to pH 6.5. The receiver well used HBSS buffer supplemented with 1% BSA. Receiver buffers had their pH adjusted to pH 7.4. The experiment was started by the addition of buffers containing dronedarone and 100 µL of solution was taken at 1 and 2 hrs from the receiver compartment. Removed buffer was replaced with fresh buffer and a correction applied to all calculations for the removed material. The compound was tested in 2 separate replicate wells for each condition. All samples were immediately collected into 400 µL 100% acetonitrile to precipitate protein and stabilize test compounds. Cells were dosed on the apical (A) or basolateral (B) side to determine forward (A to B) and reverse (B to A) permeability. Permeability through a cell free trans-well was also determined as a measure of cellular permeability through the membrane. To test for non-specific binding and compound instability the total amount of drug was quantified at the end of the experiment and compared to the material present in the original dosing solution as a percent recovery. Samples were analyzed by LC/MS/MS.

The apparent permeability, P_{app}, and % recovery were calculated as follows:

$$P_{app} = (dR/dt) \times V_r/(A \times D_0)$$

$$\% \text{ Recovery} = 100 \times ((V_r \times R_{120}) + (V_d \times D_{120}))/ (V_d \times D_0)$$

Where, dR/dt is the slope of the cumulative concentration in the receiver compartment versus time in µM/s based on receiver concentrations measured at 60 and 120 minutes.
V_r and V_d is the volume in the receiver and donor compartment in cm^3, respectively.
A is the area of the cell monolayer (0.33 cm^2).
D_0 and D_{120} is the measured donor concentration at the beginning and end of the experiment, respectively.
R_{120} is the receiver concentration at the end of the experiment (120 minutes).

Results

GS-9857 had high permeability in cell-free wells suggesting limited association with either plastic or the semi-permeable membrane in the transwell apparatus. When dosed at 10 µM, GS-9857 showed high forward and reverse permeability in caco-2 cells with P_{app} values of 4.4×10^{-6} cm/s and 13×10^{-6} cm/s, respectively. The efflux ratio of 3 suggests that GS-9857 is a substrate for intestinal efflux transporter(s) (Table 1).

Table 1: Bi-Directional Permeability of GS-9857 Through Caco-2 Cell Monolayers
(source: Study Report Table 1)

Assay #	Direction	Target Conc. (µM)	Initial Conc. (µM)	Recovery (%)	P_{app} (10^{-6} cm/s)			Efflux Ratio
					R1	R2	Average	
2012_BW020	Cell-Free	10	7.42	127.50	43.75		**43.75**	3.0
	Forward		8.21	85.47	5.23	3.48	**4.36**	
	Reverse		8.66	128.70	10.37	15.73	**13.05**	

Conclusion
GS-9857 showed high forward permeability (P_{app} = 4.36 x 10^{-6} cm/s) through monolayers of caco-2 cells with evidence of efflux transport.

Reviewer's comments:
The 0.1% BSA was added to the donor side, and 1% BSA was added to the receiver side in the HBSS buffer. Small amount BSA can help to reduce non-specific binding, and thus improve the recovery %. However, the influence on the experiment results is unknown. *(For VEL (GS-5816), which also has low solubility and high protein binding, permeability values could not be reliably obtained due to low recovery of the compound and poor reproducibility.)*

AD-338-2005: *In Vitro* Protein Binding Determination of GS-9857 by Equilibrium Dialysis

Introduction
This study evaluated the protein binding of GS-9857 in plasma from Sprague-Dawley rats, beagle dogs, cynomolgus monkeys, rhesus monkeys, and humans by equilibrium dialysis. Additionally, the relative protein binding of GS-9857 to cell culture medium (CCM) and human plasma was also determined by direct competitive dialysis.

Materials and Methods
The binding of GS-9857 in plasma from Sprague-Dawley rats, beagle dogs, cynomolgus monkeys, rhesus monkeys, and humans was performed (b) (4) The competitive dialysis studies between CCM and human plasma were performed at Gilead Sciences, Inc.

Commercially available chemicals were obtained (b) (4) The CCM was Gibco Dulbecco's Modified Eagle Medium (DMEM) with 10% (v/v) fetal bovine serum (HyClone) which was obtained (b) (4) Pooled plasma (from at least 3 males and 3 females) was from Sprague-Dawley rats, beagle dogs, cynomolgus monkeys, rhesus monkeys, and humans which was obtained (b) (4) Sodium EDTA was used as the anticoagulant. CCM had the same composition as that used for cell-based antiviral potency assays.

Plasma protein binding assay:
Sprague- Dawley rat, beagle dog, cynomolgus monkey, rhesus monkey, and human plasma were spiked with GS-9857 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.

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

$\mathbf{C_f}$ = post-dialysis buffer concentration
$\mathbf{C_t}$ = 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. Both matrices were spiked with GS-9857 to a final concentration of 2 µM. The relative binding was calculated using the following equation.

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

Results
GS-9857 was highly protein bound in plasma from all tested species (free fraction < 1%) (Table 1). The plasma to CCM concentration ratio was 12, reflecting much higher %unbound in CCM, compared to %unbound in plasma (Table 2).

Table 1: Protein binding of GS-9857 in plasma from different species
(source: Study Report Table 1)

Matrix	Conc. (µM) [a]	Unbound (%)[b]	Bound (%)[b]	Study
Sprague-Dawley Rat Plasma	2	0.36 ± 0.03	99.64 ± 0.03	(b) (4) # 60D-1287
Beagle Dog Plasma	2	0.21 ± 0.07	99.79 ± 0.07	
Cynomolgus Monkey Plasma	2	0.44 ± 0.05	99.56 ± 0.05	
Rhesus Monkey Plasma	2	0.53 ± 0.16	99.47 ± 0.16	
Human Plasma	2	0.39 ± 0.05	99.61 ± 0.05	

a: Initial concentration in protein-containing dialysis cell
b: Values represent the mean ± standard deviation (n = 3)

Table 2: Protein Binding Shift for GS-9857 in CCM to Human Plasma
(source: Study Report Table 2)

Matrix	Conc. (µM) [a]	Ratio [b]	Mean Ratio [c]	Study
CCM to Human Plasma shift	2	11.7	11.8 ± 3.0	Gilead# 120622-339
		11.2		Gilead# 120716-342
		12.4		Gilead# 120917-350

a: Initial concentration in protein-containing dialysis cell
b: Values are mean of duplicate measurements
c: Values are the mean ± standard deviation (n=3)

Conclusion
The extent of protein binding of GS-9857 in plasma was high; less than 1% free in all species tested. In the competitive dialysis study, the %unbound of GS-9857 in human plasma was 12-fold lower than in CCM. Overall, these data suggest that the CCM-human plasma protein binding adjustment to the cellular potency of GS-9857 ("protein shift") is moderate and plasma protein binding may play an important role in the pharmacokinetics and pharmacodynamics of GS-9857.

AD-338-2013: In Vitro Assessment of Blood Distribution of GS-9857

Introduction
This study determined the blood to plasma concentration ratio of GS-9857 in beagle dogs and human.

Materials and Methods
All assays were performed (b) (4) (Study: 174-R211). Heparinized blood samples from beagle dogs and humans (n ≥ 2) were obtained and the hematocrit values determined. Reference plasma and reference cell fractions were then prepared by centrifugation. GS-9857 or positive controls were incubated in triplicate, at an initial concentration of 0.5 µM, with blood or reference plasma or reference cell fraction for 60 min at 37°C and then chilled on ice. Blood samples were then centrifuged at 4°C to separate the cellular and soluble fractions. Concentrations of compound in the reference plasma samples, reference cell samples, plasma fractions from blood, and cellular fractions from blood were then determined by LC-MS/MS after de-proteination with ice cold methanol. Blood cell fractions were first lysed by three cycles of freeze-thaw.

Blood/Plasma Ratio Values: Whole blood/plasma concentration ratios (λ) and blood cell/plasma concentration ratios (CPR) were calculated using the following equations (where H is the hematocrit):

$$\lambda = CPR \bullet H + (1 - H)$$

$$CPR = \frac{\lambda - (1 - H)}{H}$$

Results

The cell/plasma concentration ratios for GS-9857 were < 1 for both species, indicating that the compound is not preferentially associated with blood cells. The dog blood/plasma ratio was the lowest ($\lambda = 0.59$), with humans showing relatively more homogeneous distribution within blood ($\lambda = 0.78$) (Table 1).

Table 1: Blood Cell/Plasma Concentration Ratios (CPR) and Whole Blood/Plasma Concentration Ratios (λ) for GS-9857 and Controls

(source: Study Report Table 1)

	GS-9857		Control	
Species	**CPR**	**λ**	**Compound**	**λ**
Beagle Dog	0.09 ± 0.04	0.59 ± 0.02	Chloroquine	3.74 ± 1.03
Human	0.51 ± 0.23	0.78 ± 0.11	Methazolamide	130 ± 15.2

Conclusion

At an initial whole blood concentration of 0.5 µM GS-9857 is relatively excluded from the cellular fraction of dog blood and shows more homogeneous distribution within human blood (whole blood/plasma concentration ratios of 0.59 and 0.78, respectively).

AD-338-2018: In Vitro Metabolic Stability of GS-9857 in Hepatic Microsomal Fractions and Human Hepatocytes

Introduction

This study evaluated the rates of metabolism of GS-9857 in BALB/c mouse, Sprague-Dawley rat, beagle dog, cynomolgus monkey and human hepatic microsomal fractions, and primary human hepatocytes to predict hepatic metabolic clearance.

Materials and Methods

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

Internal Standard/Quench (IS/Q) solution was 50 nM GS-224337 in 25% (v/v) acetonitrile, 25% (v/v) water, and 50% (v/v) methanol. 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 standard. Metabolic stability was assessed in the reaction mixture containing 3 µM test compound (GS-9857 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 100 mM potassium phosphate buffer (pH 7.4). At 0, 2, 12, 25, 45, and 65 min, 25 µl aliquots of the reaction mixture were transferred to

plates containing 225 µl of IS/Q quenching solution. After quenching, the plates were centrifuged and aliquots of the supernatant were analyzed by LC-MS.

Metabolic Stability in Cryopreserved Hepatocytes

To the cell suspension of thawed cryopreserved hepatocytes, KHB medium was added to obtain a target density of 2×10^6 cells/ml. For incubations, aliquots of hepatocyte suspension were mixed with GS-9857 or metabolic stability controls (7-hydroxycoumarin and testosterone) to achieve a final concentration of 2 µM. The incubation was carried out with gentle shaking at 37°C under a humid atmosphere of 95% air/5% CO_2 (v/v). 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 LC-MS.

Rate of GS-9857 disappearance was calculated using the following equation.

$$C_t = C_0 \bullet e^{-K \bullet t} \text{ and } T_{1/2} = \ln 2 / K \text{ where}$$

C_t: % of parent remaining at time = t
C_0: % of parent remaining at time = 0
t: time (hr)
K: first order elimination rate constant (hr^{-1})
T½: *in vitro* half-life (hr)

The hepatic clearance was predicted using the following equations.

$$CL_{int} = {}^{K \bullet V \bullet Y_P}/_{P} \text{ or } CL_{int} = {}^{K \bullet V \bullet Y_H}/_{H}$$

$$CL_h = (CL_{int} \bullet Q_h)/(CL_{int} + Q_h), \text{ where}$$

CL_h: predicted hepatic clearance (L/hr/kg body weight)
CL_{int}: intrinsic hepatic clearance (L/hr/kg body weight)
K: first order elimination rate constant (hr^{-1})
V: incubation volume (L)
Y_P: microsome protein yield (mg protein/kg body weight)
Y_H: hepatocyte yield (millions of hepatocytes/kg body weight)
P: mass of protein in the incubation (mg)
H: number of hepatocytes in the incubation (million)
Q_h: hepatic blood flow (L/hr/kg body weight)

Values used for calculation of the predicted hepatic clearance are presented in the tables below.

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Table 1: Values Used for Calculation of the Predicted Hepatic Clearance from Microsomal Stability ($Y=Y_P$)
(source: Study Report Table 1)

Species	Hepatic Microsomes			Q_h (L/kg)
	V (L)	P (mg)	Y (mg/kg)	
Mouse	0.001	0.5	3938	5.4
Rat	0.001	0.5	1800	4.2
Dog	0.001	0.5	1440	1.8
Cynomolgus Monkey	0.001	0.5	810	1.6
Human	0.001	0.5	1157	1.3

Table 2: Values Used for Calculation of the Predicted Hepatic Clearance from Hepatocyte Stability ($Y=Y_H$)
(source: Study Report Table 2)

Species	Hepatocytes			Q_h (L/kg)
	V (L)	H (million)	Y (million/kg)	
Human	0.001	1.0	3086	1.3

Results
The stability of GS-9857 in hepatic microsomal fractions and hepatocytes is expressed as half-life, predicted hepatic metabolic clearance and percent hepatic extraction and the values are given in Table 3 and Table 4. GS-9857 was stable to moderately stable in hepatic microsomal fractions and hepatocytes from all nonclinical species except for cynomolgus monkey, with predicted hepatic extraction values less than 16 % of the hepatic blood flow. GS-9857 was stable in human microsomal fractions and in human hepatocytes (< 10% loss of parent over the course of the incubation).

Table 3: *In Vitro* Rate of Metabolism of GS-9857 in Hepatic Microsomes
(source: Study Report Table 3)

Species	$t_{½}$ (min)	Predicted Hepatic Cl (L/hr/kg)	Predicted Hepatic Extraction (%)
BALB/c Mouse	341	0.82	15.1
Sprague-Dawley Rat	> 395	< 0.35	< 8.3
Beagle Dog	> 395	< 0.26	< 14.4
Cynomolgus Monkey	42	0.80	50
Human	> 395	< 0.21	< 15.8

Table 4: *In Vitro* Rate of Metabolism of GS-9857 in Primary Hepatocytes
(source: Study Report Table 4)

Species	$t_{½}$ (hr)	Predicted Hepatic Cl (L/hr/kg)	Predicted Hepatic Extraction (%)
Human	> 39.5	< 0.07	< 5.1

Conclusion
GS-9857 was stable to moderately stable in hepatic microsomal fractions from all nonclinical species except cynomolgus monkey. GS-9857 was stable in human microsomal fractions and hepatocytes.

AD-338-2006: Cytochrome P450 Metabolic Reaction Phenotyping of GS-9857

Introduction
This *in vitro* study evaluated whether GS-9857 was a substrate for particular CYP enzymes. GS-9857 was incubated with seven individual cDNA expressed human CYP enzyme preparations. Compounds known to be metabolized by each CYP450 enzyme were used as a positive control.

Materials and Methods
This study was conducted (b) (4) Bacterially expressed human CYP enzyme preparations co-expressed with human NADPH cytochrome P450 reductase were supplied (b) (4) All other chemicals were from (b) (4) equivalent vendors.

GS-9857 (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 in 0.1 M phosphate buffer pH 7.4. NADPH (final concentration at 1 mM) 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 LC-MS.

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:

$$Rate = \frac{\ln 2}{T_{1/2}} \times \frac{[S]}{[CYP]}$$

[S] = Substrate concentration (5000 pmol/mL)
[CYP] = CYP protein concentration (pmol/mL)

Results
Rates of metabolism of GS-9857 and the positive controls by individual CYP enzymes are reported in Table 1. GS-9857 was not a substrate for recombinant CYP1A2, CYP2C9, CYP2C19, or CYP2D6. Metabolism of GS-9857 with CYP2B6, CYP2C8, and CYP3A4 was detectable; however, observed rate of metabolism was low.

Table 1: Rates of metabolism of GS-9857 and control substrates by major human CYP enzymes (source: Study Report Table 1)

	Metabolism Rate (min^{-1})						
Compound	**CYP1A2**	**CYP2B6**	**CYP2C8**	**CYP2C9**	**CYP2C19**	**CYP2D6**	**CYP3A4**
GS-9857 (% Positive Control)	0.16 (1.1%)	< 0.12 (< 4.2%)	1.37 (6.2%)	< 0.47 (< 1%)	< 0.12 (< 19.5%)	< 0.23 (< 0.7%)	0.82 (8.1%)
Ethoxycoumarin	14.9	-	-	-	-	-	-
Efavirenz	-	2.76[a]	-	-	-	-	-
Amodiaquine	-	-	22.2	-	-	-	-
Diclofenac	-	-	-	44.7	-	-	-
Diazepam	-	-	-	-	0.6[b]	-	-
Dextromethorphan	-	-	-	-	-	34.5	-
Testosterone	-	-	-	-	-	-	10.2

a Efavirenz is a selective substrate for CYP2B6 but is metabolized slowly
b Diazepam is a selective substrate for CYP2C19 but is metabolized slowly

Conclusion
GS-9857 was not a substrate for recombinant human CYP2B6, CYP2C9, CYP2C19 or CYP2D6, and there was relatively slow metabolism by CYP2C8, CYP3A4, and CYP1A2, indicating that GS-9857 is a poor substrate for these enzymes.

AD-338-2027: Identification of Metabolites in Samples from In Vitro Metabolism of ^{3}H-GS-9857 by Mouse, Rat, Dog, Monkey, and Human Hepatic Microsomes

Introduction
This study identified the metabolites in samples from *in vitro* metabolism of ^{3}H-GS-9857 by mouse, rat, dog, monkey, and human hepatic microsomes.

Materials and Methods

This study was conducted [redacted] (b) (4) Samples from *in vitro* metabolism of ^{3}H-GS-9857 by mouse, rat, dog, monkey, and human hepatic microsomes were provided by the Sponsor. A 400 µL aliquot of each sample was transferred to separate micro-centrifuge tubes. The solvent was evaporated under a stream of nitrogen at ambient temperature. Each sample was reconstituted in 100 µL of acetonitrile (ACN):reverse osmosis (RO) water (95:5, v:v), sonicated, and vortex mixed. Each sample was transferred to a new tube. Duplicate aliquots were analyzed by liquid scintillation counting (LSC). Due to poor recovery, 300 µL of 0.05% trifluoroacetic acid in ACN:RO water (95:5,v:v) was added to the residue from each sample and reconstituted using the above method. The reconstituted samples from each respective sample were combined and evaporated to dryness. The samples were reconstituted in 100 µL of 0.05% trifluoroacetic acid in ACN:RO water (95:5,v:v), sonicated, and vortex mixed. Duplicate aliquots were analyzed by LSC.

Results

A summary of metabolites identified in *in vitro* incubation of hepatic microsomes from mouse, rat, dog, monkey, and human is presented in Table 1.

^{3}H-GS-9857 underwent limited metabolism in mouse, rat, dog, and human hepatic microsomes, with the majority of the radioactivity associated with unchanged GS-9857, ranging from approximately 81 to 97% of the sample radioactivity. Minor components identified in mouse, rat, dog, and human microsome incubations were oxy-GS-9857-1 (M7), dehydro-GS-9857-1 (M8), dehydro-GS-9857-2 (M9), and oxy-GS-9857-3 (M11).

Significant metabolism was observed in monkey hepatic microsomes, which showed 12 radioactive peaks. Unchanged GS-9857 was a major component in monkey microsomes, representing approximately 37 to 49% of the sample radioactivity. Oxy-GS-9857-1 (M7) and dehydro-GS-9857-2 (M9) were the major components in monkey microsomes.

Table 2: Metabolites identification of GS-9857 from incubation with mouse, rat, dog, monkey and human hepatic microsomes

(source: Study Report In-Text Table)

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Metabolite Designation	Retention Time (Minutes)[a]	$[M+H]^+$	Proposed Identification	Mouse	Rat	Dog	Monkey	Human
M13	31.80	901	Dioxy-GS-9857-1				X	
M14	33.00	901	Dioxy-GS-9857-2				X	
M15	33.70 - 33.80	865	Didehydro-GS-9857				X	
M7	36.40 - 36.50	885	Oxy-GS-9857-1	X	X		X	X
M8	37.30 - 37.40	867	Dehydro-GS-9857-1		X	X	X	X
M9	38.00 - 38.10	867	Dehydro-GS-9857-2	X	X	X	X	X
M10	40.20 - 40.30	865	Desfluoro-oxy-GS-9857			X	X	
M16	41.50 – 41.70	885	Oxy-GS-9857-2				X	
M11	45.30 - 45.50	885	Oxy-GS-9857-3	X	X	X	X	X
GS-9857	49.00	869	GS-9857	X	X	X	X	X

Note: Metabolites found in species designated with "X".
a Retention time ranges from all species (Method 1).

Conclusion

All the metabolites present in the human hepatic microsomes were present in the other species evaluated, with the exception of M7 and M8 which were not detected in dog and mouse, respectively.

AD-338-2008: In Vitro Assessment of Human Liver Cytochrome P450 Inhibition Potential of GS-9857

Introduction

This study evaluated the potential for GS-9857 to inhibit the activities of seven major human drug-metabolizing cytochrome P450 enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A), using isoform-specific probe substrates in human liver microsomal fractions.

Materials and Methods

Assays were conducted [redacted] (b) (4) Probe substrates were incubated with human liver microsomes and NADPH at 37ºC in the presence of GS-9857 (up to 25 µM) or control inhibitors (Table 1). 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 K_m values. The rates of enzyme-specific metabolite formation from probe substrates by hepatic microsomal fractions were determined in the presence and absence of test compound and, where possible, IC_{50} values were determined.

Table 1. CYP isoform-specific probe substrates and control inhibitors

CYP isoform	Probe substrate	Conc. (µM)	Incu. time (min)	Control inhibitor	Conc. range (µM)
CYP1A2	phenacetin	30	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-9857 had no effect (IC_{50} > 25 µM) on the activities of CYP1A2, CYP2C9, CYP2C19 or CYP2D6, or of CYP3A with testosterone 6β-hydroxylase as the probe activity. Inhibition of CYP2B6, CYP2C8, and CYP3A with midazolam 1'-hydroxylase as the probe activity was weak (IC_{50} 19, 11.2 and 16.7 µM respectively). The human plasma C_{max} for GS-9857 was approximately 0.22 µM based on population PK analysis when administered as SOF/VEL/VOX FDC at 400/100/100 mg under fed conditions. All IC_{50} values exceed this by >50-fold (I/K_i < 0.1). Thus GS-9857 is unlikely to cause drug interactions *in vivo* through inhibition of human CYP enzymes. In contrast, control inhibitors had low IC_{50} values, indicating potent inhibition.

Table 2: Effects of GS-9857 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-9857
CYP1A2	Phenacetin *O*-deethylase	0.06	>25
CYP2B6	Bupropion 4-hydroxylase	0.76	19
CYP2C8	Paclitaxel 6α-hydroxylase	0.35	11.2
CYP2C9	Tolbutamide 4-hydroxylase	0.52	>25
CYP2C19	*S* Mephenytoin 4'-hydroxylase	4.94	>25
CYP2D6	Dextromethorphan *O*-demethylase	0.05	>25
CYP3A	Midazolam 1'-hydroxylase	0.05	16.7
	Testosterone 6β-hydroxylase	0.11	>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-9857 had little or no inhibitory effect on the activities of human hepatic microsomal CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 or CYP3A enzymes (IC_{50} ≥ 11.2 µM) and so is unlikely to affect the pharmacokinetics of drugs metabolized by these enzymes (I/K_i < 0.1).

AD-338-2035: In Vitro Assessment of Human Hepatic Microsomal Cytochrome P450 Mechanism-Based Inhibition Potential of Voxilaprevir

Introduction

This study evaluated the potential for voxilaprevir to be a mechanism-based inhibitor of the major human hepatic microsomal cytochrome P450 drug-metabolizing enzymes, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A.

Materials and Methods

Assays were conducted [redacted] (b) (4) To test the potential for voxilaprevir to act as a mechanism-based inhibitor of human CYP enzymes a two stage incubation protocol was used, with the first stage allowing inactivation of the enzyme in the absence of substrate, and the second stage used to assay remaining enzyme activity. A 10-fold dilution was performed between the two stages to reduce the direct inhibitory effects of the test compounds.

Assays were performed in duplicate. The first stage incubation contained pooled human hepatic microsomal fractions diluted in 50 mM potassium phosphate buffer pH 7.4. Voxilaprevir was added to a final concentration of 10 µM. The incubation was carried out at 37°C for 30 minutes in the absence or presence of 1 mM NADPH as the CYP cofactor. After this time the incubation mixture was diluted 10-fold with 50 mM potassium phosphate buffer pH 7.4, plus enzyme substrate (final concentration typically ~5× the apparent K_m) and fresh NADPH (1 mM final). The second-stage incubation was carried out at 37°C and then terminated by the addition of five volumes of methanol. The enzyme-specific metabolites were quantified by LC-MS. Assay positive control mechanism-based inhibitors (Table 1) were tested in parallel at a concentration of 25 µM in the first stage incubation.

Mechanism-Based Inhibition Calculation:

Peak area ratios (PAR) for the metabolites to internal standard were used for quantification. Inhibition was calculated as percent change in the ratio of the metabolite PAR values obtained after pre-incubations performed in the absence and presence of NADPH cofactor, and corrected for the change in activity when incubated with DMSO vehicle instead of voxilaprevir or positive control inhibitor.

$$Ratio = \frac{PAR_{NADPH+}}{PAR_{NADPH-}} \bullet \frac{PAR_{DMSO,NADPH-}}{PAR_{DMSO,NADPH+}}$$

$$\%Change = (1 - Ratio) \bullet 100\%$$

The standard deviation for the %Change was calculated, using the variability between duplicates for the four values, by error propagation, assuming no covariance between the values. RMS_{CV} is the root-mean-square coefficient of variation for the four values.

$$SD = Ratio \bullet RMS_{CV} \bullet 100\%$$

Table 1. CYP isoform-specific probe substrates and control mechanism-based inhibitors

CYP isoform	Probe substrate	Conc. (µM)	Incu. time (min)	Control mechanism-based inhibitor	Conc. (µM)
CYP1A2	phenacetin	90	5	furafylline	25
CYP2B6	bupropion	550	5	ticlopidine	25
CYP2C8	paclitaxel	7.5	30	gemfibrozil 1-O-β-	25

				glucuronide	
CYP2C9	diclofenac	100	5	tienilic acid	25
CYP2C19	S-mephenytoin	250	30	ticlopidine	25
CYP2D6	dextromethorphan	25	5	paroxetine	25
CYP3A	midazolam	12.5	5	mifepristone	25
CYP3A	testosterone	100	5	mifepristone	25

Results

The positive control mechanism-based inhibitors incubated at 25 μM all achieved the expected NADPH cofactor-dependent and pre-incubation time-dependent changes in CYP inhibitory potency (%Change ≥ 51.1%) (Table 2). In contrast, at a tested concentration of 10 μM, there was no evidence for time- and cofactor-dependent inhibition of any enzyme by voxilaprevir (%Change ≤ 19.0%).

Table 2: %Change Values for Time- and Cofactor-Dependent Inhibition of Major Human Hepatic Microsomal CYP Enzymes by Voxilaprevir (source: Study Report Table 1)

CYP Enzyme	Probe Activity	Calculated %Change	
		Control Inhibitor[a]	Voxilaprevir
CYP1A2	Phenacetin O-deethylase	86.5 ± 5.1	1.5 ± 7.7
CYP2B6	Bupropion hydroxylase	85.3 ± 9.1	2.9 ± 6.5
CYP2C8	Paclitaxel 6α-hydroxylase	51.1 ± 8.5	-4.7 ± 9.2
CYP2C9	Diclofenac 4'-hydroxylase	74.1 ± 2.9	17.2 ± 11.5
CYP2C19	S-Mephenytoin 4'-hydroxylase	57.0 ± 6.7	6.1 ± 6.7
CYP2D6	Dextromethorphan O-demethylase	77.3 ± 4.4	1.5 ± 6.6
CYP3A	Midazolam 1'-hydroxylase	79.2 ± 5.3	19.0 ± 8.7
	Testosterone 6β-hydroxylase	69.6 ± 3.6	7.8 ± 3.6

a CYP1A2, furafylline; CYP2B6, ticlopidine; CYP2C8, gemfibrozil glucuronide; CYP2C9, tienilic acid; CYP2C19, ticlopidine; CYP2D6, paroxetine; CYP3A, mifepristone

Conclusion

Voxilaprevir has previously been shown to be a weak or undetectable inhibitor of human hepatic microsomal CYP enzymes (IC_{50} ≥ 11.2 μM; AD-338-2008). In the current study voxilaprevir showed no evidence for mechanism-based inhibition (pre-incubation time-dependent and cofactor-dependent change in inhibitory potency) of human hepatic microsomal CYP enzymes. Collectively these data suggest that drug interactions with substrates of these enzymes are unlikely.

AD-338-2009: *In Vitro* Assessment of Human UGT1A1 Inhibition Potential of GS-9857

Introduction

This study evaluated the potential for GS-9857 to inhibit the activity of a major human glucuronidation enzyme, UGT1A1, using microsomal fractions from baculovirus-expressed human UGT1A1 insect cells (Supersomes™).

Materials and Methods

The assay was performed [redacted] (b) (4) The UGT1A1 substrate estradiol (10 µM) was incubated with Supersomes™ (0.25 mg/mL protein), UDP-glucuronic acid (5 mM), and alamethicin (25 µg/mL) in the presence or absence of test compound (concentration range: 0.2-50 µM) for 30 min at 37ºC. The UGT1A1-selective metabolite, estradiol 3-glucuronide, was monitored by LC-MS/MS and a decrease in the formation of the metabolite compared to the vehicle control was used to calculate an IC_{50} value. The concentration of estradiol substrate is below its measured K_m value.

Results

Silybin, the positive control inhibitor reduced UGT1A1 activity as expected, with an IC_{50} of 2.99 µM, confirming satisfactory incubation conditions for the assays (Table 1). The apparent IC_{50} value for GS-9857 for human UGT1A1 was 4.75 µM. The C_{max} for GS-9857 was ~ 0.22 µM based on population PK analysis and the estimated unbound $C_{hep, inlet}$ is 0.0767 µM, and the R_1 value was ~ 0.09 and AUCR was < 1.25, and thus GS-9857 is unlikely to inhibit UGT1A1 in vivo.

Table 1. IC_{50} Values for Inhibition of Human UGT1A1 Activity for GS-9857 and Positive Control Inhibitor (Mean, n=7)

(source: Study Report Table 1)

Compound	Role	IC_{50} (µM)
GS-9857	Test compound	4.75
Silybin	Assay positive control	2.99

Estradiol 3-glucuronidation activity

Conclusion

GS-9857 was a moderate inhibitor of human hepatic microsomal UGT1A1 (IC_{50} = 4.75 µM). However, GS-9857 is unlikely to inhibit this enzyme significantly *in vivo*, with AUCR < 1.25.

AD-338-2034: Microsomal Binding of Voxilaprevir

Introduction

This study evaluated the extent of binding of GS-9857 to microsomal fraction.

Materials and Methods

Microsomal fraction was diluted to 0.5 mg protein/mL with isotonic buffer (0.133 M potassium phosphate buffer, pH 7.4). GS-9857 was added to a final concentration of 0.5-5 µM (final DMSO concentration 0.5% v/v). The microsomal solutions were then dialyzed in duplicate or triplicate against an equal volume of isotonic buffer. A quality control compound (amitriptyline at 3 µM) was tested in parallel.

Results

The extent of binding of GS-9857 in human hepatic microsomal fraction was determined and the result is summarized in Table 1. GS-9857 exhibited moderate binding (mean f_u = 20.4%) with no evidence of saturation over the concentration range tested (no trend towards a higher free fraction as the GS-9857 concentration was increased from 0.5 to 5 µM)

Table 1. Fraction Unbound of GS-9857 and Control Compound in Human Hepatic Microsomal Fraction

(source: Study Report Table 1)

Compound	Concentration (µM)	Fraction Unbound (%)	Recovery (%)
GS-9857	0.5[a]	25.5	68
	2[a]	19.4	68
	5[b]	16.2 ± 2.7	76
Amitriptyline	3[b]	31.1 ± 3.2	86

a Mean (n = 2)
b Mean ± Standard Deviation (n = 3)

Conclusion

GS-9857 exhibits moderate binding to human hepatic microsomal fraction (mean f_u = 20.4%). Though the IC_{50} values can be 5X lower for VOX inhibition on CYP enzymes ($IC_{50} \geq$ 11.2 µM without correction) after correcting microsomal binding, the unbound C_{max} is only 0.00221 µM due to high plasma protein binding, thus VOX is unlikely to affect the pharmacokinetics of drugs metabolized by CYP enzymes.

AD-338-2007: *In Vitro* Assessment of Induction Potential of GS-9857 in Humans

Introduction

This study evaluated the potential for AhR- and PXR-mediated induction of metabolizing enzymes and transporters by GS-9857.

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 µL/well containing 0.15-50 µM GS-9857 for 24 h. Positive controls including 0.1-20 µM β-naphthoflavone (AhR activator, CYP1A2-DREcells) or 0.1-20 µM 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 (b) (4) 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 (b) (4) 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-9857 and positive control compounds. Treatment with the clinically relevant inducer, rifampicin, led to up to 8.8-fold activation of reporter gene expression. In contrast, at concentrations of up to 50 μM the extent of activation of PXR by GS-9857 reached 18.8 % of the maximum achieved by the positive control. The anticipated maximum plasma concentration in human was < 1.5 μM and at this concentration there was < 5% activation of PXR. Treatment with the AhR inducer, β-naphthoflavone, resulted in up to 58.7-fold activation compared to vehicle control. Concentrations of GS-9857 up to 50 μM resulted in a maximum of 12.6% of the activation elicited by β-naphthoflavone. At 1.5 μM, the response was < 1% of the positive control.

Table 1: Human PXR Activation by GS-9857 and Positive Controls
(source: Study Report Tables 2 and 3)

Fold induction over 0.1% DMSO ctrl	PXR (CYP3A4)		AhR (CYP1A2)	
Conc. (μM)	GS-9857	rifampicin	GS-9857	α-naphthoflavone
0.1	-	1.67	-	1.77
0.15	1.02	-	1.04	-
0.5	0.98	4.47	0.93	3.40
1	-	6.53	-	5.40
1.5	1.25	-	1.06	-
5	1.79	8.40	4.73	22.4
10	-	8.38	-	43.5
15	2.47	-	7.42	-
20	-	8.80	-	58.7
50	1.87	-	4.74	-

Conclusion
These data suggest minimal potential for activation of PXR- and AhR-regulated genes by GS-9857 at concentrations that could be achieved in humans following administration of GS-9857.

AD-338-2026: Evaluation of Induction Potential of GS-9857 in Cultured Human Hepatocytes

Introduction
This study evaluated the potential of GS-9857 to induce CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP3A4, UGT1A1 and P-gp mRNA expression in primary cultures of human hepatocytes. The induction of CYP1A2, CYP2B6 and CYP3A4/5 enzyme activities by GS-9857 was also assessed by CYP-specific probe substrates.

Materials and Methods
Evaluation of the degree of cytochrome P450 induction was performed by measurement of mRNA levels by RT-PCR (TaqMan®) and by evaluating CYP enzymatic activity with specific probe substrates. Freshly isolated human hepatocytes from three separate adult donors were cultured on collagen-coated plates and incubated with vehicle alone, positive

and negative control compounds, or various concentrations of test article, for 48 hours to assess mRNA levels and for 72 hours to assess CYP activity. Test article, GS-9857, was evaluated at four (4) concentrations: 0.1, 0.3, 1, and 3 μM. Additionally, the cultures were assayed for LDH leakage from the hepatocytes as a measure of membrane stability which is an indicator of cytotoxicity. Induction was considered positive if there was a concentration dependent increase ≥ 4-fold above vehicle control for mRNA and ≥ 40% of positive control response for activity for CYP1A2, CYP2B6 and CYP3A4. Induction was considered positive if there was a concentration-dependent increase in mRNA ≥ 2-fold above vehicle control for CYP2C8, CYP2C9, UGT1A1 and P-gp mRNA. Statistically significant differences between the vehicle control and treated cultures were determined using one-way ANOVA using GraphPad Prism.

Results

The test article GS-9857 did not induce any of the enzymes assessed. There was sporadic reduction of expression of some of the mRNAs examined at the highest concentration of GS-9857 tested at 3 μM with some donors. The decreases in expression observed across multiple genes were also reflected in corresponding enzyme activity levels but did not appear to be due to overt cytotoxicity (LDH release). The only increases observed from GS-9857 were that of CYP2B6 activity. The increase in CYP2B6 activity was observed in one donor and was ~10% of the positive control response. Overall, GS-9857 is unlikely to induce the expression of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP3A4 and UGT1A1 since the *in vivo* VOX exposure level with C_{max} of 0.221 μM is within the lower range of the tested concentrations. The induction of P-gp mRNA by GS-9857 could not be reliably assessed in this study.

Conclusion

GS-9857 was not an enzyme inducer for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP3A4, and UGT1A1.

AD-338-2012: Bi-Directional Permeability of GS-9857 Through Monolayers of P-glycoprotein and BCRP Over-expressing Cells

Introduction

This study evaluated if GS-9857 is a substrate for either P-glycoprotein (P-gp) or Breast Cancer Resistance Protein (BCRP).

Materials and Methods

Experiments were run using HBSS donor buffer from (b) (4) containing additional 10mM HEPES, 15mM Glucose adjusted to pH 6.5. The receiver well had HBSS buffer supplemented with 1% BSA and the pH was adjusted to 7.4. The experiment was started by the addition of dosing solutions containing test compounds. At 1 and 2 hr time points, 100 μl samples were taken from the receiver compartment. Removed buffer was replaced with fresh buffer and a correction was applied to all calculations for the removed material. Each compound was tested in 2 separate replicate wells for each condition. Cells were dosed on the apical or basolateral side to determine forward (A to B) and reverse (B to A) permeability.

Permeability through a cell free transwell was also determined as a measure of membrane passive permeability and non-specific binding. To test for non-specific binding and compound instability, the total amount of drug was quantified at the end of the experiment and compared to the material present in the original dosing solution as a percentage of recovery. Samples were analyzed by LC/MS/MS.

The apparent permeability, P_{app}, and % recovery were calculated as follows:

$$\mathbf{P_{app} = (d_R / d_t) \times V_r / (A \times D_0)}$$

$$\mathbf{\% \ Recovery = 100 \times ((V_r \times R_{120}) + (V_d \times D_{120})) / (V_d \times D_0)}$$

Where,
$\mathbf{d_R / d_t}$ is the slope of the cumulative concentration in the receiver compartment versus time in μM/s based on receiver concentrations measured at 60 and 120 minutes.
$\mathbf{V_r}$ and $\mathbf{V_d}$ is the volume in the receiver and donor compartment in cm^3, respectively.
A is the area of the cell monolayer (0.33 cm^2).
$\mathbf{D_0}$ and $\mathbf{D_{120}}$ is the measured donor concentration at the beginning and end of the experiment, respectively.
$\mathbf{R_{120}}$ is the receiver concentration at the end of the experiment (120 minutes).

Results
The efflux ratio of GS-9857 in P-gp over-expressing cells increased 6.4-fold relative to the results in wild type cells, reflecting a decrease in forward permeability and an increase in reverse permeability of GS-9857 (Table 1). The efflux ratio was decreased from 7.1 to 3.6 in MDCKII-MDR1 cells in the presence of P-gp inhibitor cyclosporin A (10 μM). The bi-directional permeability of the Pgp substrate vinblastine was tested on the same assay plates and showed a ~5-fold increase in efflux ratio in MDCKII-MDR1 cells relative to results in MDCKII-WT cells.

In BCRP over-expressing cells a 3.9-fold increase in the efflux ratio of GS-9857 was observed compared to results in wild type cells. The increased efflux ratio reflected a decrease in forward permeability and an increase in reverse permeability of GS-9857 (Table 2) in the MDCKII-BCRP cells. The presence of BCRP inhibitor Ko134 (10 μM) reduced the efflux ratio of GS-9857 from 4.3 to 3.1 in MDCKII-BCRP cells. The bidirectional permeability of the BCRP substrate prazosin was tested on the same assay plates and showed a 38-fold increase in efflux ratio in MDCKII-BCRP cells relative to results in MDCKII-WT cells.

Table 1: Bi-Directional Permeability of GS-9857 in Wild Type and MDR1 Transfected MDCKII Cells (source: Study Report Table 1)

Cell Type	Direction	Initial Conc. (µM)	Recovery (%)	P_{app} (10-6 cm/s)			Efflux Ratio
				R1	R2	Avg.	
MDCKII-WT	Cell-Free	3.9	129	39.2		39.2	
	Forward	3.3	55	21.4	17.8	19.6	1.1
	Reverse	4.4	64	16.4	25.9	21.2	
MDCKII-MDR1	Forward	4.2	53	7.0	6.9	7.0	7.1
	Reverse	2.8	151	38.9	60.0	49.5	
MDCKII-MDR1 (10 µM CsA)	Forward	4.7	56	8.1	9.3	8.7	3.6
	Reverse	3.9	125	26.5	36.4	31.5	

Table 2: Bi-Directional Permeability of GS-9857 in Wild Type and BCRP Transfected MDCKII Cells (source: Study Report Table 2)

Cell Type	Direction	Initial Conc. (µM)	Recovery (%)	P_{app} (10-6 cm/s)			Efflux Ratio
				R1	R2	Avg.	
MDCKII-WT	Cell-Free	3.9	129	39.2		**39.2**	
	Forward	3.3	55	21.4	17.8	**19.6**	1.1
	Reverse	4.4	64	16.4	25.9	**21.2**	
MDCKII-BCRP	Forward	4.2	36	7.4	11.8	**9.6**	4.3
	Reverse	2.9	131	34.1	47.9	**41.0**	
MDCKII-BCRP (10 µM Ko134)	Forward	3.9	47	8.0	12.4	**10.2**	3.1
	Reverse	3.2	117	22.5	40.0	**31.2**	

Conclusion

GS-9857 was found to be a substrate for P-gp and BCRP transport based on the increases in efflux ratios in MDR1 and BCRP over-expressing cells compared to wild type cells. Consistent with P-gp and BCRP dependent transport, the efflux ratios of GS-9857 were decreased in the presence of the P-gp inhibitor cyclosporin A (CsA;10 μM) and the BCRP inhibitor Ko134 (10 μM).

AD-338-2011: *In Vitro* Assessment of GS-9857 Uptake in Human Hepatocytes

Introduction
This study evaluated if GS-9857 is a substrate for hepatic uptake transporters.

Materials and Methods
Cells were equilibrated at 37ºC for 30 minutes prior to assay. GS-9857 (50 nM) was incubated in the presence or absence of rifampicin (40 μM) and cyclosporin A (2.5 μM) with 2 x 10^6 fresh human hepatocytes per mL. The cell suspension containing test compounds was incubated at 37ºC for 0.5, 1, 3, 5, 7, and 10 minutes in KHB buffer containing 0.5% BSA. The suspension was then centrifuged through an oil layer, which allows the cells to pass through but excludes the solution containing the free test articles. The resulting cell layer was then lysed and extracted using organic solvents and analyzed by LC/MS/MS. Controls atorvastatin and antipyrine were assayed at 0.1 μM and 10 μM, respectively.

The uptake into hepatocytes was determined by the following formula:
Uptake amount = (concentration of compound in cell lysate) * volume of sample / millions of cells in sample

Results
GS-9857 showed time-dependent increases in cell associated compound under all assay conditions over the first 7 minutes of incubation followed by decreasing concentration over 7 to 10 minute interval. The drop-off in cellular concentration is most likely due to loss in cell viability and/or in transporter function. The presence of inhibitors caused a consistent decrease in cell-associated GS-9857 over the first 7 minutes of incubation, suggesting that transporter mediated uptake of GS-9857 is involved in the hepatic uptake of the compound. Positive control atorvastatin showed similar uptake in cells. For the passive permeability control antipyrine, included in the second experiment, there was no marked difference between compound dosed alone and dosed with inhibitors. The results for GS-9875 and controls are summarized in Table 1 and Table 2.

Table 1: Uptake of 50 nM GS-9857 into Fresh Human Hepatocytes in the Presence and Absence of Inhibitors Rif (40 μM) and CsA (2.5 μM)
(source: Study Report Tables 1)

Incubation Time (min)	Donor 1 GS-9857 Uptake (pmol per million cells)		Donor 2 GS-9857 Uptake (pmol per million cells)	
	Alone	+ Inhibitors	Alone	+Inhibitors
0.5	1.0	0.28	3.6	2.3
1	1.8	1.3	3.2	2.4
3	2.8	1.9	4.9	3.4
5	3.8	2.3	6.0	4.3
7	3.5	2.6	8.9	3.4
10	2.2	3.1	7.1	4.5

Table 2: Uptake of 50 nM Atorvastatin and 10 µM Antipyrine in Fresh Human Hepatocytes in the Presence and Absence of Inhibitors Rif (40 µM) and CsA (2.5 µM)
(source: Study Report Tables 2)

Incubation Time (min)	Donor 1 Atorvastatin Uptake (pmol per million cells)		Donor 2 Atorvastatin Uptake (pmol per million cells)		Donor 2 Antipyrine Uptake (pmole per million cells)	
	Alone	+ Inhibitors	Alone	+Inhibitors	Alone	+Inhibitors
0.5	1.3	0.25	2.2	2.6	26	28
1	1.6	1.2	6.0	2.6	28	28
3	3.0	2.2	7.4	5.2	32	26
5	4.1	2.5	7.6	9.3	28	31
7	5.1	2.9	10.0	7.7	30	30
10	4.5	4.3	11.0	7.8	30	32

Conclusion
GS-9857 showed time-dependent increases in cell associated compound in hepatocytes from two individual donors. The presence of Rif and CsA decreased the amount of cell-associated GS-9857, suggesting a role for transporter mediated pathway(s) in the uptake of GS-9857 in human hepatocytes.

AD-338-2037: *In Vitro* Assessment of GS-9857 as Substrate for Human OCT1 Transporter

Introduction
This study investigated if GS-9857 is a substrate for human OCT1 transporter using transfected cell lines.

Materials and Methods
GS-9857 (lot #3) was synthesized at the Medicinal Chemistry Department at Gilead Sciences. Radiolabeled ^{14}C-GS-9857 (lot G-01805-4, specific activity 56.8mCi/mmol) was synthesized (b) (4) All other chemicals used in this

experiment were purchased [redacted] (b) (4) were of analytical grade. The studies were performed [redacted] (b) (4) GS-9857 was studied at 1 µM and 10 µM in control and OCT1-overexpressing cells and time points of 2 and 20 min. Compound accumulation in the cell types was compared. TEA was used as positive control substrate, and verapamil was reference inhibitor.

Results

GS-9857 was found not to inhibit OCT1-mediated uptake of TEA at test concentrations up to 23µM. The OCT1 substrate assay was conducted at concentrations 1 and 10 µM for GS-9857; these test concentrations are at least three fold less than the IC_{50} values. Fold accumulation values of GS-9857 remained below the arbitrary threshold of 2-fold in the OCT1-expressing cells, compared with the control cells at the applied concentrations and time points (2 and 20 min) in the substrate uptake experiments (Table 1). GS-9857 was determined not to be a substrate for OCT1. In contrast, positive control substrate TEA had a 21 fold accumulation in OCT1 transfected cells when compared to control cells; its accumulation in OCT1 was reduced by more than 90% in the presence of OCT1 inhibitor verapamil.

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Table 1. Ratio of GS-9857 and TEA Accumulation in OCT1-overexpressed Cells over Control Cells
(source: Study Report Table 3)

Test article	Transporter	Fold accumulation	Conditions (µM / min)
GS-9857	OCT1	1.0	1.0 / 2
		0.9	1.0 / 20
		0.9	10 / 2
		0.8	10 / 20
TEA (control)	OCT1	21	5 / 10
		1.1	5/10 (+ 100µM Verapamil)

Conclusion
GS-9857 was determined not to be a substrate for OCT1 uptake based on the lack of the transporter specific accumulation in OCT1 transfected cells.

AD-338-2010: *In Vitro* Inhibition Assessment of GS-9857 with Human P-gp and BCRP

Introduction
This study evaluated the inhibition of the ATP-Binding Cassette (ABC) efflux transporters P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP) by GS-9857 *in vitro* using cell lines transfected with the individual transporters and fluorescent model substrates.

Materials and Methods
MDCKII cells were maintained in Dulbecco's Modification of Eagle's Medium (DMEM) with sodium pyruvate, Glutmax, 1% Pen/Strep, and 10% fetal bovine serum in an incubator set at 37º C, 90% humidity and 5% CO_2. MDCKII cells were seeded in 96-well black cell culture plates with clear bottoms at densities of 5 x 10^4 cells/well for P-gp and 2 x 10^4 cells/well for BCRP and grown to confluence.

For the P-gp assay, test compounds were serially diluted in DMSO and then spiked into cell culture medium (without fetal bovine serum (FBS)) containing 10 µM calcein AM and incubated for 1 hour. Following the removal of media containing calcein AM and test compound, cells were washed five times with 1M PBS buffer containing magnesium and calcium. Wells were immediately analyzed for calcein fluorescence at an excitation of 494 nm and an emission of 517 nm.

For the BCRP assay, test compounds were serially diluted with DMSO and then spiked in cell culture medium (without FBS) containing 1 µM pheophorbide A (PhA) and incubated for

18 hours with MDCKII-BCRP cells. Following the removal of media containing PhA and test compound, cells were then washed five times with 1M PBS buffer containing magnesium and calcium. Wells were immediately analyzed for PhA fluorescence at an excitation of 415 nm and an emission of 675 nm.

The % inhibition was calculated as follows:

$$\textbf{Ratio (R)} = \textbf{TF}_{\textbf{P-gp}}/\ \textbf{TF}_{\textbf{WT}}$$

$$\textbf{Ratio (R)} = \textbf{TF}_{\textbf{BCRP}}/\ \textbf{TF}_{\textbf{WT}}$$

$$\textbf{\% Inhibition} = ((R^{I}-R^{NI})/\ (1-R^{NI}))\ \textbf{x}\ \textbf{100\%}$$

Where,
TF is total fluorescence
$\mathbf{R^{I}}$ and $\mathbf{R^{NI}}$ represent the ratio observed in the presence and absence of test compound, respectively.

Results
The dose-dependent inhibition of P-gp-mediated efflux of calcein-AM and BCRP-mediated efflux of pheophorbide A (PhA) by GS-9857 was tested at six concentrations ranging from 0.04 to 10 µM, and the study results are summarized in Table 1. GS-9857 showed no inhibition of P-gp at up to the highest concentration tested (10 µM). The P-gp positive control verapamil had an IC_{50} value of 1.6 ± 0.27 µM. GS-9857 showed a 6.7 ± 1.4% inhibition in PhA accumulation in the BCRP cell line. It is unclear if this was due to inhibition of transport or a slight effect on cell viability that was observed at the 10 µM concentration of GS-9857. The BCRP positive control FTC had an IC_{50} value of 0.86 ± 0.03 µM.

Table 1. Inhibition of BCRP-Mediated Transport of Pheophorbide A and P-gp-Mediated Transport of Calcein AM by GS-9857 and Control Compounds
(source: Study Report Table 1)

	Efflux Transporters IC50 (µM)	
Test Compounds	**Pgp**	**BCRP**
GS-9857	> 10	> 10
Verapamil	1.6 ± 0.27	N/A
Fumitremorgin C (FTC)	N/A	0.86 ± 0.03

Conclusion
GS-9857 showed no dose-dependent inhibition of P-gp-mediated calcein AM transport at up to the highest concentration tested (10 µM). GS-9857 showed a slight (6.7 %) decrease in BCRP-mediated pheophorbide A transport at 10 µM.

Reviewer's comment:
There is no percentage of recovery data from this transporter inhibition assay to validate the actual drug concentrations for the inhibition. It is uncertain if the IC_{50} values are accurate, especially considering the low solubility of GS-9857 with potential for non-specific binding or precipitation. Thus those IC_{50} values need to be interpreted with caution for comparison between compounds or for *in vitro-in vivo* extrapolation.

AD-230-2075: *In Vitro* Assessment of GS-9857 Inhibition of Human OATP1B1 and OATP1B3

Introduction

This study evaluated the inhibition of the Solute Carrier (SLC) influx transporters organic anion-transporting polypeptide 1B1 and 1B3 (OATP1B1 and OATP1B3) by GS-9857 *in vitro* using cell lines transfected with the individual transporters and fluorescent model substrates.

Materials and Methods

OATP1B1 and OATP1B3 overexpressing cells were seeded in BioCoat Poly-D-Lysine coated 96 well black cell culture plates with clear bottoms at a density of 1 x 10^5 cells / well. Sodium butyrate (10 mM) was added to the OATP1B1 and OATP1B3 cells to increase the protein expression level, and the cells were grown to confluence overnight. The assay buffer contained 142 mM NaCl, 5 mM KCl, 1 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1.5 mM $CaCl_2$, 5 mM Glucose and 12.5 mM HEPES (pH 7.4). After removal of the media and before adding test compounds, the cells were washed twice with 37ºC assay buffer followed by a 0.5 h pre-incubation with assay buffer. Test compounds were serially diluted in DMSO at 250 fold of final test concentrations to create the compound spiking solutions. Compounds were then spiked into assay buffer containing 2 µM Fluo 3 and incubated with cells for 1 h. Following removal of assay buffer containing Fluo 3 and test compound, cells were washed 3 times with 200 µl of ice cold assay buffer and then lysed at room temperature for 15 minutes in a lysis buffer containing 0.05 % SDS in a 1 mM $CaCl_2$ solution. Wells were analyzed for Fluo 3 fluorescence at an excitation of 485 nm and emission of 530 nm.

The % transport inhibition by test article was calculated as follows:

$$\% \text{ inhibition} = (1- ((OATP_I – WT_{NI}) / (OATP_{NI}-WT_{NI}))*100$$

Where,

$OATP_I$ represents the fluorescence in the presence of test article for either OATP1B1 or OATP1B3 overexpressing cells.

$OATP_{NI}$ represents the fluorescence in the absence of test article for either OATP1B1 or OATP1B3 overexpressing cells.

WT_{NI} represents the fluorescence in the absence of test article for wild type cells

Results

The dose-dependent inhibition OATP1B1 and OATP1B3-mediated transport of Fluo 3 by GS-9857 was tested at seven concentrations ranging from 0.014 to 30 µM. GS-9857 showed dose-dependent inhibition of OATP1B1 and OATP1B3 with IC_{50} of 0.18 ± 0.03 µM and IC_{50} of 0.70 ± 0.11 µM, respectively (Table 1). The positive control rifampicin had an IC_{50} value of 2.7 ± 1.7 µM for OATP1B1 and 2.5 ± 1.8 µM for OATP1B3 assay.

Table 1. Inhibition of OATP1B1/ 1B3-Mediated Transport of Fluo3 by GS-9857 and Control Compound Rifampicin
(source: Study Report Table 1)

	Uptake Transporters IC_{50} (μM)	
Transporters	**OATP1B1**	**OATP1B3**
GS-9857	0.18 ± 0.03	0.70 ± 0.11
Rifampicin	2.7 ± 1.7	2.5 ± 1.8

Conclusion
GS-9857 showed dose-dependent inhibition of OATP1B1 and OATP1B3 with IC_{50} values of 0.18 ± 0.03 μM and 0.70 ± 0.11μM, respectively. Based on an estimated VOX unbound hepatic inlet concentration of 0.0767 μM and IC_{50} value at 0.18 and 0.70 μM, the R value is 1.43 and 1.11 for OATP1B1 and OATP1B3, respectively. The R values are greater or close to 1.25, and thus VOX (GS-9857) showed some potential to inhibit the hepatic uptake transporters OATP1B1 and OATP1B3 during first pass, which is consistent with the result from clinic DDI study with OATP substrate pravastatin.

Reviewer's comment:
There is no percentage of recovery data from this transporter inhibition assay to validate the actual drug concentrations for the inhibition. It is uncertain if the IC_{50} values are accurate, thus those IC_{50} values need to be interpreted with caution for comparison between compounds or for *in vitro-in vivo* extrapolation.

AD-338-2020: *In Vitro* Assessment of GS-9857 Inhibition of Human MRP2 and BSEP

Introduction
This study evaluated the inhibition of human bile salt export pump (BSEP; ABCB11), and multidrug resistance protein 2 (MRP2; ABCC2) efflux transporters by GS-9857 *in vitro* using vesicles transfected with the individual transporters and radiolabeled model substrates.

Materials and Methods
The studies were performed (b) (4) Vesicles used in the assay were Sf9 cell membrane vesicles. The model substrates were taurocholate and estradiol-17-beta-glucuronide ($E_2$17ßG), and the positive control inhibitors were cyclosporin A (CsA) and benzbromarone, for BSEP and MRP2 assay, respectively.

Test compounds were incubated with membrane vesicle preparations (total protein: 50 μg/well) and probe substrates, taurocholate (2 μM) for BSEP or Estradiol-17-beta-glucuronide (0.2 μM) for MRP2, in the absence or presence of ATP. Reaction mixtures were pre-incubated for 15 minutes at 37°C. Reactions were started by the addition of 25 μL of 12 mM MgATP or assay buffer (for background controls), pre-incubated separately. Reactions were stopped after 5 min by the addition of 200 μL of ice-cold washing buffer and immediate filtration via glass fiber filters mounted to a 96-well plate (filter plate). The filters were

washed, dried and the amount of substrate inside the filtered vesicles determined by liquid scintillation. CsA (20 μM) or benzbromarone (100 μM), for BSEP or MRP2 respectively, were used as positive control inhibitors and tested in parallel. Control membranes lacking transporter expression were used as negative control. All assays were performed in duplicate.

Fractional transport activities were calculated from the equation:

$$\textit{Activity \%} = \textbf{(A-B)/(C-D) x 100}$$

Legend:
A: translocated amount of substrate in the presence of TA and ATP
B: translocated amount of substrate in the presence of TA
C: translocated amount of substrate in the presence of solvent and ATP
D: translocated amount of substrate in the presence of solvent

Results

The positive controls, CsA (20 μM) for BSEP and benzbromarone (100 μM) for MRP2, showed 89% and 96% inhibition, respectively.

The dose-dependent inhibition of MRP2-mediated $E_2$17βG transport and BSEP-mediated taurocholate transport by GS-9857 was tested at seven concentrations ranging from 0.054 to 40.0 μM and the result is presented in Table 1. GS-9857 showed moderate dose-dependent inhibition of BSEP with an IC_{50} value of 1.5 μM. GS-9857 showed weak dose-dependent inhibition of MRP2 with 45% inhibition at the highest concentration tested (40 μM) (Table 1).

Cyclosporin A (CsA), a known inhibitor of BSEP, was tested in parallel in the same study at concentrations up to 10 μM and the result is presented in Table 2. CsA showed potent dose-dependent inhibition of BSEP with an IC_{50} value of 0.33 μM. Weak inhibition of MRP2 transport was observed for CsA with 30% inhibition at the highest concentration tested (10 μM).

Table 1. Inhibitory Effect of GS-9857 on Vesicular Efflux Transporters
(source: Study Report Table 1)

	Vesicular Transport Inhibition	
Transporter	**IC_{50} (μM)**	**Maximum inhibition (% of control)**
BSEP	1.5	100% inhibition at 40 μM
MRP2	>40	45% inhibition at 40 μM

Table 2. Inhibitory Effect of Cyclosporin A on Vesicular Efflux Transporters
(source: Study Report Table 2)

	Vesicular Transport Inhibition	
Transporter	**IC_{50} (μM)**	**Maximum inhibition (% of control)**
BSEP	0.33	97% inhibition at 10 μM
MRP2	>10	30% inhibition at 10 μM

Conclusion
GS-9857 inhibited BSEP-mediated taurocholate transport with an IC_{50} value of 1.5 μM. GS-9857 showed weak inhibition of MRP2-mediated $E_2$17βG transport with 45% inhibition at the highest concentration tested (40 μM).

Reviewer's comment:
There is no percentage of recovery data from this transporter inhibition assay to validate the actual drug concentrations for the inhibition. It is uncertain if the IC_{50} values are accurate, although GS-9857 showed inhibition of BSEP. Thus those IC_{50} values need to be interpreted with caution for comparison between compounds or for *in vitro-in vivo* extrapolation.

AD-338-2036: *In Vitro* Assessment of GS-9857 Inhibition of Human OCT1, OCT2, OAT1, OAT3, and MATE1 Transporters

Introduction
This study investigated if GS-9857 is an inhibitor of human MATE1, OAT1, OAT3, OCT1, and OCT2 transporters using model substrates and transfected cell lines.

Materials and Methods
The studies were performed (b) (4) The probe substrates were p-aminohippuric acid (PAH), tetraethylammonium chloride (TEA), TEA, metformin, and estrone-3-sulfate (E3S), and the reference inhibitors were benzbromarone, verapamil, verapamil, pyrimethamine, and probenecid, for human OAT1 (CHO), OCT1 (CHO), OCT2 (CHO), MATE1 (MDCKII), and OAT3 (HEK293) assay, respectively.

Transporter expressing and control cells were cultured according to the corresponding (b) (4) SOPs. Before experiments the medium were removed and cells rinsed with Henseleit-Krebs buffer pH 7.4 or pH 8.0 in case of MATE1. Uptake experiments were carried out at 37°C in Henseleit-Krebs buffer (pH 7.4 or pH 8.0 in case of MATE1) containing the probe substrate and the TA or the solvent. Following the experiment, cells were rinsed with Henseleit-Krebs buffer and lysed with 0.1 M NaOH. The amount of substrate inside the cells were determined by liquid scintillation counting.

Fractional transport activities were calculated from the equation:

Activity % = (A-B)/(C-D) x 100

A. translocated amount of substrate in the presence of test article (TA) on transfected cells
B. translocated amount of substrate in the presence of TA on parental cells
C. translocated amount of substrate in the presence of solvent on transfected cells
D. translocated amount of substrate in the presence of solvent on parental cells

Results
All transporter inhibition data are summarized in Table 1. Results at 70μM were excluded due to the observed precipitation in dosing solutions for OAT1, OAT3, OCT1, OCT2, and MATE1 inhibition assays.

GS-9857 showed no inhibition of OAT1-mediated transport of aminohippuric acid (PAH) at concentrations up to 23µM. The positive control inhibitor benzbromarone showed almost complete inhibition of OAT1 transport at 200 µM. GS-9857 showed no inhibition of OAT3-mediated transport of estrone-3-sulfate (E3S) at concentrations up to 23µM. The positive control inhibitor probenecid showed almost complete inhibition of OAT3 transport at 500 µM. GS-9857 showed no inhibition of OCT1-mediated transport of TEA at concentrations up to 23 µM. The positive control inhibitor verapamil showed almost complete inhibition of OCT1 transport at 100 µM. GS-9857 showed no inhibition of OCT2-mediated transport of TEA at concentrations up to 23µM. The positive control inhibitor verapamil showed almost complete inhibition of OCT2 transport at 100 µM.
GS-9857 showed no inhibition of MATE1-mediated transport of metformin at concentrations up to 23µM. The positive control inhibitor pyrimethamine showed almost complete inhibition of MATE1 transport at 1 µM.

Table 1. Inhibition of Human OCT1, OCT2, OAT1, OAT3, and MATE1 Transporters by GS-9857 (source: Study Report Table 2)

Uptake transporter inhibition		
Transporter	**Maximum inhibition (% of control)**	**IC_{50} (µM)**
OAT1	No inhibition up to 23µM	NA
OAT3	No inhibition up to 23µM	NA
OCT1	No inhibition up to 23µM	NA
OCT2	No inhibition up to 23µM	NA
MATE1	No inhibition up to 23µM	NA

Conclusion
GS-9857 showed no inhibition of OAT1, OAT3, OCT1, OCT2 or MATE1 mediated transport at concentrations up to 23µM. Thus VOX is unlikely to affect the pharmacokinetics of substrates of those transporters.

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/s/

QIN SUN
05/08/2017

HUIMIN ZHENG
05/08/2017

FANG LI
05/08/2017

JEFFRY FLORIAN
05/08/2017

SHIRLEY K SEO
05/08/2017

JOHN A LAZOR
05/08/2017