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APPLICATION NUMBER:

204671Orig1s000

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

OFFICE OF CLINICAL PHARMACOLOGY REVIEW: ADDENDUM

NDA: 204671	Submission Date(s): April 8, 2013
Brand Name	To be determined
Generic Name	Sofosbuvir (GS-7977)
Primary Clinical Pharmacology Reviewer	Jenny H. Zheng, Ph.D.
Primary Pharmacometrics Reviewer	Jeff Florian, Ph.D.
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OCP Division	Division 4
OND division	DAVP
Applicant	Gilead Sciences
Relevant IND(s)	106739; 112681; 111572
Submission Type	Priority
Formulation; Strength(s)	Tablets; 400 mg
Indication	Treatment of chronic hepatitis C (CHC) as a component of a combination antiviral treatment regimen

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1. EXECUTIVE SUMMARY

Gilead Sciences is currently seeking approval of sofosbuvir (SOF) 400 mg tablets in combination with other agents for the treatment of chronic hepatitis C (CHC) in adults. The original NDA Application was submitted on April 6, 2013 and is currently under priority review. Breakthrough therapy designation was granted for SOF on October 10, 2013 under the IND 106739.

At the time of the initial Clinical Pharmacology Review, the primary safety and efficacy data supporting the use of SOF was based on safety and efficacy data from 4 pivotal Phase 3 trials in a total of 1296 subjects: P7977-1231 (FISSION), GS-US-334-0107 (POSITRON), GS-US-334-0108 (FUSION), and GS-US-334-0110 (NEUTRINO). Study GS-US-334-0110 was conducted in treatment-naïve subjects with genotypes 1, 4, 5 or 6 CHC in combination with peginterferon alfa 2a (PEG) and ribavirin (RBV). The other three trials were conducted in subjects with genotype 2 or 3 CHC in combination with ribavirin including one in treatment-naïve subjects, one in interferon intolerant, ineligible or unwilling subjects and one in subjects previously treated with an interferon-based regimen. These Phase 3 trials included subjects who had compensated liver disease, including cirrhosis.

In advance of the American Association for the Study of Liver Diseases (AASLD) annual meeting on November 1st-5th, the Applicant provided top-line data to the Agency from two studies (GS-US-334-0133 [VALENCE] and GS-US-334-123 [PHOTON-1]) which would be presented at AASLD. The top-line information from GS-US-334-0133 shared by the Applicant appeared to support longer treatment duration (24-weeks of SOF/RBV) in genotype 3 subjects. Analyses from Applicant's initial submission package indicated that the studied SOF/RBV treatment duration in genotype 3 subjects (16-weeks) may have been suboptimal. As there were already data from 24 week treatment duration in genotype 3 subjects showing increased response rates, a decision was made to review the currently available data from VALENCE trial during the current review cycle. Likewise, top-line information from GS-US-334-0123 appeared to support a treatment option (24 weeks of SOF/RBV) for interferon-ineligible genotype 1 patients and safety and efficacy data to support the use of SOF/RBV in the HIV-HCV co-infected patient population. These assessments led to submission and review of data from the two ongoing trials, GS-US-334-0133 (VALENCE) and GS-US-334-0123 (PHOTON-1) discussed in this review.

1.1 Recommendation

The Office of Clinical Pharmacology has determined that there is sufficient clinical pharmacology information provided in the NDA to support a recommendation of approval of SOF for use in hepatitis C virus (HCV)/HIV-1 co-infected patients. In addition, the results of GS-US-334-0133 support the use of 24-weeks SOF/RBV in genotype 3 subjects instead of a 16-week duration as was recommended in the original Clinical Pharmacology Review. Finally, based on analyses conducted by the Office of Clinical Pharmacology and Office of Biostatistics (see Addendum Biostatistics review by Karen Qi and the original Clinical Pharmacology Review) as well as discussion at the SOF Advisory Committee Meeting on October 25th, 2013, the Office of Clinical Pharmacology also recommends extending the SOF indication to include prior genotype 1 PEG/RBV treatment-failures. The updated dosage and administration

information for SOF, including treatment duration and treatment combinations based on HCV genotype, are shown below in Table 1.

Table 1 Recommended Dose and Treatment Duration for SOF Combination Therapy

HCV Mono-infected and HCV/HIV-1 Co-infected	Treatment	Duration
Patients with genotype 1 or 4 CHC	SOF+peg-interferon alfa ^a + ribavirin ^b	12 weeks
Patients with genotype 2 CHC	SOF+ribavirin ^b	12 weeks
Patient with genotype 3 CHC	SOF+ribavirin ^b	24 weeks

a. See peginterferon alfa prescribing information for dosing recommendation for patients with genotype 1 or 4 CHC.

b. Dose of ribavirin is weight-based (<75 kg = 1000 mg and ≥ 75 kg = 1200 mg). The daily dose of ribavirin is administered orally in two divided doses with food. Patients with renal impairment (CrCl ≤ 50 mL/min) require ribavirin dose reduction; refer to ribavirin prescribing information.

1.2 Post-Marketing Commitments/Post-Marketing Requirements

- [PMR]: Submit the final study report and datasets for the ongoing trial GS-US-334-0154, entitled, “A Phase 2b, Open-Label Study of 200 mg or 400 mg Sofosbuvir + RBV for 24 Weeks in Genotype 1 or 3 HCV-Infected Subjects with Renal Insufficiency”, in order to provide dosing recommendations for chronic hepatitis C patients with severely impaired renal function.
- [PMR]: Submit the final study report and datasets for the ongoing trial GS-US-334-0154, entitled, “A Phase 2b, Open-Label Study of 200 mg or 400 mg Sofosbuvir + RBV for 24 Weeks in Genotype 1 or 3 HCV-Infected Subjects with Renal Insufficiency”, in order to provide dosing recommendations for chronic hepatitis C patients with ESRD.

1.3 Summary of Important Clinical Pharmacology Findings

Sofosbuvir (SOF) is a first in class, pan-genotypic inhibitor of the Hepatitis C virus (HCV) NS5B RNA-dependent RNA polymerase, which is essential for viral replication. Based on emerging data from two trials (GS-US-334-0123 [HCV/HIV-1 co-infected patients] and GS-US-334-0133 [24-week treatment duration in genotype]), the sponsor was advised to submit data and study reports from these trials for review. Clinical pharmacology conclusions for these trials are summarized below:

GS-US-334-0123

- There was no evidence of altered SOF or GS-331007 pharmacokinetics when coadministered with the following antiretroviral regimens: efavirenz (EFV), rilpivirine (RPV), raltegravir (RAL), darunavir boosted with ritonavir (DRV/RTV), and atazanavir boosted with ritonavir (ATV/RTV) each in combination with emtricitabine and tenofovir (FTC/TDF). No dose adjustment for SOF is needed when administered with the above antiretroviral regimens.

- Subjects infected with genotype 1b had a lower overall SVR rate (54% [13/24]) compared to subjects infected with genotype 1a (82% [74/90]) when treated with 24-weeks of SOF/RBV. On-treatment viral kinetics support that subjects infected with genotype 1b may be less responsive to treatment with SOF/RBV. At week 1, 31% genotype 1a and 21% of genotype 1b subjects had HCV RNA <25 IU/mL. At week 2 and week 4 subjects infected with genotype 1a were more likely to have HCV RNA target not detected (TND) (39%, and 72%, respectively) compared to subjects infected with genotype 1b (17% and 54%, respectively). Achieving HCV RNA TND was associated with an increased likelihood of achieving SVR. The SVR rate was 91% (59/65) and 77% (10/13) in genotype 1a and genotype 1b subjects with HCV RNA TND by week 4 in GS-US-334-0123 compared to 60% (15/25) and 27% (3/11) in genotype 1a and genotype 1b subjects who did not achieve HCV RNA TND by week 4.
- *IFNL3* (referred to as IL28B in this addendum) non-CC genotype was associated with modestly lower SVR12 rates in subjects with HCV genotype 1 infection after 24 weeks of combination treatment with sofosbuvir and ribavirin, though interpretation of these results is limited by the small sample size in GS-US-334-0123.

GS-US-334-0133

- Genotype 3 treatment naïve and prior PEG/RBV treatment failures administered SOF/RBV for 24-weeks had SVR12 rates of 93% (98/105) and 77% (112/145), respectively. These SVR12 rates were higher than those previously obtained for 12-weeks SOF/RBV in genotype 3 treatment naïve subjects (SVR12: 61% [60/98]) and 16-weeks SOF/RBV in genotype 3 prior PEG/RBV treatment failures (SVR12: 62% [39/63]). These updated results for genotype 3 treatment naïve and prior PEG/RBV treatment failures supports the use of 24-weeks SOF/RBV in this population.
- A higher SVR rate was observed in genotype 3 subjects in both treatment naïve and prior PEG/RBV treatment failures regardless of treatment duration if a subject achieved HCV RNA <25 IU/mL earlier on treatment.
- The highest SVR rates were observed in subjects with HCV RNA <25 IU/mL by week 1 of treatment. By week 2, 90% of subjects achieved HCV RNA <25 IU/mL regardless of prior treatment history. A lower percentage of subjects achieved HCV RNA TND at the week 2 and 4 visits, but this virologic assessment did provide increased sensitivity for identifying on treatment response differences between treatment naïve and prior PEG/RBV treatment failures. It cannot be ruled out that on-treatment assessments at week 2, week 3, or week 4 may be useful in identifying subjects who would benefit from longer treatment durations or the addition of a third drug.
- Among genotype 2 subjects, both 12-weeks and 24-weeks treatment with sofosbuvir resulted in similar SVR12 rates. There appeared to be no benefit to extending treatment duration in genotype 2 subjects, though the available data is not sufficient to determine if certain subgroups may benefit from longer treatment duration.

2. APPENDICES

2.1 Pharmacogenomics Review (by Dr. Sarah Dorff)

AMENDMENT TO GENOMICS GROUP REVIEW

NDA/BLA Number	204671
Submission Date	04/08/2013
Applicant Name	Gilead Sciences Inc.
Generic Name	Sofosbuvir
Proposed Indication	Treatment of chronic hepatitis C (CHC) as a component of a combination antiviral treatment regimen
Primary Reviewer	Sarah Dorff, Ph.D.
Secondary Reviewer	Michael Pacanowski, Pharm.D., M.P.H.

1 Additional Submission Contents Related to Genomics

The applicant provided *IFNL3* genotype data for two Phase 3 trials (PHOTON-1 and VALENCE), the designs of which are summarized in table 1. Trial endpoint measurements were sustained virologic response at week 12 (SVR12) post-treatment. Because of the interim nature of these reports, SVR12 data was not available for all subjects.

Table 1. Phase 3 trials assessed for *IFNL3* genotype

Study	N	Genotype	Prior HCV Treatment	Treatment
GS-US-334-0123 (PHOTON-1)	223	1, 2, 3	Treatment-naïve and Treatment-experienced, HIV-1 Co-Infected	SOF+RBV for 12 weeks or SOF+RBV for 24 weeks
GS-US-334-0133 (VALENCE)	419	2, 3	Treatment-naïve and Treatment-experienced	Placebo+RBV for 12 weeks or SOF+RBV for 12 weeks or SOF+RBV for 24 weeks

SOF: sofosbuvir; RBV: ribavirin.

2 Does *IFNL3* genotype influence SVR rates to sofosbuvir-containing regimens across HCV genotypes 1-3?

2.1 Distribution of *IFNL3* genotype (rs12979860) by trial

The frequency of *IFNL3* genotypes is summarized in Table 2. The study populations were predominantly white (68.6% for PHOTON-1 and 93.8% for VALENCE). The proportion of individuals with *IFNL3* CC genotype in these two studies was similar to those in the studies previously reviewed from the original NDA submission. *IFNL3* genotype was not balanced across treatment arms within the HCV genotype strata, such that the C/C genotype tended to be more prevalent in the sofosbuvir/ribavirin arms.

Table 2. Distribution of *IFNL3* genotype by trial

<i>IFNL3</i> genotype, N (%)	PHOTON-1		VALENCE		
	Treatment-Naïve and Treatment-Experienced		Treatment-Naïve and Treatment-Experienced		
	SOF+RBV 12 weeks	SOF+RBV 24 weeks	SOF+RBV 12 weeks	SOF+RBV 24 weeks	Placebo+RBV 12 weeks
Genotype 1					
		N = 113 ^a			
CC	-	30 (26.5%)	-	-	-
CT	-	57 (50.4%)	-	-	-
TT	-	26 (23.0%)	-	-	-
Genotype 2					
	N = 26	N = 24	N = 73		N = 18
CC	10 (38.5%)	10 (41.7%)	24 (32.9%)	-	3 (16.7%)
CT	15 (57.7%)	10 (41.7%)	41 (56.2%)	-	12 (66.7%)
TT	1 (3.8%)	4 (16.7%)	8 (11.0%)	-	3 (16.7%)
Genotype 3					
	N = 42	N = 17	N = 11	N = 250	N = 67
CC	15 (35.7%)	10 (58.8%)	4 (36.4%)	86 (34.4%)	19 (28.4%)
CT	22 (52.4%)	7 (41.2%)	4 (36.4%)	131 (52.4%)	37 (55.2%)
TT	5 (11.9%)	-	3 (27.3%)	33 (13.2%)	11 (16.4%)

SOF: sofosbuvir; RBV: ribavirin.

^a N = 114, 1 subject missing genotype. All genotype 1 subjects were treatment-naïve.

Data Source: PHOTON-1 and VALENCE 'adeffout' datasets.

Reviewer Comment: Imbalances in IFNL3 genotype could influence the observed treatment effects. As such, results within each IFNL3 genotype stratum were reviewed.

2.2 SVR12 by *IFNL3* genotype

The SVR12 results for all HCV genotypes by *IFNL3* are shown in Table 3 below.

Genotype 1: In PHOTON-1, 24-weeks of treatment with sofosbuvir and ribavirin was assessed in treatment-naïve subjects with HCV genotype 1 infection. In this interferon-free regimen, SVR12 rates were 82.8% in CCs and 74.7% in non-CCs. In the non-CC subjects, SVR12 rates were 78.9% in CTs (N = 57) and 65.4% in TTs (N = 26). These results are consistent with previous findings of the influence of *IFNL3* genotype on HCV genotype 1 treatment.

Genotype 2: Subjects with HCV genotype 2 treated with sofosbuvir for 12 weeks had SVR12 rates above 90% in all *IFNL3* genotype subgroups.

Genotype 3: Subjects with HCV genotype 3 infection tended to have higher SVR12 rates after 24-weeks of treatment with sofosbuvir (83-100%) compared to 12-weeks of treatment with sofosbuvir (17-73%). Non-CCs tended to have lower SVR12 rates, though few subjects were treated for 24 weeks in PHOTON-1. In VALENCE, however, after stratification for treatment experience, responses were similar between CCs and non-CCs. Specifically, treatment-naïve subjects had SVR12 rates of 97.6% for CCs (N = 42) and 93.4% for non-CCs (N = 61), whereas for treatment-experienced subjects, SVR12 was 82.9% for CCs (N = 41) and 77.2% for non-CCs (N = 101). Consistent with results from the previously reviewed trials, no trends for differences in SVR12 were observed based on *IFNL3* CC versus non-CC genotype in subjects infected with HCV genotype 2 and 3.

Reviewer comment: Conclusions and interpretation of the directionality of effect with respect to IFNL3 genotype is limited in these trials because of the small sample sizes.

Table 3. SVR12 rates by HCV genotype, *IFNL3* genotype, treatment arm, and trial.

<i>IFNL3</i> genotype n/N [missing SVR] (%)	PHOTON-1		VALENCE	
	Treatment-Naïve and Treatment-Experienced		Treatment-Naïve and Treatment-Experienced	
	SOF+RBV 12 weeks	SOF+RBV 24 weeks	SOF+RBV 12 weeks	SOF+RBV 24 weeks
Genotype 1				
		N = 113		
CC	-	24/29 [1] (82.8%)	-	-
95% CI	-	(64.2%-94.2%)	-	-
Non-CC	-	62/83 (74.7%)	-	-
95% CI	-	(60.6%-85.9%)	-	-
Genotype 2				
	N = 26	N = 24	N = 73	
CC	8/9 [1] (88.9%)	5/6 [4] (83.3%)	24/24 (100.0%)	-
95% CI	(51.8%-99.7%)	(35.9%-99.6%)	(85.8%-100.0%)	-
Non-CC	15/15 [1] (100.0%)	9/9 [5] (100.0%)	44/49 (89.8%)	-
95% CI	(78.2%-100.0%)	(66.4%-100.0%)	(77.8%-96.6%)	-
Genotype 3				
	N = 42	N = 17	N = 11	N = 250
CC	9/14 [1] (64.3%)	6/6 [4] (100.0%)	2/3 [1] (66.7%)	75/83 [3] (90.4%)
95% CI	(35.1%-87.2%)	(54.1%-100.0%)	(9.4%-99.2%)	(81.9%-95.8%)
Non-CC	19/26 [1] (73.1%)	6/7 (85.7%)	1/6 [1] (16.7%)	135/162 [2] (83.3%)
95% CI	(52.2%-88.4%)	(42.1%-99.6%)	(0.4%-64.1%)	(76.7%-88.7%)

Data Source: PHOTON-1 and VALENCE 'adeffout' datasets.

Reviewer Comment: Because of the small sample size in most subgroups and high SVR rates overall, it is difficult to draw firm conclusions with respect to differences in response by IFNL3 genotype.

3 Summary and Conclusions

The applicant evaluated the effect of *IFNL3* genotype on SVR12 in subjects infected with HCV. *IFNL3* non-CC genotype was associated with modestly lower SVR12 rates in subjects with HCV genotype 1 infection after 24 weeks of combination treatment with sofosbuvir and ribavirin, albeit not significantly different. Among genotype 2 subjects, both 12-weeks and 24-weeks treatment with sofosbuvir and ribavirin resulted in similar SVR12 rates. Genotype 3 subjects treated with sofosbuvir and ribavirin for 24-weeks had higher SVR12 rates compared to the 12-week regimen. No consistent difference in SVR12 was observed among *IFNL3* genotypes in HCV genotype 2 and 3 infected subjects. Review of the additional data from PHOTON-1 and VALENCE support the conclusions reached in the original Genomics Group review with respect to the influence of *IFNL3* on SVR12 after treatment with sofosbuvir-containing regimens.

4 Recommendations

Because the effect of *IFNL3* genotype is typically most pronounced in genotype 1-infected patients, results for PHOTON-1 should be depicted by *IFNL3* genotype in labeling.

4.1 Post-marketing studies

None.

4.2 Labeling Recommendations

Please refer to final labeling.

2.2 Pharmacometrics Review (by Dr. Florian)

AMENDMENT TO PHARMACOMETRICS REVIEW

NDA/BLA Number	204671
Submission Date	04/08/2013
Applicant Name	Gilead Sciences Inc.
Generic Name	Sofosbuvir
Proposed Indication	Treatment of chronic hepatitis C (CHC) as a component of a combination antiviral treatment regimen
Primary Reviewer	Jeffry Florian, Ph.D.
Secondary Reviewer	Yaning Wang, Ph.D.

Summary of Findings

Are the sofosbuvir (SOF) and GS-331007 PK parameters from GS-US-334-0123 (PHOTON) in HCV/HIV-1 co-infected patients similar to the SOF and GS-331007 PK observed in HCV mono-infected patients?

The pharmacokinetic parameters for 400 mg sofosbuvir (SOF) q.d. resulted in similar pharmacokinetic parameters between HCV mono-infected patients and HCV/HIV-1 co-infected patients. SOF AUC, GS-331007 AUC, and GS-331007 C_{min} were similar between the mono-infected HCV population from Phase III and the co-infected HCV/HIV-1 population from GS-US-334-0123. GS-331007 C_{max} was higher (75%) in the co-infected population, but was lower than the GS-331007 C_{max} observed from healthy volunteers. This difference in GS-331007 C_{max} may have resulted from the PK sampling schedules used in these populations (sparse sampling with limited or no data for C_{max}).

SOF and GS-331007 PK parameters (AUC and C_{max}) were obtained by simulating concentration time courses from individual post-hoc population PK parameter estimates, then performing noncompartmental analysis on the simulated profiles using WinNonLin v.5. GS-331007 C_{min} was obtained from observed data where the sample was obtained within 20-28 hours of the last dose at steady state. The mono-infected analyses presented below in Table 1 were limited to the Phase III population (GS-US-334-107, GS-US-334-0110, P7977-1231, GS-US-334-0108) as both these studies and GS-US-334-0123 utilized sparse sampling. Two subjects from GS-US-334-0123 were excluded from the PK analysis based on the sponsor's provided datasets (pknca.xpt). One of these subjects (GS-US-334-0123-1691-8720) was removed from the analysis by the sponsor due to document non-adherence to study drug. The other subject (GS-

US-334-0123-1961-8783) discontinued treatment at week 4 due to HCV virologic breakthrough. This subject had the lowest non-compartment analysis GS-331007 AUC, 67% lower than the geometric mean and may also have been reflective of poor adherence. At the start of SOF/RBV treatment, both of these subjects were not on an antiretroviral treatment for their HIV-1. As an additional comparison the PK parameters for healthy volunteers are also presented in Table. Based on these summarized results, no clear difference in the SOF and GS-331007 PK were observed between HCV mono-infected or HCV/HIV-1 co-infected subjects.

Table 1: Summary of SOF and GS-331007 PK Parameters from HCV-infected Subjects from Phase III, HCV/HIV-1 Co-infected Subjects from GS-US-334-0123, and Healthy Volunteers

		HCV, mono-infected (SOF+RBV or SOF/PEG/RBV) [only Phase 3]	HCV/HIV-1 co-infected (SOF+RBV)	Healthy Volunteers (SOF)
SOF	N	760	221	272
	AUC, [CV%] ng/mL·hr (Median) [Range]	814 (67%) (817) [245; 3312]	868 (79%) (819) [286; 4600]	595 (63%) (583) [205; 1548]
GS-331007	N	986	221	272
	AUC, [CV%] ng/mL·hr (Median) [Range]	6864 (61%) (6970) [1781; 17375]	7721 (58%) (7683) [3569; 22021]	11147 (50%) (11204) [5494; 22229]
	C _{max} , ng/mL (Median) [Range]	540 (70%) (565) [92; 1795]	946 (69%) (1018) [227; 1514]	1079 (52%) (1089) [523; 1781]
	C _{min} , ng/mL (Median) [Range] {n=405, 85}*	196 (84%) (192) [26; 1188]	214 (89%) (211) [45; 874]	- -

*There were only 405 HCV-infected subjects in Phase III and 85 HCV/HIV-1 co-infected subjects from GS-US-334-0123 with one or more GS-331007 C_{min} measurements available where the last dose was between 20-28 hours. Only these subjects were used in the C_{min} calculation above.

The PK data from GS-US-334-0123 was further explored by summarized SOF AUC and GS-331007 AUC and C_{max} by background antiretroviral treatment regimen (Table 2). GS-331007 C_{min} was not included in the table as the number of subjects with PK sampling 20-28 hours following the last dose for the background antiretroviral groups was too small to permit comparisons. The background regimens included in the table were efavirenz (EFV), rilpivirine (RPV), raltegravir (RAL), darunavir boosted with ritonavir (DRV/RTV), and atazanavir boosted

with ritonavir (ATV/RTV) each in combination with emtricitabine and tenofovir (FTC/TDF). Nine subjects who were not on antiretroviral therapy and 11 subjects who were on antiretroviral combinations not listed above were not included among the background regimens listed below. No clear difference in SOF AUC or GS-331007 AUC and C_{max} were identified with respect to any of these antiretroviral regimens, though GS-331007 C_{max} was consistently higher across all treatment groups compared to the GS-331007 C_{max} in HCV mono-infected subjects.

Table 2: Summary of SOF and GS-331007 PK Parameters from HCV/HIV-1 Co-infected Subjects in GS-US-334-0123, Grouped by Antiretroviral Background Regimen

	HCV/HIV-1 coinfectd (SOF+RBV)						
	Background Regimen	Any	EFV+ FTC/TDF	RPV+ FTC/TDF	RAL+ FTC/TDF	DRV/RTV+ FTC/TDF	ATV/RTV+ FTC/TDF
SOF	n	221	78	14	36	34	39
	AUC, [CV%] ng/mL·hr [Median]	868 (79%) [819]	797 (65%) [813]	873 (77%) [824]	941 (85%) [901]	759 (83%) [700]	1058 (80%) [1132]
	C _{max} , ng/mL [Median]	946 (69%) [1018]	915 (74%) [997]	815 (50%) [800]	886 (68%) [931]	1035 (64%) [1123]	1019 (69%) [1141]
GS-331007	n	221	78	14	36	34	39
	AUC, [CV%] ng/mL·hr [Median]	7721 (58%) [7683]	7321 (54%) [7380]	6726 (57%) [6709]	7635 (56%) [7762]	7873 (57%) [7713]	8780 (61%) [8279]
	C _{max} , ng/mL [Median]	946 (69%) [1018]	915 (74%) [997]	815 (50%) [800]	886 (68%) [931]	1035 (64%) [1123]	1019 (69%) [1141]

Are genotype 1 treatment-naïve patients with three poor baseline predictive factors who are treated with PEG/RBV representative of prior PEG/RBV treatment failures?

Yes, a majority of the genotype 1 treatment naïve patients with three poor baseline predictive factors (metavir fibrosis score of F3/4, IFNL3 host genotype non-CC, and baseline viral load >800,000 copies/mL) were classified as treatment failures following their first course of treatment and would subsequently be prior relapsers, partial, and null responders for future studies.

The analyses in this addendum focus on evaluation of the baseline factor analysis that was described in the Clinical Pharmacology Review (September 5th, 2013). The initial analyses looked at three factors listed above in NEUTRINO. A similar analysis was performed in the context of other development programs and indicated that the response rate obtained from

treatment-naïve patients with these three baseline factors was between the observed SVR rate observed for prior PEG/RBV treatment failures. The updated analysis below applies this same baseline factor analysis to previously submitted PEG/RBV treatment-naïve control arms where IL28B host genotype information was available.

In total, there were 481 genotype 1 treatment naïve subjects administered PEG/RBV in control arms who also had IL28B host genotype information available for analysis. Of these subjects, 47% were classified as responders (achieved SVR), 17% were relapsers, 30% were partial/null responders, and 6% discontinued treatment or were lost to follow-up (Table 3). In partial/null responders, 17% of these patients had all three baseline factors and 85% of patients had 2 or more factors. In contrast, only 2% of all responders had all three baseline factors and 27% had 2 or more of the listed baseline factors. Of all the patients with the three baseline factors (n=42), 57% (n=24) were classified as partial/null responders at the end of treatment and 90% (n=38) were classified at treatment failures. Responders comprised 10% (4/42) of this subset, which is a similar SVR rate to that observed for prior non-responders retreated with PEG/RBV. This analysis continues to support that selection of patients with above listed three baseline factors in a treatment-naïve population may be representative of prior PEG/RBV treatment failures, though selection of alternative factors may permit increased sensitivity for identify patients who, if treated with PEG/RBV, would have failed initial therapy as partial or null responders.

Table 3: Summary of PEG/RBV Treatment Outcomes Based on the Number of Poor Baseline Predictive Factors Present (Assessment Limited to Three Baseline Factors Listed Above)

	Other	Partial/Null	Relapser	Responder
Number of Baseline Factors	N=31	N=145	N=80	N=225
0	0 (0%)	0 (0%)	1 (1%)	20 (9%)
1	9 (29%)	23 (16%)	33 (41%)	145 (64%)
2	16 (52%)	98 (68%)	38 (48%)	56 (25%)
3	6 (19%)	24 (17%)	8 (10%)	4 (2%)

In addition to the above analysis, the reviewer also updated the baseline factor analysis conducted in the original Clinical Pharmacology Review (pg 253-255). The updated analysis used identical methodology to that detailed in the previous review but combines treatment-naïve results for a direct-acting antiviral (DAA) in the situations where multiple trials were available (Table 4).

Table 4: Comparison of SVR12 Rate From Various Direct-Active Antiviral Treatment-Naïve and Treatment-Experienced Studies. The SVR Rates Shown for the Treatment-Naïve Studies are Based on the Subset of Subjects with Multiple Poor Baseline Predictive Factors (non-CC, baseline viral load >800K, F3 or F4 fibrosis staging) [Adapted from Clinical Pharmacology Review, September 5th, 2013]

Drug	DAA+PEG/RBV		
	Treatment-naïve Subjects	Prior Null Responders	Prior Partial Responders
Telaprevir	44% (61/138)	32% (47/147)	59% (57/97)
Boceprevir	45% (32/71)	38% (20/52)	46% (53/115)
Simeprevir	51% (36/73)	49% (49/101)	66% (91/137)

Does the on treatment viral kinetics indicate a difference in response for genotype 1b subjects compared to genotype 1a subjects from GS-US-334-0110 or GS-US-334-0123?

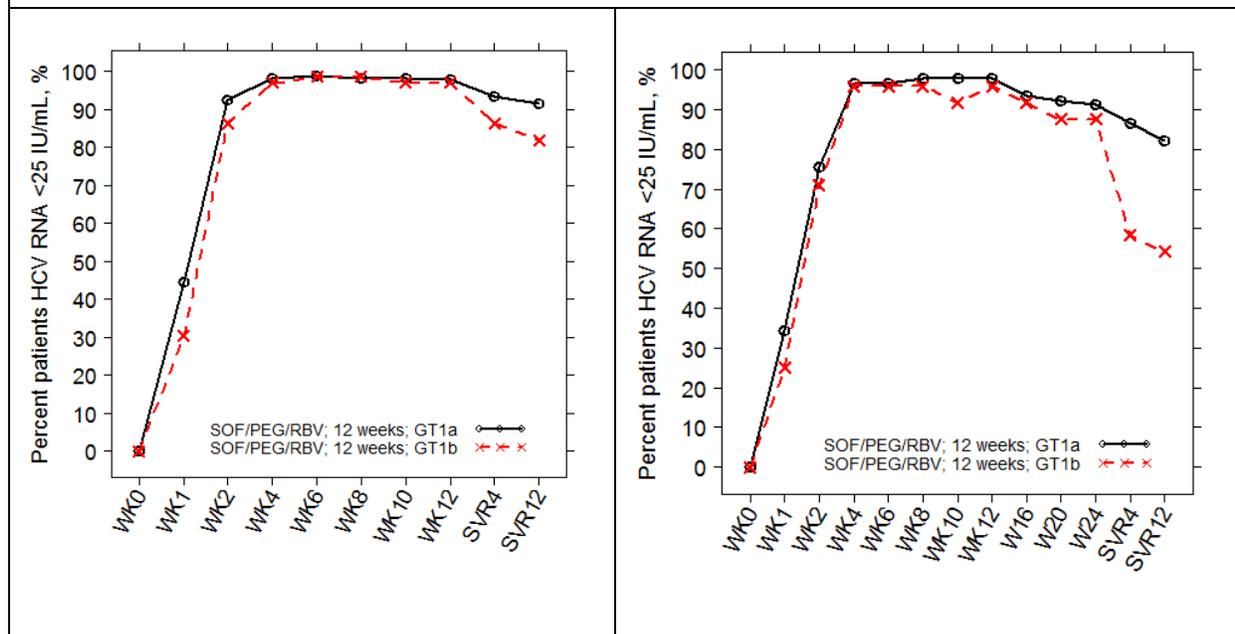
On treatment viral kinetics from both GS-US-334-0110 and GS-US-334-0123 support that clinical observation of decreased response in subjects infected with HCV genotype 1b compared to subjects infected with HCV genotype 1a.

In both GS-US-334-0110 and GS-US-334-0123, which evaluated treatment-naïve subjects infected with genotype 1 HCV or co-infected with genotype 1 HCV/HIV-1, a difference in sustained virologic response (SVR) rate between subjects infected with HCV genotype 1b and genotype 1a was observed, with a lower SVR rate observed for subjects infected with genotype 1b. While GS-US-334-0123 included subjects infected with genotype 2 and 3, these subjects were not included in this analysis of the impact of genotype 1 subtype on SOF treatment outcome. In GS-US-334-0110 (regimen: 12-weeks SOF/PEG/RBV), subjects infected with genotype 1b had an SVR rate of 82% (54/66) compared to 92% (206/225) in subjects infected with HCV genotype 1a. Similarly, in GS-US-334-0123 (regimen: 24-weeks SOF/RBV), subjects infected with HCV genotype 1b had an SVR rate of 54% (13/24) compared to 82% (74/90) in subjects infected with HCV genotype 1a. To assist in determining whether this observed difference in SVR rate was reflective of true differences in response between genotype 1b and 1a, on-treatment viral kinetics from both studies were evaluated.

The percentage of genotype 1 subjects with HCV RNA <25 IU/mL at each visit from GS-US-334-0110 and GS-US-334-0123 grouped by genotype subtype is shown below in Figure 1. These analyses identified a difference in the percentage of subjects with on-treatment HCV RNA <25 IU/mL at week 1 of treatment (43% genotype 1a and 27% genotype 1b from GS-US-334-110 and 31% genotype 1a and 21% genotype 1b from GS-US-334-0123), that decreased by week 2 of treatment (92% genotype 1a and 86% genotype 1b from GS-US-334-110 and 77% genotype 1a and 71% genotype 1b from GS-US-334-0123), and was no longer evident from the week 4 assessment onward (96% or more with HCV RNA <25 IU/mL). Neither of these studies included a week 3 virologic assessment or virologic assessments between week 1 and week 2 of treatment. Besides the week 1 and week 2 on-treatment differences, the only other noticeable differences between these groups was in the frequency of relapse, which occurred more frequently in subjects infected with HCV genotype 1b. In GS-US-334-0110, 65 subjects infected with HCV genotype 1b had HCV RNA target not detected at the end of treatment and 9

subjects relapsed for a relapse rate of 14%. By comparison, 225 subjects infected with HCV genotype 1a had HCV RNA target not detected at the end of treatment and 18 subjects relapsed for a relapse rate of 8%. Similar trends were observed in GS-US-334-0123 where the genotype 1b relapse rate was 42% (10 out of 24 subjects) compared to 17% (15 out of 90 subjects) in subjects infected with HCV genotype 1a.

Figure 1: Virologic Time Course for Subjects Infected with HCV Genotype 1a or Genotype 1b from GS-US-334-0110 (left) or GS-US-334-0123 (right)



Next, data from GS-US-334-0110 and GS-US-334-0123 were summarized to determine if on treatment assessments at week 1, week 2, and week 4 were predictive of whether a patient would achieve SVR (subjects with missing assessments were removed for that visit from this analysis). Summary results for SVR rate based on on-treatment virologic response and time to first HCV RNA target not detected are shown below in Table 4. In this table the percentage of subjects from each study and each genotype subtype who first achieved HCV RNA <25 IU/mL at the listed visit is shown (N (%)) as well as the SVR rate for those subjects [SVR Rate; n/N].

As would be expected from the profiles shown in Figure 1, most of the subjects achieved HCV RNA <25 IU/mL by week 2 of treatment in both studies regardless of HCV genotype 1 subtype. There was a trend towards higher SVR rates in genotype 1a subjects from GS-US-334-0110 who achieved HCV RNA <25 IU/mL at week 1 of treatment compared to week 2 or later, though similar trends could not be identified for genotype 1b subjects from GS-US-334-0110 or either genotype subtype in GS-US-334-0123 (Table 5). In addition, a decrease in SVR rate was observed for genotype 1a and genotype 1b subjects from GS-US-334-0123 who achieved HCV RNA <25 IU/mL after week 2 compared to those who achieved HCV RNA <25 IU/mL at week

2 or earlier. However, given the small number of genotype 1b subjects in GS-US-334-0110 (n=66) and GS-US-334-0123 (n=24), it is difficult to draw any conclusions about relationships between on-treatment response and SVR rate. The observations from GS-US-334-0110 are consistent with previous observations from interferon-containing regimens where on-treatment response (e.g., how early on treatment a patient achieves viral suppression) is associated with SVR rate.

Table 5: Summary of SVR Rates in Genotype 1 Subjects from GS-US-334-0110 and GS-US-334-0123, Group By Subtype, Based on Time to First Assessment with HCV RNA <25 IU/mL

Time until <25 IU/mL	Genotype 1			
	GS-US-334-0110, NEUTRINO [12-weeks SOF/PEG/RBV]		GS-US-334-0123, PHOTON [24-weeks SOF/RBV]	
	Genotype 1a	Genotype 1b	Genotype 1a	Genotype 1b
	N (%) [SVR Rate; n/N]	N (%) [SVR Rate; n/N]	N (%) [SVR Rate; n/N]	N (%) [SVR Rate; n/N]
Week 1	96 (43%) [SVR: 98%; 94/96]	18 (27%) [SVR: 83%; 15/18]	28 (31%) [SVR: 86%; 24/28]	5 (21%) [SVR: 80%; 4/5]
Week 2	111 (49%) [SVR: 87%; 97/111]	39 (59%) [SVR: 85%; 33/39]	42 (47%) [SVR: 88%; 37/42]	12 (50%) [SVR: 67%; 8/12]
Week 4	16 (7%) [SVR: 88%; 14/16]	8 (12%) [SVR: 75%; 6/8]	18 (20%) [SVR: 61%; 11/18]	6 (25%) [SVR: 17%; 1/6]
>Week4	2 (1%) [SVR: 50%; 1/2]	0 (0%) [SVR: NA]	2 (2%) [SVR: 100%; 2/2]	1 (4%) [SVR: 0%; 0/1]

An alternative assessment of on-treatment response for these studies was also performed based on percentage of subjects with HCV RNA target not detected (TND) at week 1, week 2, and week 4 assessments (Table 6). Similar to the trends that have been described above between genotype 1a and genotype 1b on-treatment response, genotype 1a subjects were more likely to have HCV RNA target not detected at week 2 of treatment in GS-US-334-0123 on SOF/PEG/RBV (52% genotype 1a with TND compared to 33% genotype 1b with TND at week 2). Likewise, a greater proportion of genotype 1a subjects achieved TND at week 2 (39%) and week 4 (72%) in GS-US-334-0123 compared to genotype 1b subjects (17% and 54% at week 2

and week 4, respectively). Achieving TND by week 4 of treatment was a significant predictor of SVR rate in GS-US-334-0123, and subjects infected with HCV genotype 1b were less likely to achieve HCV RNA TND by week 4. Similarly, achieving HCV RNA TND by week 4 in GS-US-334-0110 was a significant predictor of achieving SVR for subjects infected with HCV genotype 1a, though it was not a significant predictor of SVR rate for genotype 1b (p-value=0.07). These were all univariate analyses and did not account for additional baseline factors that could further influence these analyses including cirrhosis, baseline viral load, and IL28B host genotype. SVR rate was 91% (59/65) and 77% (10/13) in genotype 1a and genotype 1b subjects with HCV RNA TND by week 4 in GS-US-334-0123 compared to 60% (15/25) and 27% (3/11) in genotype 1a and genotype 1b subjects who did not achieve HCV RNA TND by week 4. Given the small number of subjects in these subgroups and that interferon-ineligible genotype 1 subjects would not have any alternative treatment option at this time, the utility of using this on-treatment information to guide clinical decisions is uncertain. Similar trends were observed in GS-US-334-0110 with genotype 1a and 1b subjects with HCV RNA TND at week 4 achieving SVR rates of 93% (187/201) and 86% (48/56) compared to 79% (19/24) and 60% (6/10) in genotype 1a and 1b subjects with HCV RNA that was not yet TND. Again, there are small numbers in among subjects who did not achieve HCV RNA TND at week 4, but the decrease in SVR rate and previous observations regarding on-treatment response and treatment duration from interferon-containing regimens suggests that SVR rate may be improved by extending the treatment duration in such subjects, particularly genotype 1b subjects with HCV RNA detectable at week 4 of treatment.

Table 6: Summary of Percentage of Genotype 1a and 1b Subjects with HCV RNA TND from GS-US-334-0110 and GS-US-334-0123 By Assessment

Target Not Detected	Genotype 1			
	GS-US-334-0110, NEUTRINO [12-weeks SOF/PEG/RBV]		GS-US-334-0123, PHOTON [24-weeks SOF/RBV]	
	Genotype 1a	Genotype 1b	Genotype 1a	Genotype 1b
	n/N (%)	n/N (%)	n/N (%)	n/N (%)
Week 1	16/225 (7%)	4/66 (6%)	4/90 (4%)	1/24 (4%)
Week 2	118/225 (52%)	22/66 (33%)	35/90 (39%)	4/24 (17%)
Week 4	201/225 (89%)	56/66 (85%)	65/90 (72%)	13/24 (54%)

In total, these on-treatment assessments demonstrate that subjects infected with HCV genotype 1a were more likely to have HCV RNA <25 IU/mL or TND earlier on treatment than genotype 1b subjects in both studies. The relationship between earlier on-treatment response and SVR rate demonstrated that earlier on treatment response may be associated with increased likelihood of achieving SVR with higher SVR rates observed in those subjects who achieved HCV RNA <25

IU/mL at week 2 or earlier on treatment compared to those subjects who first negative HCV/RNA assessment was after week 2.

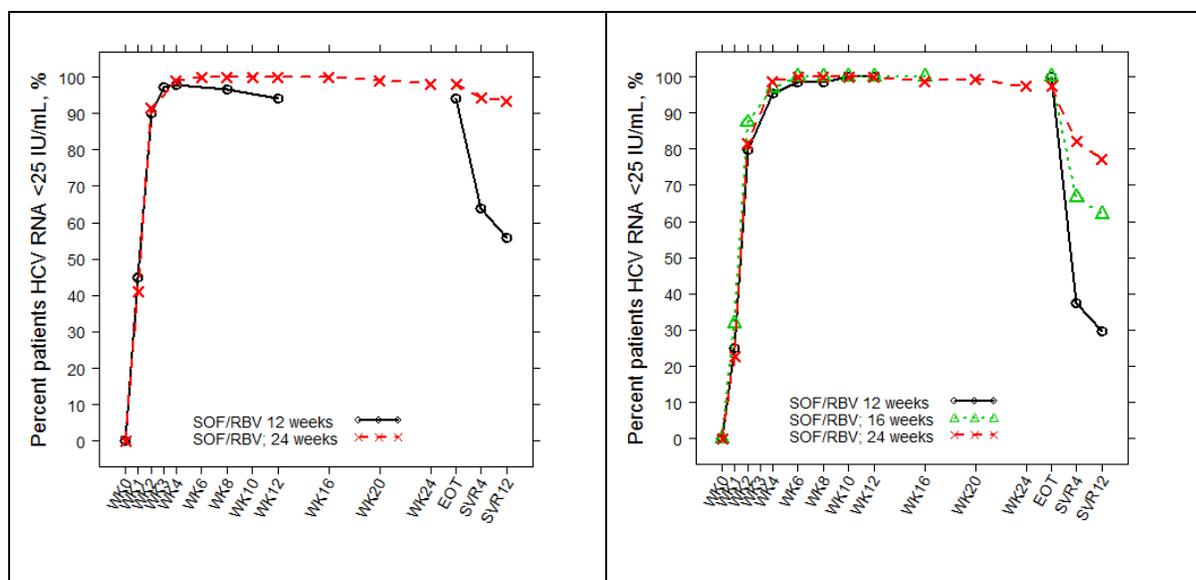
Does the on treatment viral kinetics indicate a difference in response for genotype 3 treatment-naïve or previous PEG/RBV treatment failures from P7977-1231, GS-US-334-0108, and GS-US-334-0133?

On treatment viral kinetics for genotype 3 treatment naïve and prior PEG/RBV treatment failures from P7977-1231, GS-US-334-0108, and GS-US-334-0133 does not indicate an on treatment difference between different treatment durations. However, the impact of the longer treatment duration is evident in the increase SVR rate (decreased relapse rate) and supports extending the treatment duration to 24-weeks in genotype 3 patients.

Previous analyses in the Clinical Pharmacology Review (September 5th, 2013) evaluated the viral kinetics and impact of treatment duration of response/relapse in genotype 3 subjects treated with SOF/RBV. The sponsor has recently updated the Agency with materials from an additional study evaluating SOF/RBV for 24-weeks in genotype 3 treatment naïve and prior PEG/RBV treatment failures. Together, these studies permit a cross-study comparison of the impact of treatment duration on viral kinetics and response in this population. All genotype 3 subjects from GS-US-334-0108 were included in this analysis. Only genotype 3 subjects administered SOF/RBV from P7977-1231 were included in this analysis. Finally, while GS-US-334-133 included data in genotype 3 subjects from a 12-week (n=11) and 24-week (n=250) treatment duration, only the data from the 24-week duration was used in the analysis below.

No discernible differences in the on-treatment response were identified with respect to treatment duration, which is expected as the subjects would have received identical treatments through the first 12 weeks for all three regimens (Figure 2). The most notable difference was a decrease in relapse rate in both treatment-naïve and prior PEG/RBV treatment failures with 24-week SOF/RBV treatment duration (black line). The relapse rate was 40% compared to 5% in genotype 3 treatment-naïve subjects administered 12-weeks and 24-weeks of SOF/RBV, respectively. Similarly, the relapse rate was 66%, 38%, and 20% in genotype 3 prior PEG/RBV treatment failures administered 12-weeks, 16-weeks, and 24-weeks of SOF/RBV, respectively.

Figure 2: Virologic Time Course for Treatment Naïve Subjects (left) and prior PEG/RBV Treatment Failures (right) Infected with HCV Genotype 3 Administered 12 or 24-weeks (left) or 12, 16, or 24 weeks (right) of SOF/RBV. These Results Were Obtained from: P7977-1231, GS-US-334-0108, and GS-US-334-0133



Data from P7977-1231, GS-US-334-0108, and GS-US-334-0133 were summarized to determine if on treatment assessments at week 1, week 2, or week 4 (week 3 in P7977-1231) were predictive of whether a patient would achieve SVR. Summary results for these three trials are shown below in Table 7. In this table the percentage of subjects from each treatment duration for each population (treatment naïve or prior PEG/RBV treatment failure) who first achieved HCV RNA <25 IU/mL at the listed visit is shown (N (%)) as well as the SVR rate for those subjects [SVR Rate; n/N]. In general, a higher SVR rate was observed in both treatment naïve and prior PEG/RBV treatment failures regardless of treatment durations if a subjects achieved HCV RNA <25 IU/mL earlier on treatment. Similar trends were observed when the analysis was conducted for HCV RNA TND (analysis not shown). By week 2, 90% of genotype 3 subjects achieved HCV RNA <25 IU/mL regardless of prior treatment history. In contrast, 34-47% of treatment naïve subjects had HCV RNA TND at week 2 compared to 21-40% of prior PEG/RBV treatment failures (analysis not shown). The utility of on treatment response in genotype 3 treatment-naïve subjects is unclear given the overall high SVR rate regardless of when subjects first achieved HCV RNA <25 IU/mL. On-treatment assessments may be useful in identifying subjects who would benefit from longer treatment durations or the addition of a third drug based on week 2 and week 4 virologic response.

Table 7: Summary of SVR Rates in Genotype 3 Subjects from P7977-1231, GS-US-334-0108, and GS-US-334-0133, Group By Treatment Duration, Based on Time to First Assessment with HCV RNA <25 IU/mL

Population	Genotype 3, Treatment Naïve		Genotype 3, Prior PEG/RBV Treatment Failures			
	Study	P7977-1231	GS-US-334-0133	GS-US-334-0108	GS-US-334-0108	GS-US-334-0133

Regimen	12-weeks SOF/RBV	24-weeks SOF/RBV	12-weeks SOF/RBV	16-weeks SOF/RBV	24-weeks SOF/RBV
Time until <25 IU/mL	N (%) [SVR Rate; n/N]	N (%) [SVR Rate; n/N]	N (%) [SVR Rate; n/N]	N (%) [SVR Rate; n/N]	N (%) [SVR Rate; n/N]
Week 1	81 (44%) [SVR Rate: 69%; 56/81]	41 (39%) [SVR Rate: 100%; 41/41]	16 (25%) [SVR Rate: 38%; 6/16]	19 (30%) [SVR Rate: 74%; 14/19]	32 (22%) [SVR Rate: 97%; 31/32]
Week 2	84 (46%) [SVR Rate: 48%; 40/84]	54 (51%) [SVR Rate: 89%; 48/54]	34 (53%) [SVR Rate: 35%; 12/34]	36 (57%) [SVR Rate: 56%; 20/36]	85 (59%) [SVR Rate: 79%; 67/85]
Week 3	13 (7%) [SVR Rate: 46%; 6/13]	-	-	-	-
Week 4	-	10 (10%) [SVR Rate: 90%; 9/10]	11 (17%) [SVR Rate: 9%; 1/11]	6 (10%) [SVR Rate: 83%; 5/6]	25 (17%) [SVR Rate: 52%; 13/25]
>Week4	5 (3%) [SVR Rate: 0%; 0/5]	0 (0%)	3 (4%) [SVR Rate: 0%; 0/3]	2 (3%) [SVR Rate: 0%; 0/2]	3 (2%) [SVR Rate: 33%; 1/3]

Recommendations

Based on the submitted data from GS-US-334-0133, a 24-week SOF/RBV treatment duration offers increased SVR rates in genotype 3 treatment naïve and prior PEG/RBV treatment failures and should be a preferred regimen compared to shorted SOF/RBV treatment durations.

Based on the submitted data from GS-US-334-0123 in HCV/HIV co-infected patients, no discernible differences in SOF or GS-331007 PK were identified, and no SOF dose adjustments are necessary when used in combination with the following antiretroviral regimens: efavirenz (EFV), rilpivirine (RPV), raltegravir (RAL), darunavir boosted with ritonavir (DRV/RTV), and atazanavir boosted with ritonavir (ATV/RTV) each in combination with emtricitabine and tenofovir (FTC/TDF).

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/s/

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11/22/2013

Addendum review to primary clinical pharmacology review 9/5/2013

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BIOPHARMACEUTICS REVIEW			
Review#2 - Addendum			
Office of New Drug Quality Assessment			
Application No.:	NDA 204671	Reviewer: Minerva Hughes, Ph.D.	
Submission Date: (Orig)	6 April 2013		
Received Date:	8 April 2013	Team Leader: Angelica Dorantes, Ph.D. Acting Supervisor: Richard Lostritto, Ph.D.	
Division:	Division of Anti-viral Products		
Sponsor:	Gilead	Secondary Reviewer: Angelica Dorantes, Ph.D.	
Trade Name:	TBD	Date Assigned:	8 April 2013
		GRMP Date:	6 September 2013
		PDUFA Date:	8 December 2013
Generic Name:	Sofosbuvir	Date of Review:	05 November 2013
Indication:	Hepatitis C infection	Type of Submission: Original NDA Review (NME – Priority) Review#2 –Addendum to Review dated 30 Aug 2013	
Formulation/strengths	Tablet, 400 mg		
Route of Administration	Oral		
Biopharmaceutics Review Topics: Refer to Review #1 for full details.			
<ul style="list-style-type: none"> ○ Dissolution test method and acceptance criterion, ○ Formulation/process attributes impacting dissolution, and ○ Dissolution stability 			
<p>SUBMISSION: NDA 204671 was submitted in accordance with Section 505(b)(1) of the FDC Act for the use of sofosbuvir, 400 mg tablets, to treat chronic hepatitis C infection in adults with compensated liver disease, including cirrhosis. Sofosbuvir (SOF) is a novel nucleotide prodrug that potently inhibits genotype 1 to 6 HCV RNA replicons in vitro and has demonstrated sustained virologic response (SVR) rates when administered with ribavirin to subjects with chronic genotype 2 and 3 HCV infection and with pegylated interferon + ribavirin (PEG+RBV) to subjects with chronic genotype 1, 2, 3, 4, and 6 HCV infection.</p> <p>The Phase 3 program for SOF includes 4 clinical studies: P7977-1231 (FISSION), GS-US-334-0107 (POSITRON), GS-US-334-0108 (FUSION), and GS-US-334-0110 (NEUTRINO), which evaluated the efficacy and safety of SOF +RBV or SOF+PEG+RBV for various durations and genotypes.</p> <p>If approved, sofosbuvir will be the first nucleotide-analog HCV therapy.</p>			
<p>BIOPHARMACEUTIC INFORMATION: NDA 204671 was amended several times after finalization of the 30 August 2013 Biopharmaceutics Quality Review (see eCTD sequence 21 through 48). There are no data in any of these submissions pertaining to Biopharmaceutics.</p>			
<p>CONCLUSIONS AND RECOMMENDATION: From the perspective of Biopharmaceutics, NDA 204671 for Sofosbuvir Tablets is recommended for approval.</p>			
APPROVAL SIGNATURES: { see electronic signature page }			
Minerva Hughes, Ph.D.		Angelica Dorantes, Ph.D.	
Biopharmaceutics Reviewer/ONDQA		Biopharmaceutics Team Leader/ONDQA	

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11/05/2013

ANGELICA DORANTES
11/05/2013

OFFICE OF CLINICAL PHARMACOLOGY REVIEW

NDA: 204671	Submission Date(s): April 8, 2013
Brand Name	To be Determined
Generic Name	Sofosbuvir (GS-7977)
Primary Reviewer	Jenny H. Zheng, Ph.D.
In vitro Study Reviewer	Su-Young Choi, Pharm.D., Ph.D.
Secondary Reviewer	Shirley Seo, Ph.D.
PM Reviewer/Team Leader	Jeff Florian, Ph.D./ Yaning Wang, Ph.D.
PG Reviewer/Team Leader	Sarah Dorff, Ph.D./ Michael Pacanowski, Pharm.D., M.P.H.
OCP Division	Division 4
OND division	DAVP
Applicant	Gilead Sciences
Relevant IND(s)	106739; 112681; 111572
Submission Type	Priority
Formulation; Strength(s)	Tablets; 400 mg
Indication	Treatment of chronic hepatitis C (CHC) in adults in combination with other agents

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LIST OF ABBREVIATIONS and NOMENCLATURES

ATR:	efavirenz/emtricitabine/tenofovir disoproxil fumarate (Atripla®)
CatA:	cathepsin A
CES1:	carboxyl esterase 1
CHC:	chronic hepatitis C
CI:	confidence interval
CrCL:	creatinine clearance
CsA:	cyclosporine (cyclosporin A)
CYP:	cytochrome P450 enzyme(s)
DAA:	direct-acting antiviral
DDI:	drug-drug interaction
DRV:	darunavir
EFV:	efavirenz

eGFR:	estimated glomerular filtration rate
eq:	equivalent
ESRD:	end-stage renal disease
FMO:	flavin monooxygenase
FTC:	emtricitabine
GLSM:	geometric least-squares mean
HCC:	hepatocellular carcinoma
HCV:	hepatitis C virus
HINT1:	histidine triad nucleotide-binding protein 1
HIV, HIV-1:	human immunodeficiency virus, type 1
IFN:	interferon
IL28B:	IL28B gene
OATP:	organic anion transporting polypeptide
OCT:	organic cation transporter
PD:	pharmacodynamic(s)
PEG:	pegylated interferon or Peg-IFN-alfa-2a
Pgp:	p-glycoprotein
PI:	protease inhibitor
PK:	pharmacokinetic(s)
pTVR:	posttransplant virologic response 12 weeks after liver transplant
QT:	electrocardiographic interval between the beginning of the Q wave and termination of the T wave, representing the time for both ventricular depolarization and repolarization to occur
QTc:	QT interval corrected for heart rate
QTcF:	QT interval corrected for heart rate using the Fridericia formula
RAL:	raltegravir
RBV:	ribavirin
RNA:	ribonucleic acid
RPV:	rilpivirine
RTV:	ritonavir
SD:	standard deviation
SVR, SVRxx:	sustained virologic response, sustained virologic response at "xx" weeks following completion of all treatment
TDF:	tenofovir disoproxil fumarate (Viread®)
UGT:	uridine diphosphate glucuronosyltransferase
ULN:	upper limit of the normal range
UMP-CMP:	uridine monophosphate-cytidine monophosphate
SOF:	sofosbuvir (GS-7977; formerly PSI-7977); nucleotide prodrug, S-diastereomer at phosphorous
GS-491241:	formerly PSI-7976; nucleoside prodrug; R-diastereomer at phosphorus
GS-9851:	formerly PSI-7851; nucleotide prodrug, isomeric mixture at phosphorous containing GS-7977 and GS-491241
GS-566500:	formerly PSI-352707; nucleoside analog monophosphate alanine
GS-331007:	formerly PSI-6206; nucleoside analog
GS-461203:	formerly PSI-7409; nucleoside analog triphosphate; pharmacologically active metabolite

1. EXECUTIVE SUMMARY

Gilead Sciences is seeking approval of sofosbuvir (SOF) 400 mg tablets in combination with other agents for the treatment of chronic hepatitis C (CHC) in adults.

SOF is a pan-genotypic inhibitor of the hepatitis C virus (HCV) NS5B RNA-dependent RNA polymerase, which is essential for viral replication. SOF is a nucleotide prodrug that undergoes intracellular metabolism to form the pharmacologically active uridine analog triphosphate (GS-461203), which can be incorporated by HCV NS5B and acts as a chain terminator.

The proposed SOF dose is one 400 mg tablet, taken orally, once daily with or without food. SOF should be used in combination with peginterferon/ribavirin (PEG/RBV) for the treatment of chronic HCV in patients with genotypes 1 or 4 and in combination with ribavirin only for the treatment of chronic HCV in patients with genotypes 2 or 3. The recommended dose and treatment duration for SOF combination therapy is provided in [Table 1](#).

Table 1 Recommended Dose and Treatment Duration for SOF Combination Therapy

	Total Treatment Duration	SOF Dose (daily)	Peginterferon alfa Dose	Ribavirin Dose (daily)
Treatment-naïve patients with genotype 1, or 4 CHC	12 weeks	400 mg	See peginterferon alfa prescribing information	See ribavirin prescribing information
Patients with genotype 2 CHC	12 weeks		NA	<75 kg =1000 mg ^a ≥75 kg =1200 mg ^a
Patients with genotype 3 CHC	16 weeks			

NA = not applicable

- a. Ribavirin dose for genotypes 2 and 3 are different from ribavirin prescribing information. The daily dose of ribavirin is administered orally in two divided doses with food.

The consideration for approval of this NDA is based on safety and efficacy data from 4 pivotal Phase 3 trials in a total of 1296 subjects: P7977-1231 (FISSION), GS-US-334-0107 (POSITRON), GS-US-334-0108 (FUSION), and GS-US-334-0110 (NEUTRINO). Study GS-US-334-0110 was conducted in treatment-naïve subjects with genotypes 1, 4, 5 or 6 CHC in combination with peginterferon alfa 2a (PEG) and ribavirin (RBV). The other three trials were conducted in subjects with genotype 2 or 3 CHC in combination with ribavirin including one in treatment-naïve subjects, one in interferon intolerant, ineligible or unwilling subjects and one in subjects previously treated with an interferon-based regimen. These Phase 3 trials included subjects who had compensated liver disease, including cirrhosis.

SOF (400 mg once daily) in combination with ribavirin is also recommended for patients with hepatocellular carcinoma awaiting liver transplantation to prevent post-transplant HCV reinfection. The duration of administration of SOF in patients awaiting liver transplantation should be guided by an assessment of the potential benefits and risks for

the individual patient and should not continue past liver transplantation. The dose recommendation for patients with CHC awaiting liver transplantation is based on limited safety (n=61) and efficacy (n=28) data from a Phase 2 open-label study (P7977-2025), where SOF+RBV was administered prior to liver transplantation to prevent HCV infection recurrence post-liver transplant in subjects with genotypes 1 through 6 HCV infection and hepatocellular carcinoma (HCC) who were within 1 year of an anticipated liver transplantation.

No dose adjustment of SOF is recommended for patients with mild or moderate renal impairment based on the pharmacokinetics of SOF and its metabolites, as well as clinical and animal toxicity data. The safety of SOF has not been assessed in patients with severe renal impairment (estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m² or creatinine clearance (CrCL) <30 mL/min) or end stage renal disease (ESRD) requiring hemodialysis. At the time of this review, only Copegus® (not Rebetol®) is approved for use in patients with CrCL<50 mL/min. Thus, for patients with moderate renal impairment taking Copegus with SOF, the ribavirin dose should be based on the recommended dose in the Copegus prescribing information, where a dose reduction to alternating doses, 200 mg and 400 mg every other day is recommended.

Note: At the time of this review, the following issue was under discussion within the review team:

While prior PEG/RBV nonresponders (nulls and partial responders) with genotype 1 were not explicitly evaluated by the sponsor, high response rates were observed in treatment-naïve patients with genotype 1 HCV from NEUTRINO (study GS-US-334-0110). Additionally, previous analyses demonstrated that PEG/RBV nonresponders are represented within the treatment-naïve population and thus supports the use of SOF/PEG/RBV in prior PEG/RBV nonresponders with genotype 1. Extending the indication to all HCV genotype 1 patients, rather than just the treatment-naïve population, is being considered.

1.1 Recommendation

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

1.2 Phase IV Commitments

None.

1.3 Summary of Important Clinical Pharmacology Findings

Sofosbuvir (SOF) is a first in class, pan-genotypic inhibitor of the Hepatitis C virus (HCV) NS5B RNA-dependent RNA polymerase, which is essential for viral replication. SOF is a nucleotide prodrug that undergoes intracellular metabolism to form the pharmacologically active uridine analog triphosphate (GS-461203).

A comprehensive range of clinical studies was conducted to characterize the PK of SOF and its predominant circulating metabolite GS-331007, because GS-461203 is not measurable in plasma. The results are summarized below:

- Following oral administration of SOF, SOF was absorbed with peak plasma concentration reached within 0.5-2 hours post-dose, regardless of dose level. Peak plasma concentration of GS-331007 was observed between 2 and 4 hours post-dose.
- Steady-state GS-331007 and SOF pharmacokinetic (PK) parameters after once-daily administration of SOF are similar between HCV-infected subjects and healthy subjects.
- Relative to fasting conditions, the administration of a single dose of SOF with a standardized high fat meal slowed the rate of absorption of SOF but did not substantially affect the extent of absorption. The exposure of GS-331007 was not altered in the presence of a high-fat meal. Therefore, SOF can be administered without regard to food (as instructed in phase 3 trials).
- SOF is approximately 61-65% bound to human plasma proteins and the binding is independent of drug concentration over the range of 1 µg/mL to 20 µg/mL. Protein binding of GS-331007 was minimal in human plasma. After a single 400 mg dose of [¹⁴C]-SOF in healthy subjects, the blood to plasma ratio of ¹⁴C-radioactivity was approximately 0.7.
- SOF is extensively metabolized in the liver to form the pharmacologically active, intracellular nucleoside analog triphosphate GS-461203. The metabolic activation pathway involves sequential hydrolysis of the carboxyl ester moiety catalyzed by human cathepsin A (CatA) or carboxylesterase 1 (CES1) and phosphoramidate cleavage by histidine triad nucleotide-binding protein 1 (HINT1) followed by phosphorylation by the pyrimidine nucleotide biosynthesis pathway. Dephosphorylation of the active metabolite results in the formation of the nucleoside metabolite GS-331007, which cannot be efficiently rephosphorylated and lacks anti-HCV activity in vitro.
- After a single 400 mg oral dose of [¹⁴C]-SOF, 4%, 7.0% and 91% of the mean circulating plasma total radioactivity (24,979 ng eq-h/g) were accounted for by SOF, GS-566500 and GS-331007, respectively. These results indicate GS-331007 is the major circulating metabolite of SOF.
- Following a single 400 mg oral dose of [¹⁴C]-SOF, mean total recovery of the dose was greater than 92%, consisting of approximately 80%, 14%, and 2.5% recovered in urine, feces, and expired air, respectively. The majority of the SOF dose recovered in urine was GS-331007 (78%) while 3.5% was recovered as SOF. These data indicate that renal clearance is the major elimination pathway for GS-331007.
- The median terminal half-lives of SOF and GS-331007 were 0.4 and 27 hours, respectively.
- Population pharmacokinetics analysis in HCV-infected subjects indicated that race had no clinically relevant effect on the exposures of SOF or GS-331007.

- No clinically relevant pharmacokinetic differences have been observed between men and women for SOF and GS-331007.
- Population pharmacokinetic analysis in HCV-infected subjects showed that within the age range (19 to 75 years) analyzed, age did not have a clinically relevant effect on the exposures of SOF or GS-331007.
- The pharmacokinetics of SOF were studied in HCV negative subjects with mild (eGFR ≥ 50 and < 80 mL/min/1.73m²), moderate (eGFR ≥ 30 and < 50 mL/min/1.73m²), severe renal impairment (eGFR < 30 mL/min/1.73m²) and subjects with end stage renal disease (ESRD) requiring hemodialysis following a single 400 mg dose of SOF. Relative to subjects with normal renal function (eGFR > 80 mL/min/1.73m²), the SOF AUC_{0-inf} was 61%, 107% and 171% higher in subjects with mild, moderate and severe renal impairment, while the GS-331007 AUC_{0-inf} was 55%, 88% and 451% higher, respectively. In subjects with ESRD (relative to subjects with normal renal function), SOF and GS-331007 AUC_{0-inf} was 28% and 1280% higher when SOF was dosed 1 hour before hemodialysis compared with 60% and 2070% higher when SOF was dosed 1 hour after hemodialysis. No dose adjustment is required for patients with mild or moderate renal impairment. The safety of SOF has not been assessed in patients with severe renal impairment or ESRD and dose recommendation cannot be given in these populations at this time.
- The pharmacokinetics of SOF was studied following 7-day dosing of 400 mg SOF in HCV-infected subjects with moderate and severe hepatic impairment (Child-Pugh Class B and C). Relative to subjects with normal hepatic function, the SOF AUC₀₋₂₄ were 126% and 143% higher in subjects with moderate and severe hepatic impairment, while the GS-331007 AUC₀₋₂₄ were 18% and 9% higher, respectively. Population pharmacokinetics analysis in HCV-infected subjects indicated that cirrhosis had no clinically relevant effect on the exposure of SOF and GS-331007. No dose adjustment of SOF is recommended for patients with mild, moderate and severe hepatic impairment.
- In vitro studies indicated that SOF and its metabolites:
 - are not inhibitors (IC₅₀ > 50 – 100 μ M) of human CYP isozymes CYP3A4, CYP1A2, CYP2C19, CYP2C9, CYP2C8, and CYP2D6.
 - show no significant inhibition (IC₅₀ > 50 μ M) of UGT1A1
 - show no induction of CYP enzymes
 - show no inhibition of the transport of probe substrates by P-gp, breast cancer resistance protein (BCRP), OATP1B1, OATP1B3, OCT1, and BSEP
- GS-331007 showed little or no inhibition of the renal transporters OAT1, OAT3, OCT2, and MATE1 (IC₅₀ values > 100 μ M).
- SOF and its metabolites GS-566500 and GS-331007 were minimally metabolized by FMO, UGT, or CYP. In human liver microsomes, CYP- and UGT-related metabolism represents a minor contribution to SOF and GS-606965 disappearance.
- SOF is a substrate of drug transporters P-gp and BCRP, while GS-331007 is not. Drugs that are potent P-gp inducers in the intestine (e.g., rifampin or St. John's

wort) may decrease SOF plasma concentration leading to reduced therapeutic effect of SOF and thus should not be used with SOF. Coadministration of SOF with drugs that inhibit P-gp and/or BCRP would likely increase SOF plasma concentration (e.g., cyclosporine).

- The effects of coadministered drugs on the exposure of SOF and GS-331007 have been studied for cyclosporine, darunavir/ritonavir, emtricitabine, efavirenz, raltegravir, rilpivirine, tacrolimus, and tenofovir disoproxil fumarate. No significant effects of coadministered drugs on the exposure of SOF and GS-331007 have been observed except cyclosporine (CsA, [Table 2](#)).

Table 2 Drug Interactions: Changes in Pharmacokinetic Parameters for SOF and the Predominant Circulating Metabolite GS-331007 in the Presence of the Coadministered Drug

Co-administered Drug	Dose of Coadministered Drug (mg)	SOF Dose (mg)	N	Mean Ratio (90% CI) of SOF and GS-331007 PK With/Without Coadministered Drug No Effect=1.00		
					C _{max}	AUC
Cyclosporine	600 single dose	400 single dose	19	SOF	2.54 (1.87, 3.45)	4.53 (3.26, 6.30)
				GS-331007	0.60 (0.53, 0.69)	1.04 (0.90, 1.20)

- Coadministration of SOF with the potent P-gp and BCRP inhibitor CsA (administered as single dose at a high dose of 600 mg), resulted in an increase (approximately 4-fold) in SOF exposure, but the exposure of GS-331007 was unchanged in the presence of CsA. Limited safety data from an ongoing post-transplant study (GS-US-334-0126) indicate that the safety of SOF+RBV is similar between subjects not taking CsA (n=30) and subjects taking CsA (n=10). Furthermore, the safety margins for SOF (and metabolites), after coadministration with cyclosporine, are adequate (AUC safety margin ranges from 1.9 to 16.0) compared with exposures obtained in toxicology studies. Therefore, dose modification of SOF is not warranted when coadministered with CsA.
- No drug interaction study has been formally conducted for SOF and PEG/RBV or RBV. However, Study P7977-0523 shows that GS-331007 exposures were higher in monotherapy as compared to when SOF is coadministered with PEG/RBV or RBV alone. GS-331007 exposure is similar when SOF is coadministered with PEG/RBV or RBV alone. An interaction between GS-331007 and RBV is plausible since both compounds are mainly renal eliminated.
- The effects of SOF on the exposure of coadministered drugs were studied for cyclosporine, darunavir/ritonavir, emtricitabine, efavirenz, methadone, raltegravir, rilpivirine, tacrolimus, and tenofovir disoproxil fumarate. No clinically significant effect of SOF has been observed on these drugs.
- An ongoing Phase 1 study (GS-US-334-0146) is evaluating the effect of SOF on the PK of a representative hormonal contraceptive medication,

norgestimate/ethinyl estradiol. Results from this study were not available for this submission. Thus, the sponsor's proposed recommendation for pregnancy prevention is two non-hormonal methods of contraception during treatment with concomitant ribavirin due to the known teratogenic effects of ribavirin.

- Exposure-response (efficacy) analyses:
The Phase III SOF dose of 400 mg once daily was selected based on on-treatment virologic response data observed from P7977-0221. Subjects were administered one of three SOF doses (100, 200, and 400 mg once daily) in combination with PEG/RBV and change from baseline in HCV RNA was assessed at day 3 of treatment. An E_{max} model based on GS-331007 AUC_{tau} fit to the virologic response data supported that change from baseline in HCV RNA at day 3 increased with increasing SOF dose up to 400 mg once daily.

SOF and GS-331007 exposure-response analyses for efficacy in genotype 1, genotype 2, and genotype 3 subjects were based on sparse pharmacokinetic sampling from 991 subjects who received either SOF/RBV or SOF/PEG/RBV in P7977-1231, GS-US-334-0107, GS-US-334-0108, and GS-US-334-0110. Due to the high overall response rates and small number of genotype 2 subjects from P7977-1231 (n=70, 97%) and GS-US-334-0108 (treatment-experience; SOF/RBV 12 weeks: n=36, 86%; SOF/RBV 16 weeks: n=32, 94%) exposure-response analyses were not conducted in these subjects. Univariate analyses in genotype 1 and genotype 3 subjects identified a trend of higher sustained virologic response at week 12 of follow-up (SVR12) in subjects with higher GS-331007 AUC_{tau} , though no relationship was identified between SOF AUC_{tau} and SVR12. GS-331007 AUC_{tau} was not retained during multivariate analysis, which suggests that other factors such as cirrhosis, IL28B, and weight-based ribavirin dose may be more important factors for predicting response.

In addition, data from GS-US-334-0108 demonstrated that increasing the treatment duration from 12-weeks to 16-weeks in genotype 3 subjects improved SVR12 from 30% to 62%. Given the improvement in response observed by extending treatment to 16-weeks, the observation that all the treatment failures in both durations were relapsers, and a similar observation of lower SVR12 and high relapse among genotype 3 treatment-naïve subjects in P7977-1231, it is likely that extending the treatment duration in all genotype 3 subjects to 16-weeks may improve response. In addition, even longer (e.g., 24 weeks) treatment duration may result in further SVR12 improvements in this population and should be considered as potential treatment arms in future studies.

- Exposure-response (safety) analyses:
The exposure-response safety analyses for SOF and GS-331007 evaluated whether there were any adverse event relationships between SOF and GS-331007 AUC_{tau} and the most common adverse events observed during the Phase III trials (e.g., headaches, diarrhea, nausea) as well as cardiac adverse events and dyspnea. For SOF and GS-331007, no relationship was observed between predicted AUC_{tau} and common adverse events of interest. Exposure-response safety analyses identified that any grade dyspnea and any grade cardiac events were more likely in subjects with higher GS-331007 exposures. However, the significance of these adverse events relationships should be interpreted with caution as event rate was less than that observed in PEG/RBV control arm. In addition, these adverse events may be confounded by

concomitant administration of ribavirin to all patients during the Phase III trials, which is known to cause anemia.

- Pharmacogenomics:
The single nucleotide polymorphism rs12979860 near the IFNL3 (IL28B) gene encoding interferon-lambda 3 has been shown to be a strong predictor of SVR in HCV genotype 1 patients receiving peginterferon/ribavirin-based therapies, with a less pronounced effect in HCV genotypes 2 and 3. After 12-week treatment with SOF, treatment-naïve genotype 1, 4, 5 and 6 infected subjects with non-CC genotypes had modestly lower SVR rates compared to subjects with the CC genotype (87.1% vs. 97.9%). In genotypes 2 and 3, no consistent correlation was found between IFNL3 genotype and the rate of SVR12. Longer treatment durations (i.e., 16 weeks) tended to increase SVR rates in treatment-experienced patients and could be considered in genotype 2 or 3 treatment-naïve or interferon ineligible patients who are likely to have lower responses rate. However, IFNL3 genotype alone would not be sufficient to identify these patients.

2. QUESTION BASED REVIEW

2.1 General Attributes

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

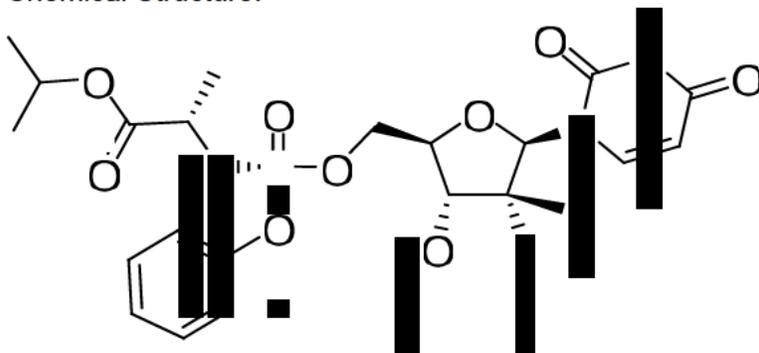
SOF is a nucleotide prodrug of 2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate that is converted to the active uridine triphosphate form (GS-461203) within the hepatocyte and is a HCV NS5B-directed inhibitor that has displayed potent inhibition of HCV replicon RNA replication in vitro.

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

Empirical Formula: C₂₂H₂₉FN₃O₉P

Molecular Weight: 529.45 g/mol

Chemical Structure:



pKa: 9.3

(b) (4)

Solubility: Slightly soluble in water and acidic media (approximately 2 mg/mL), see Section 2.5.1.

The following are conversion for SOF

(b) (4)

SOF 1 μ M = 0.529 μ g/mL

(b) (4)

The quantitative composition of to-be-marketed SOF 400 mg tablets ([Table 3](#)):

Table 3: Composition of to-be-marketed SOF 400 mg tablets

Component	Quality Standard	Composition		Function
		% w/w	mg/unit	
(b) (4)				
Sofosbuvir	In-house	(b) (4)	400.0 ^a	(b) (4)
Mannitol	USP, Ph. Eur.		(b) (4)	
Microcrystalline Cellulose	NF, Ph. Eur.			
Croscarmellose Sodium	NF, Ph. Eur.			
Colloidal Silicon Dioxide	NF, Ph. Eur.			
Magnesium Stearate	NF, Ph. Eur.			
(b) (4)				
Microcrystalline Cellulose	NF, Ph. Eur.	(b) (4)		
Croscarmellose Sodium	NF, Ph. Eur.	(b) (4)		
Colloidal Silicon Dioxide	NF, Ph. Eur.	(b) (4)		
Magnesium Stearate	NF, Ph. Eur.	(b) (4)		
Total		100	1200	
(b) (4)				
(b) (4)	In-house	(b) (4)		
(b) (4)	USP, Ph. Eur.	(b) (4)		
(b) (4)				

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

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

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

The proposed dose of SOF is one 400 mg tablet, taken orally, once daily with or without food. SOF should be used in combination with other agents. The proposed dose and treatment duration for SOF combination therapy is based on genotype ([Table 4](#)).

Table 4: Proposed sofosbuvir dose regimens

	Total Treatment Duration	SOF Dose (daily)	Peginterferon alfa Dose	Ribavirin Dose (daily)
Treatment-naïve patients with genotype 1, or 4 CHC	12 weeks	400 mg	See peginterferon alfa prescribing information	See ribavirin prescribing information
Patients with genotype 2 CHC	12 weeks		NA	
Patients with genotype 3 CHC	16 weeks			

NA = not applicable

- a. Ribavirin dose for genotypes 2 and 3 are different from ribavirin prescribing information. The daily dose of ribavirin is administered orally in two divided doses with food.

SOF (400 mg once daily) in combination with ribavirin is also proposed for patients with hepatocellular carcinoma awaiting liver transplantation to prevent post-transplant HCV reinfection. The duration of administration of SOF in patients awaiting liver transplantation should be guided by an assessment of the potential benefits and risks for the individual patient.

2.2 General Clinical Pharmacology

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

A comprehensive range of clinical studies was conducted to characterize the PK of SOF and its predominant circulating metabolite GS-331007. In some early Phase 1 studies, the PK for another circulating metabolite, GS-566500, was also characterized. This submission includes 22 studies with biopharmaceutic or clinical pharmacology data as follows:

- 1 mass balance study in healthy subjects (P7977-0312)
- 1 single-dose PK study in healthy subjects (P7851-1101)
- 2 multiple-dose studies with PK and/or PK/pharmacodynamics (PD) in HCV-infected subjects following SOF monotherapy for 3 or 7 days (P7851-1102 and P2938-0212 [NUCLEAR])
- 1 Phase 2a dose-ranging study that investigated safety, tolerability, PK, and PD following SOF for 28 days in combination with PEG+RBV in treatment-naïve subjects with genotype 1 HCV infection (P7977-0221)
- 1 single-dose study that investigated the effect of SOF at therapeutic and suprathreshold doses on QT/QTc interval in healthy subjects (P7977-0613)
- 1 single-dose PK study in subjects with various degrees of renal impairment and matched healthy control subjects (P7977-0915)
- 1 multiple-dose PK/PD study in subjects with HCV infection with various degrees of hepatic impairment (P2938-0515)

- 3 DDI studies in healthy subjects (1 study conducted with SOF and methadone [P7977-0814], 1 study conducted with SOF and cyclosporine (cyclosporin A [CsA]) and tacrolimus [P7977-1819], and 1 study conducted with SOF and ARV combinations [GS-US-334-0131])
- 1 DDI study between SOF and ARV combinations in HCV/HIV-coinfected subjects (P7977-1910)
- 2 bioavailability and food effect studies (P7977-0111 and P7977-1318) (note, Cohort 5 from the DDI Study GS-US-334-0131 assessed the PK equivalence between SOF Forms I and II)
- 4 Phase 2 clinical studies (P7977-0422 [PROTON], P7977-0523 [ELECTRON], P7977-0724 [ATOMIC], and P2938-0721 [QUANTUM])
- Population PK and PK/PD analyses for SOF and GS-331007 from 4 Phase 3 studies (GS-US-334-0107 [POSITRON], P7977-1231 [FISSION], GS-US-334-0108 [FUSION], and GS-US-334-0110 [NEUTRINO])

In addition to the studies described above, an ongoing Phase 1 study (GS-US-334-0146) is evaluating the effect of SOF on the PK of a representative hormonal contraceptive medication, norgestimate/ethinyl estradiol. Results from this study were not available for this submission.

The diastereoisomeric mixture, GS-9851 (50:50 diastereomeric mixture of SOF and GS-491241), was used in 3 clinical studies (P7851-1101, P7851-1102, and P7977-0111), but the single diastereoisomer (SOF) was eventually chosen for further development and registration. Because the metabolites and active moiety of GS-9851 and SOF were similar, the use of GS-9851 in initial studies informs the clinical PK of SOF. The enriched (98:2; considered a single isomer) diastereoisomer, SOF, will be the registered entity.

Dose Selection: Dose, duration, and combination regimens of SOF were explored in 5 Phase 2 clinical studies: P7977-0221, P7977-0422 (PROTON), P7977-0523 (ELECTRON), P7977-0724 (ATOMIC), and P2938-0721 (QUANTUM).

Data from dose-ranging studies conducted within the development program as either monotherapy or combination therapy with PEG+RBV revealed exposure-response relationships that supported the dose selection of SOF 400 mg for the treatment of HCV infection. Study P7851-1102 (multiple-ascending dose) assessed GS-9851 once-daily doses from 50 to 400 mg administered for 3 consecutive days to treatment-naïve subjects with chronic genotype 1 HCV infection. The GS-9851 400-mg dose had the earliest and most potent antiviral effect in the greatest percentage of subjects, with the majority of subjects having a continued reduction in HCV RNA ($\geq 1.0 \log_{10}$) 2 days after the last dose of GS-9851. The Phase 2 dose-finding Studies P7977-0221 and P7977-0422 confirmed a SOF dose of 400 mg administered once daily is appropriate to be evaluated in Phase 3.

Pivotal Phase 3 Studies: The clinical program includes 4 pivotal Phase 3 studies: P7977-1231 (FISSION), GS-US-334-0107 (POSITRON), GS-US-334-0108 (FUSION), and GS-US-334-0110 (NEUTRINO). Three of these studies assessed SOF+RBV in genotype 2 or 3 HCV-infected subjects (Studies P7977-1231, GS-US-334-0107, and GS-US-334-0108), and Study GS-US-334-0110 assessed SOF+PEG+RBV in treatment-naïve genotype 1, 4, 5, or 6 HCV-infected subjects. An overview of these Phase 3 studies is presented in [Table 5](#).

Table 5 Overview of Sofosbuvir Pivotal Phase 3 Studies

Study Number	Design	Study Objectives	Population	Number of Subjects by Treatment	Duration of Treatment
Genotypes 2 or 3					
P7977-1231 (FISSION)	Phase 3, randomized, active-controlled, open-label, multicenter study	Assess the efficacy (proportion of subjects with SVR12) and safety of SOF+RBV administered for 12 weeks compared with PEG+RBV administered for 24 weeks in treatment-naïve subjects with genotype 2 or 3 HCV infection	Treatment-naïve adult subjects with chronic genotype 2 or 3 HCV infection; up to 20% of subjects may have the presence of cirrhosis.	Overall: 499 treated; 464 completed through SVR12 assessment SOF+RBV group: 256 treated; 239 completed PEG+RBV group: 243 treated; 225 completed	SOF+RBV group: 12 weeks PEG+RBV group: 24 weeks
GS-US-334-0107 (POSITRON)	Phase 3, randomized, double-blind, placebo-controlled, multicenter study	Assess the efficacy (proportion of subjects with SVR12) and safety of SOF+RBV compared with placebo administered for 12 weeks in subjects with genotype 2 or 3 HCV infection who are IFN intolerant, IFN ineligible, or unwilling to take IFN	Adult subjects with chronic genotype 2 or 3 HCV infection who were IFN intolerant, IFN ineligible, or unwilling to take IFN; up to 20% of subjects may have the presence of cirrhosis	Overall: 278 treated; 171 completed through SVR12 assessment SOF+RBV group: 207 treated; 171 completed Placebo group: 71 treated; 0 completed	12 weeks
GS-US-334-0108 (FUSION)	Phase 3, randomized, double-blind, multicenter study	Assess the efficacy (proportion of subjects with SVR12) and safety of SOF+RBV administered for 12 weeks compared with 16 weeks in subjects with genotype 2 or 3 HCV infection who failed prior treatment with IFN	Treatment-experienced adult subjects with chronic genotype 2 or 3 HCV infection; up to 30% of subjects may have the presence of cirrhosis	Overall: 201 treated; 127 completed through SVR12 assessment SOF+RBV 12-week group: 103 treated; 54 completed SOF+RBV 16-week group: 98 treated; 73 completed	SOF+RBV 12-week group: 12 weeks SOF+RBV 16-week group: 16 weeks
Genotypes 1, 4, 5, or 6					
GS-US-334-0110 (NEUTRINO)	Phase 3, open-label, multicenter study	Assess the efficacy (proportion of subjects with SVR12) and safety of treatment with SOF+PEG+RBV in treatment-naïve subjects with genotype 1, 4, 5, or 6 HCV infection	Treatment-naïve adult subjects with chronic genotype 1, 4, 5, or 6 HCV infection; up to 20% of subjects may have the presence of cirrhosis.	Overall: 327 treated; 301 completed through SVR12 assessment	12 weeks

Table 6 presents the overall SVR12 rate and the SVR12 rate by genotype (2 or 3) and cirrhosis status in Studies P7977-1231, GS-US-334-0107, and GS-US-334-0108.

Table 6 P7977-1231, GS-US-334-0107, and GS-US-334-0108: Percentages of Subjects with SVR12 by HCV Genotype and Presence of Cirrhosis

	Number of Subjects with SVR12 n, %				
	P7977-1231 (FISSION)		GS-US-334-0107 (POSITRON) ^a	GS-US-334-0108 (FUSION)	
	Treatment Naive		Interferon Ineligible, Intolerant, Unwilling	Treatment Experienced	
	SOF+RBV 12 Weeks	PEG+RBV 24 Weeks	SOF+RBV 12 Weeks	SOF+RBV 12 Weeks	SOF+RBV 16 Weeks
	N = 253	N = 243	N = 207	N = 100	N = 95
Overall SVR12	170/253 (67.2%)	162/243 (66.7%)	161/207 (77.8%)	50/100 (50.0%)	69/95 (72.6%)
No Cirrhosis	147/204 (72.1%)	143/193 (74.1%)	142/176 (80.7%)	39/64 (60.9%)	48/63 (76.2%)
Cirrhosis	23/49 (46.9%)	19/50 (38.0%)	19/31 (61.3%)	11/36 (30.6%)	21/32 (65.6%)
Genotype 2	68/70 (97.1%)	52/67 (77.6%)	101/109 (92.7%)	31/36 (86.1%)	30/32 (93.8%)
No Cirrhosis	58/59 (98.3%)	44/54 (81.5%)	85/92 (92.4%)	25/26 (96.2%)	23/23 (100.0%)
Cirrhosis	10/11 (90.9%)	8/13 (61.5%)	16/17 (94.1%)	6/10 (60.0%)	7/9 (77.8%)
Genotype 3	102/183 (55.7%)	110/176 (62.5%)	60/98 (61.2%)	19/64 (29.7%)	39/63 (61.9%)
No Cirrhosis	89/145 (61.4%)	99/139 (71.2%)	57/84 (67.9%)	14/38 (36.8%)	25/40 (62.5%)
Cirrhosis	13/38 (34.2%)	11/37 (29.7%)	3/14 (21.4%)	5/26 (19.2%)	14/23 (60.9%)

^a None of the subjects in the placebo group in Study GS-US-334-0107 achieved SVR12.

In the Phase 3 SOF clinical program, subjects with genotype 2 or 3 HCV infection were studied together and using the same SOF+RBV treatment, which was consistent how these two genotypes are treated with PEG/RBV (24-week duration). The RBV dose used in these trials was 1000 mg for subjects with body weight <75 kg and 1200 mg for subjects with body weight ≥75 mg. This RBV dose is analogous to that approved for genotype 1 subjects but differs from the approved RBV dose for genotype 2 and 3 subjects (800 mg). However, based on review of the SVR12 data from these Phase 3 studies, it was clear that response to SOF treatment differs substantially between HCV genotype 2 and 3 and, therefore, it is appropriate to review the results of each genotype separately.

- Genotype 2: For genotype 2 HCV-infected subjects, the overall SVR12 rates for subjects who received SOF+RBV were high across the Phase 3 studies, with SVR12 rates ranging from 86.1% (treatment experienced; SOF+RBV 12 weeks) to 97.1% (treatment naive; SOF+RBV 12 weeks), compared with the SVR12 rates for treatment-naive genotype 2 HCV-infected subjects who received 24 weeks of PEG+RBV (77.6%) (Table 6). Within each treatment group, noncirrhotic subjects had a similar or higher SVR12 rates than cirrhotic subjects. In addition, the SVR12 response rates was higher in genotype 2 treatment-naïve subjects in

the SOF+RBV 12 Week group (Studies P7977-1231) compared to the SVR12 response rates in genotype 2 treatment-experienced subjects administered SOF+RBV 12 Week (GS-US-334-0108). Numerically higher SVR12 rates were observed in genotype 2 treatment-experienced subjects by extending the treatment duration from 12- to 16-weeks. The sponsor insists that the benefit of a longer SOF+RBV treatment duration (16 vs. 12 week) in treatment-experienced subjects with genotype 2 infection (93.8% vs. 86.1%) is minimal, though the small number of genotype 2 subjects in each arm and that all treatment failures were relapses do not rule out that a subset of subjects may benefit from a 16-week treatment duration.

- Genotype 3: For genotype 3 HCV-infected subjects, the overall SVR12 rates for subjects who received SOF+RBV ranged from 29.7% (treatment experienced; SOF+RBV 12 weeks) to 61.9% (treatment experienced; SOF+RBV 16 weeks) (Table 6). The SVR12 rate for treatment-naïve genotype 3 HCV-infected subjects who received 12 weeks of SOF+RBV was 55.7%, while it was 62.5% for 24 weeks of PEG+RBV. Within each treatment group, noncirrhotic subjects had higher SVR12 rates than cirrhotic subjects, except for treatment-experienced subjects in the SOF+RBV 16 Week group who had similar SVR12 rates for subjects with or without cirrhosis (60.9% and 62.5%, respectively).

Results from Study GS-US-334-0108 showed SVR12 rates were higher following a longer SOF+RBV treatment duration (12 vs. 16 weeks) in treatment-experienced subjects with genotype 3 HCV infection (29.7% vs. 61.9%). Since a 16-week treatment of SOF+RBV was not studied in genotype 3 treatment-naïve subjects, modeling and simulation was performed to predict the response rate in these patients. Based on the Bayesian logistic regression model, the predicted SVR12 rate for treatment-naïve subjects following 16 weeks of SOF+RBV treatment was 78.2% (95% Credible Set: 62.5%, 89.6%). The results of this bridging analysis indicate that increasing the SOF+RBV treatment duration from 12 to 16 weeks may increase the SVR12 rate by up to 22.5% (55.7% vs. 78.2%) for treatment-naïve subjects with genotype 3 HCV infection.

- Genotype 1, 4, 5, and 6: Table 7 presents the overall SVR12 response rate and SVR12 by genotype and cirrhosis status in Study GS-US-334-0110 (NEUTRINO). Study GS-US-334-0110 met its primary efficacy endpoint of superiority of 12 weeks of SOF+PEG+RBV compared with a predefined historic control SVR rate of 60%, with 90.2% of genotype 1, 4, 5, or 6 HCV-infected subjects achieving SVR12 (295 subjects; 95% CI: 86.5–93.2%, $p < 0.001$). Rates of SVR12 were numerically lower in genotype 1b subjects compared to genotype 1a subjects: 91.6% (95% CI: 87.1–94.8%) for subjects with genotype 1a, 81.8% (95% CI: 70.4–90.2%) for those with genotype 1b. An overall high SVR12 rate was observed in genotype 4, 5, and 6 subjects (97.1% [95% CI: 85.1–99.9%]), though the number of subjects with these HCV genotypes included in the study was small (28 with genotype 4, 1 with genotype 5, and 6 with genotype 6) (Table 7). The data from GS-US-334-0110 supports the use of 12 weeks of SOF (400 mg once daily) in combination with PEG+RBV in genotype 1 and 4 subjects, though the small number of genotype 5 and 6 subjects included in the study hinder generalization of the results to the overall population. In addition, the lower response rate in genotype 1 cirrhotics and the observation that all treatment failures were relapsers suggests that a subgroup of genotype 1

subjects may benefit from longer, treatment duration. However, that available data did not support any on-treatment virologic response measures (e.g., time to HCV RNA undetected) for identifying subjects who may require a longer treatment duration.

Table 7 GS-US-334-0110: Percentages of Subjects with SVR12 by HCV Genotype and Presence of Cirrhosis (Full Analysis Set)

	Number of Subjects with SVR12 n, %
	GS-334-0110 (NEUTRINO)
	Treatment Naive
	SOF+PEG+RBV 12 Weeks (N = 327)
Overall SVR12	295/327 (90.2%)
No Cirrhosis	252/273 (92.3%)
Cirrhosis	43/54 (79.6%)
Genotype 1 (1a, 1b, 1a/1b)	261/292 (89.4%)
Genotype 1a	206/225 (91.6%)
Genotype 1b	54/66 (81.8%)
Genotypes 4, 5, or 6	34/35 (97.1%)

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

The goal of treatment of chronic HCV infection is long-lasting viral eradication, generally defined as SVR (i.e., undetectable virus [LLOQ or limit of detection for assay] 12 [SVR12] or 24 [SVR24] weeks after the completion of therapy). Previously, achieving SVR24 has been proven as a reliable predictor of long-term clearance of HCV RNA for PEG+RBV treatment and is generally accepted as a cure of infection. For the Phase 2 Study P7977-0724, SVR24 was selected as the primary efficacy endpoint. More recently, analyses of large datasets demonstrated a high concordance between SVR12 and SVR24. Therefore, in the Phase 2 Study P2938-0721 and the Phase 3 Studies GS-US-334-0110, P7977-1231, GS-US-334-0107, GS-US-334-0108, and GS-US-334-0123, SVR12, defined as HCV RNA < LLOQ 12 weeks after completing treatment, was selected as the primary endpoint. For Study P7977-2025, the primary efficacy endpoint was proportion of subjects with pTVR (defined as HCV RNA < LLOQ at Week 12 after transplant).

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

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

2.2.4 Exposure-response

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

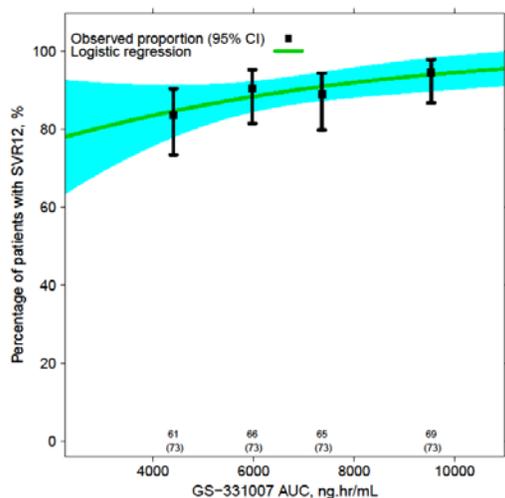
Exposure-response analyses were based on SOF and GS-331007 AUC_{τ} for genotype 1, genotype 2, and genotype 3 subjects from the following Phase III trials: GS-US-334-0108 (treatment-experienced genotype 2/3, SOF/RBV for 12 or 16 weeks), GS-US-334-0110 (treatment naïve genotype 1, SOF/RBV/PEG for 12 weeks), and P7977-1231 (treatment-naïve genotype 2/3, SOF/RBV for 12 weeks). The exposure variable was calculated using population pharmacokinetic modeling of SOF and GS-331007 separately. Subjects in Phase III had only sparse sampling, and samples were obtained pre-dose on days of on-treatment virologic assessment. The primary endpoint evaluated in these analyses was sustained virologic response at week 12 of follow-up (SVR12). Also evaluated were various on-treatment virologic assessments at weeks 1, 2, and 4 of treatment based on the percentage of subjects with virologic measurements not detected.

Genotype 1

Univariate analysis of the results from GS-US-334-0110 (n=292 genotype 1 subjects with pharmacokinetic data) identified an exposure-response relationship between GS-331007 AUC_{τ} and SVR12 ([Figure 1](#)), but no relationship between SOF AUC_{τ} and SVR12. Subjects with GS-331007 in the lowest exposure quartile had an SVR12 rate of 84% compared to 95% in the highest exposure quartile. Similar analyses performed based on on-treatment virologic response at week 1, 2, and 4, however, indicated that the percentage of subjects with virologic measurements not detected were more likely in those subjects in the lowest exposure quartile (8%, 52%, and 88%, respectively) compared to subjects in the highest exposure quartile (1%, 36%, and 84%, respectively). In addition, multivariate analysis including GS-331007 AUC_{τ} as well as other predictive factors such as genotype subtype, cirrhosis status, IL28B genotype (CC versus non-CC), ribavirin dose (mg/kg), resulted in rejection of GS-331007 AUC_{τ} as a significant predictor for SVR12. Finally, as displayed above in the metabolic pathway, GS-331007 is the end-step metabolite from SOF and it is uncertain how GS-331007 exposures may

be related to concentrations of the active triphosphate compound. Altogether, while an exposure-response efficacy relationship was identified for GS-331007, it cannot be concluded that subjects on the lower range of exposures observed in GS-US-334-0110 would be less likely to have a response compared to subjects in the highest exposure quartile.

Figure 1 Percentage of Patients Achieving Sustained Virologic Response (SVR12) Versus GS-331007 AUC_{tau} from GS-US-334-0110 (univariate analysis)



Genotype 2

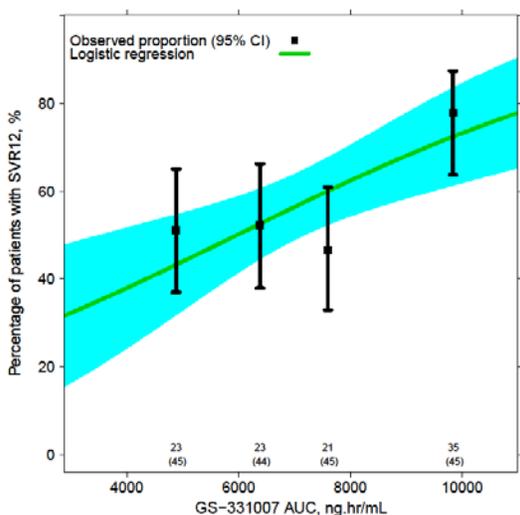
Exposure-response analyses using GS-331007 AUC_{tau} were conducted for genotype 2 subjects based on the data from P7977-1231 (treatment-naïve, n=70) and GS-US-334-0108 (treatment-experience; SOF/RBV 12 weeks: n=36; SOF/RBV 16 weeks: n=32). Due to the small number of subjects in each of these treatment arms numeric comparisons were performed between subjects above and below the median SOF and GS-331007 AUC_{tau}. In the treatment-naïve study there was no difference in response between subjects below (97% [34/35]) and above (97% [34/35]) the median GS-331007 exposures (7000 ng.hr/mL). In the treatment experienced study, numeric trends were observed based on GS-331007 AUC_{tau} for 12-weeks (below median: 83% [15/18]; above median: 88% [16/18]) and 16-weeks (below median: 88% [14/16]; above median: 100% [16/16]). Similar to the conclusions for genotype 2 treatment-naïve subjects, the small sample size and low number of treatment failures hinders interpreting these numeric trends as a result of GS-331007 exposures. Finally, no clear relationship between GS-331007 AUC_{tau} and on-treatment virologic response at weeks 1, 2, or 4 could be determined from the available data for either treatment-naïve or treatment-experienced genotype 2 subjects.

Genotype 3

Exposure-response analyses using GS-331007 AUC_{tau} was conducted for genotype 3 subjects based on the data from P7977-1231 (treatment-naïve, n=179) and GS-US-334-0108 (treatment-experience; SOF/RBV 12 weeks: n=64; SOF/RBV 16 weeks: n=63).

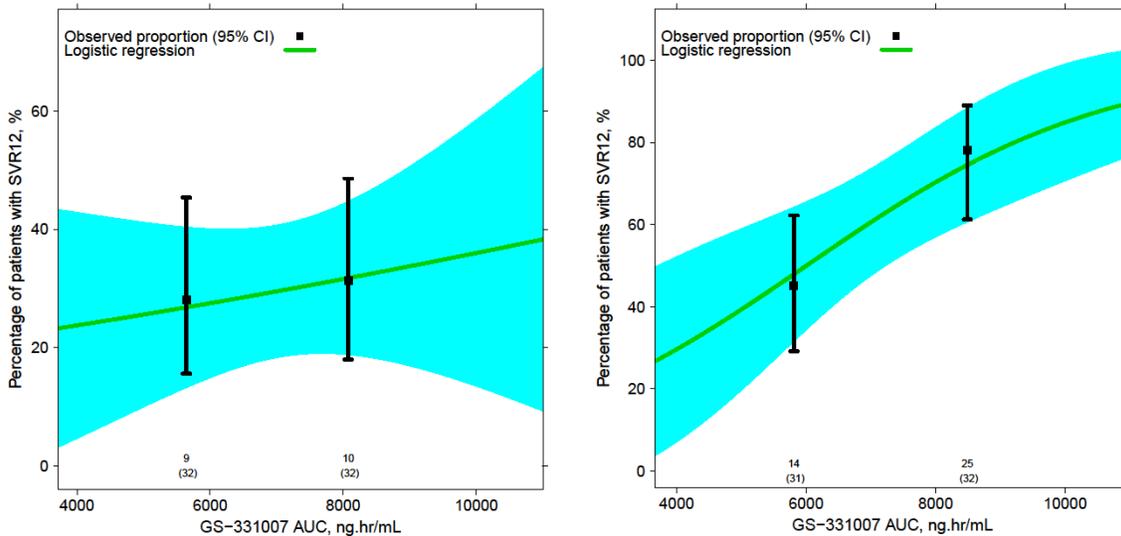
Univariate analysis of the results from P7977-1231 identified exposure-response relationship between GS-331007 AUC_{tau} and SVR12 ([Figure 2](#)). The response in the lowest quartile for GS-331007 AUC_{tau} was 51% compared to 78% in the highest quartile. Similar to the results for genotype 1 subjects, multivariate analysis of factors impacting genotype 3 response (IL28B, cirrhosis, ribavirin mg/kg, baseline viral load) resulted in removal of GS-331007 as a significant predictor of response. Furthermore, no relationship was identified between GS-331007 AUC_{tau} and on-treatment virologic response at week 1, 2 and 4.

Figure 2 Percentage of Genotype 3 Patients Achieving Sustained Virologic Response (SVR12) Versus GS-331007 AUC_{tau} from P7977-1231 (univariate analysis)



GS-331007 AUC_{tau} was also identified as a significant factor for response in treatment-experienced subjects administered SOF/RBV 16-weeks ([Figure 3](#), right) but not for SOF/RBV 12-weeks ([Figure 3](#), left). SVR12 in subjects with GS-331007 exposures less than the median (7062 ng·hr/mL) was 28% and 45% for 12- and 16-weeks compared to 31% and 78% in subjects with exposures above the median. Multivariate analysis retained GS-331007 AUC_{tau} as a predictor of response, but this was primarily driven by the higher response rate observed for 16-weeks in subjects with exposures above the median (response rate in subjects below the median had only modest improvement). These observations, as well as the lack of any on-treatment differences in virologic response at weeks 1, 2, or 4 with respect to GS-331007 AUC_{tau} suggest that treatment duration may be a confounding factor for this exposure response relationship analysis. In addition, the increase in SVR12 rate with a 16-week treatment duration compared to a 12-week treatment duration in those subjects with exposures above the median GS-331007 AUC_{tau} supports that longer treatment durations in genotype 3 subjects may result in higher SVR response rates, particularly those subjects with GS-331007 exposure below the median.

Figure 3 Percentage of Genotype 3 Patients Achieving Sustained Virologic Response (SVR12) Versus GS-331007 AUC_{tau} for 12-weeks (left) and 16-weeks (right) (univariate analysis)



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

Two separate exposure-response safety analyses were conducted based on: i) a pooled analysis of Phase III subjects administered SOF 400 mg and ribavirin in GS-US-334-0108, GS-US-334-0107, and P7977-1231; and ii) subjects administered SOF 400 mg, ribavirin, and pegylated interferon in GS-US-334-0110.

In each of these analyses, exposure-response relationships could not be identified for the most common adverse events observed during the Phase III SOF trials (e.g., headaches, diarrhea, and nausea). Logistic regression models were evaluated for SOF and GS-331007 AUC_{tau} and no significant relationships were identified.

Exposure-response safety analyses were also evaluated for dyspnea and system organ class cardiac disorders to identify if the SOF or GS-331007 exposures from the Phase III trials were associated with any cardiac adverse events. This analysis was based on the pooled Phase III population and identified that any grade dyspnea and any grade cardiac events were more likely in subjects with higher GS-331007 exposures. The significance of these adverse events relationships should be interpreted with caution. First, the overall number of cardiac events in the Phase III population administered SOF was 19 out of 991 patients with PK data available (6 of 327 in SOF/P/R [1.8%] and 13 of 664 in SOF/R [1.9%]). This event rate was lower than the cardiac event rate observed the P/R control arm from P7977-1231 (11 of 243 [4.2%]). In addition, the adverse event listings under this system organ class were predominantly grade 1 and include palpitations, tachycardia, bradycardia, and ventricular extrasystoles (see review by the Medical Officer, Dr. Poonam Mishra for additional details). These adverse events could also be confounded by concomitant administration of ribavirin to all patients during the Phase III trials which is known to cause anemia. Additional analyses looking for associations between creatinine kinase elevations exceeding the upper limit of normal (>336 U/L for

males and >176 U/L for females) and SOF or GS-331007 exposures also did not demonstrate any drug-exposure association with these elevations.

Exposure-response safety analyses could not be performed for either pegylated-interferon or ribavirin as pharmacokinetic data for these compounds were not collected during the Phase III trials. However, a weight-based exposure-response safety analysis was performed for the two pooled populations described above to assess whether increased mg/kg ribavirin dosing was associated with increased likelihood of anemia.

Anemia adverse events occurred in 6.1% (10 of 163) of subjects administered SOF/RBV with the lowest mg/kg ribavirin dosing (6.4-12.6 mg/kg) compared to 14.4% (24 of 166) in subjects with the highest mg/kg ribavirin dosing (15-20 mg/kg). Ribavirin dose reductions were also more frequent in the quartile with the highest mg/kg ribavirin dosing (15 of 166 [9.0%]) compared to the lowest quartile (8 of 163 [4.9%]). These trends remain despite the use of the weight based ribavirin dosing approved for genotype 1 subjects (1000 mg for body weight <75 kg and 1200 mg for body weight >75 kg) in the Phase III trials rather than the approved 800 mg ribavirin dose for use with genotype 2 and 3 subjects (in combination with pegylated interferon).

A similar relationship between ribavirin mg/kg dosing and both anemia and ribavirin dose reduction was observed in GS-US-334-0110 where genotype 1, 4, 5, and 6 subjects were administered SOF/RBV/PEG. Anemia adverse events (9 of 82 [11.0%]) and ribavirin dose reductions (8 of 82 [9.8%]) were less frequent in the lowest mg/kg ribavirin dosing quartile (7-12 mg/kg) compared to anemia adverse events (22 of 82 [26.8%]) and ribavirin dose reductions (19 of 82 [23.2%]) in the highest mg/kg ribavirin dosing quartile (15-20 mg/kg). The increase in anemia event rate between these two populations is likely due to the addition of PEG to the SOF/RBV in GS-US-334-0110.

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

Following subsections describe the PK characteristics of SOF and its metabolites.

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

Study P7977-0221 evaluated the PK of single and multiple ascending doses of SOF (100, 200, and 400 mg for 28 days; 100-mg tablet formulation) in treatment-naive subjects with genotype 1 HCV infection. SOF was absorbed with the C_{max} occurring within 1 hour (median T_{max}) of dosing following single and multiple oral doses. Although SOF demonstrated a trend of decreased CL_r with increased dose at steady-state, CL_r is similar after single dose or multiple dose, and SOF exposure is near dose proportional. GS-331007 is slightly increased with increased sofosbuvir dose and relatively higher at steady-state as compared to the matching single dose ([Table 8](#)). However, no significant accumulation ($\leq 21\%$) of SOF or GS-331007 was observed at the 400-mg dose (accumulation ratios approached 1).

Nonlinearity in PK as a function of time was explored in the time invariance analyses. sofosbuvir and metabolites exhibit time independent linear pharmacokinetic characteristics because GLSM ratio approached 1.

In addition, Dose proportionality (across the range of sofosbuvir doses evaluated in this study) was examined using a power model and ANOVA analyses. Across the doses evaluated, the GLSM ratio and the 90% CI for sofosbuvir on Day 0 and Day 27 were greater than 1; suggesting more than dose proportional increases. However, these

results should be interpreted with caution, given the significant variability in the estimate. For GS-331007, increasing doses of sofosbuvir resulted in dose proportional increases in their exposure (AUC_{inf} and AUC_{tau}). The GLSM ratio for GS-331007 on Day 0 and Day 27 approached 1.

Table 8 P7977-0221: Plasma Sofosbuvir and GS-331007 Pharmacokinetic Parameters Following Single and Multiple Doses of Sofosbuvir in HCV-Infected Subjects

Plasma PK Parameters ^a	SOF 100 mg (N = 16)		SOF 200 mg (N = 17) ^b		SOF 400 mg (N = 15)	
	Day 1	Day 27	Day 1	Day 27	Day 1	Day 27
SOF						
AUC (ng·h/mL) ^c	280.20 (71.21)	375.70 (54.07)	585.48 (58.51)	732.27 (40.58)	1864.84 (51.35)	2011.23 (49.14)
C _{max} (ng/mL)	218.08 (83.25)	253.54 (67.49)	332.26 (64.87)	475.14 (75.53)	1257.50 (58.56)	1355.65 (62.98)
T _{max} (h)	1.00 (0.5, 1.31)	0.53 (0.50, 1.01)	1.00 (0.5, 1.58)	1.00 (0.63, 1.27)	1.00 (0.5, 1.03)	1.00 (0.50, 1.50)
t _{1/2} (h)	0.54 (0.40, 0.73)	0.63 (0.49, 0.98)	0.73 (0.52, 1.08)	0.74 (0.47, 1.03)	0.75 (0.46, 1.10)	0.68 (0.53, 1.00)
CL _r (L/min)	0.081 (46.8)	0.104 (36.0)	0.083 (43.5)	0.085 (43.2)	0.082 (40.1)	0.072 (47.8)
GS-331007						
AUC (ng·h/mL) ^c	1766.67 (39.17)	2256.81 (43.53)	3338.48 (22.89)	3389.23 (24.55)	6492.71 (39.37)	7398.99 (35.60)
C _{max} (ng/mL)	197.32 (44.94)	233.79 (44.92)	357.33 (27.95)	357.38 (30.75)	662.13 (32.43)	717.23 (29.09)
T _{max} (h)	3.50 (3.00, 4.00)	3.03 (3.00, 4.00)	4.00 (3.92, 4.25)	4.00 (2.55, 6.00)	4.00 (4.00, 4.25)	4.00 (1.58, 6.00)
t _{1/2} (h)	9.18 (6.30, 12.81)	9.93 (8.19, 12.70)	7.68 (6.51, 12.99)	12.23 (10.70, 15.67)	7.37 (6.26, 10.06)	13.00 (7.17, 16.34)
CL _r (L/min)	0.128 (35.8)	0.173 (33.4)	0.153 (27.7)	0.192 (22.9)	0.175 (27.9)	0.211 (29.7)

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

b Subject 1005-1043 was excluded from the PK analysis set due to an inadequate PK profile. Subject 1005-1085 was excluded from Day 27 summary statistics because this subject only received 15 days of SOF+PEG+RBV before being lost to follow-up.

c AUC represents AUC₀₋₂₄ for Day 1 and AUC_{tau} for Day 27.

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

Results from Study P2938-0212 indicated that the exposures of SOF and GS-331007 in HCV-infected subjects (Table 9) are similar to healthy subjects. Similar GS-331007 exposures were observed in Phase 2 study P7977-0523 (ELECTRON) following administration of SOF in HCV-infected patients for 4 weeks. Study P7977-0523 shows that GS-331007 exposures were higher in monotherapy as compared to when SOF is coadministered with PEG/RBV or RBV. GS-331007 exposure is similar when SOF is coadministered with PEG/RBV or RBV, and is similar to population PK analysis for HCV-infected subjects (Table 10). Therefore, the exposures for SOF and its metabolites appear comparable between patients and healthy subjects.

Table 9 Study P2938-0212: Pharmacokinetic Parameters after Once-Daily Administration of Sofosbuvir Alone in HCV-infected Subjects

Analyses	Mean (%CV) PK parameters	SOF 400 mg once daily (alone) for 7 days in HCV-infected patients (n = 8)
Sofosbuvir	AUC ₀₋₂₄ (ng.h/mL)	538 (39.0)
	C _{max} (ng/mL)	603 (47.1)
GS-331007	AUC ₀₋₂₄	9639 (18.7)
	C _{max}	1378 (19.2)

Table 10 Steady-State GS-331007 and Sofosbuvir Pharmacokinetic Parameters after Once-Daily Administration of Sofosbuvir in HCV-Infected Subjects (Population Pharmacokinetic Analysis from Phase 3 Studies) or in Healthy Subjects (Population Pharmacokinetic Analysis from Phase 1 Studies)

Mean (%CV) PK Parameter ^a	HCV-Infected Subjects (Population PK)	Healthy Subjects ^b (Population PK)
	SOF 400 mg (N = 986)	SOF 400 mg (N = 284)
GS-331007 AUC ₀₋₂₄ (ng.h/mL)	7200 (30.5)	11,900 (32.0)
GS-331007 C _{max} (ng/mL)	582 (37.1)	1140 (27.9)
SOF AUC ₀₋₂₄ (ng.h/mL)	860 (36.3)	634 (35.9)

a Pharmacokinetic parameters are presented as mean (%CV) and are shown to 3 significant digits.

b Subjects with severe renal impairment (n = 6) or ESRD (n = 6) were excluded in this summary (P7977-0915).

Population PK-derived mean GS-331007 and SOF exposures were comparable across all HCV genotypes in the Phase 3 population.

2.2.5.3 What are the characteristics of drug absorption?

SOF was stable in simulated gastric and intestinal fluids with half-lives of > 20 hours. Assessment of SOF permeability (concentration: 10-2800 µM) through Caco-2 cell monolayers revealed partially saturable efflux with an efflux ratio decreasing from 49.7 at 10 µM to 7.3 at 2800 µM. In vitro screening for interaction with various membrane transporters revealed that SOF is a substrate for p-glycoprotein (P-gp) and breast cancer resistance protein (BCRP).

Following oral administration of SOF, peak SOF concentrations were generally observed approximately 0.5 to 2 hours postdose, regardless of the dose administered to subjects with HCV infection and in healthy subjects. Peak plasma concentrations of GS-331007 were generally observed between 2 to 4 hours after SOF administration. Following a single-oral dose of [14C]-SOF to healthy male subjects, SOF was absorbed and subsequently eliminated in the urine as GS-331007. The sponsor indicates because approximately 80% of the administered dose was recovered in urine, ≥ 80% of the administered dose was absorbed into systemic circulation. This conclusion is not necessarily true because 80% may include metabolites formed from metabolism in the epithelia cells in the GI tract and from first pass metabolism, thus the real proportion that is absorbed into systemic circulation could be much less.

2.2.5.4 What are the characteristics of drug distribution?

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

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

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

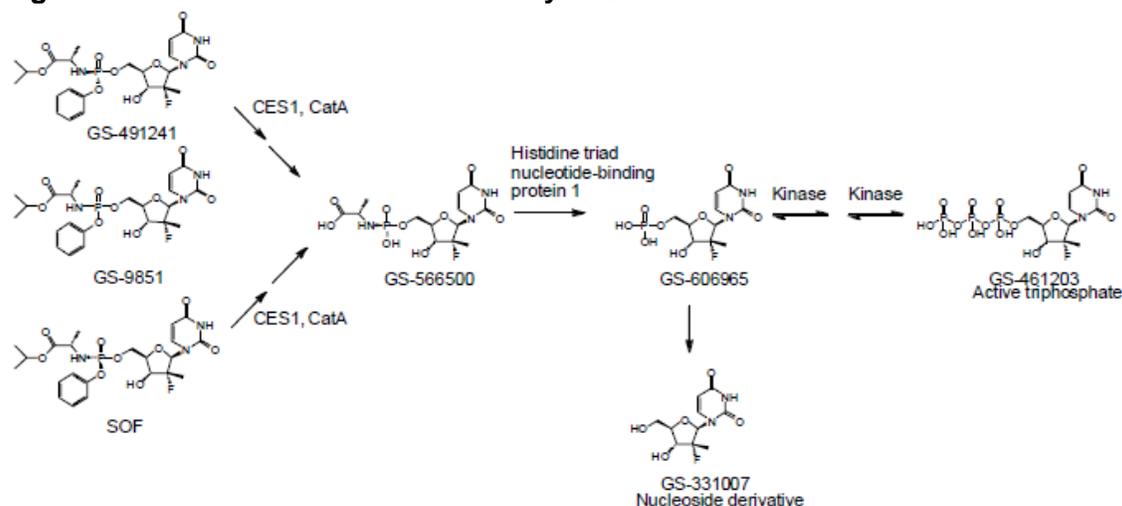
Elimination is primarily hepatic for SOF and renal for GS-331007. Following administration of [^{14}C]-SOF, mean total recovery of the radioactive dose was > 92%, consisting of approximately 80%, 14%, and 2.5% recovered in urine, feces, and respired air, respectively. The majority (78%) of the dose recovered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

2.2.5.6 What are the characteristics of drug metabolism?

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

The primary metabolic route of SOF is via hydrolase cleavage, which ultimately results in the formation of GS-331007. Sequential intracellular activation by generally low affinity and high capacity hydrolase ([carboxyl esterase 1 [CES1], cathepsin A [CatA], histidine triad nucleotide binding protein 1[HINT1]) and nucleotide phosphorylation (uridine monophosphate-cytidine monophosphate [UMP-CMP] kinase, nucleoside diphosphate [NDP] kinase) pathways resulted in the formation of the pharmacologically active nucleoside analog triphosphate GS-461203 ([Figure 4](#)).

Figure 4 Intracellular Metabolic Pathway of Sofosbuvir



2.2.5.7 What are the characteristics of drug excretion?

See Section 2.2.5.5.

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

A cross-study analysis of SOF and GS-331007 AUC_{inf} and C_{max} was performed to investigate the dose linearity of SOF (power model regression) using data from Study P7977-0613 that evaluated the PK of single therapeutic (400 mg) and suprathreshold (1200 mg) doses of SOF in fasted healthy subjects and Study P7977-0111 that evaluated the 200-mg single-dose SOF in fasted healthy subjects. The power model mean slope and 90% CIs indicated that near dose linearity was observed for SOF AUC_{inf} and C_{max}, and GS-331007 AUC_{inf}, with GS-331007 C_{max} showing modestly less than dose proportional increases (Table 11). Similar results were observed in HCV-infected subjects following single and multiple doses (once daily) of SOF 100-400 mg in Study P7977-0221 (Section 2.2.5.1).

Table 11 Summary of Sofosbuvir and GS-331007 Single Dose Pharmacokinetic Parameters Dose Linearity in Healthy Subjects

PK Parameters ^a		P7977-0111	P7977-0613		Power Model Mean Slope (90%CI)
		SOF 200 mg (N = 24)	SOF 400 mg (N = 59)	SOF 1200 mg (N = 59)	
SOF	AUC _{inf} (ng·h/mL)	263 (41.3)	629 (44.9)	2480 (42.5)	1.25 (1.16, 1.34)
	C _{max} (ng/mL)	267 (47.7)	622 (56.1)	2250 (47.5)	1.18 (1.08, 1.27)
GS-331007	AUC _{inf} (ng·h/mL)	6260 (25.3)	11,100 (22.6)	27,600 (24.1)	0.83 (0.78, 0.88)
	C _{max} (ng/mL)	781 (32.8)	1110 (28.0)	2100 (28.5)	0.56 (0.50, 0.62)

^a Pharmacokinetic parameters are presented as mean (%CV) and are shown to 3 significant digits.

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

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

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

SOF

Overall, in both healthy and HCV-infected subjects, moderate to high inter-individual variability was observed for SOF. Based on the population pharmacokinetic analysis, the inter-individual variability for the apparent oral clearance (CL/F) and apparent volume of distribution (V/F) was 51% and 173%. A significant difference between apparent oral clearance was identified between healthy subjects and HCV-infected patients (27% lower CL/F in HCV-infected patients); however, the available data is not sufficient to determine if this difference in clearance is due to disease status, differences in concomitant treatments (no additional drugs; administered with ribavirin; administered with pegylated-interferon and ribavirin), or a result of the different sampling schedules within the healthy subject (intensive) versus the HCV-infected (sparse) trials. No additional major causes of SOF variability were identified from the population pharmacokinetic analysis. There was insufficient data available to characterize the intra-subject variability of SOF from the available pharmacokinetic data.

In healthy subjects, based on single dose SOF pharmacokinetic data from P7977-0111 (200 mg) and P7977-0613 (400 and 1200 mg), the mean inter-individual variability values (% coefficient of variation) for AUC₀₋₂₄ and C_{max} were 41-45% and 48-56%. These inter-individual variability values were similar to that observed in HCV-infected subjects administered SOF 100, 200, and 400 mg at day 1 (AUC₀₋₂₄ and C_{max}: 51-71% and 59-83%, respectively) and steady state (AUC_{tau} and C_{max}: 49-54% and 63-76%, respectively). C_{tau} could not be calculated as no SOF was detectable at the end of the dosing interval.

GS-331007:

The inter-individual variability for GS-331007 was low (11%) for apparent oral clearance (CL/F) and high (80%) for apparent volume of distribution based on a population pharmacokinetic analysis. This analysis identified a significant impact of creatinine clearance (3.4 L/h decrease in GS-331007 clearance for a 10 mL/min decrease in creatinine clearance) and disease status (29-39% lower CL/F in HCV-infected patients) on the clearance of CL/F. However, similar to the comments for SOF above, the available data is not sufficient to determine if this difference in clearance is due to disease status, differences in concomitant treatments (no additional drugs; administered with ribavirin; administered with pegylated-interferon and ribavirin), or a result of the different sampling schedules within the healthy subject (intensive) versus the HCV-infected (sparse) trials. No additional major causes of GS-331007 variability were identified from the population pharmacokinetic analysis. There was insufficient data available to characterize the intra-subject variability of GS-331007 from the available pharmacokinetic data.

In healthy subjects, based on single dose GS-331007 pharmacokinetic data from P7977-0111 (200 mg) and P7977-0613 (400 and 1200 mg), the mean inter-individual variability values (% coefficient of variation) for AUC_{tau} and C_{max} were 23-25% and 28-33%. These inter-individual variability values were similar to that observed in HCV-infected subjects administered SOF 100, 200, and 400 mg at day 1 (AUC_{0-24} and C_{max} : 23-39% and 25-44%, respectively) and steady state (AUC_{tau} and C_{max} : 23-44% and 29-45%, respectively).

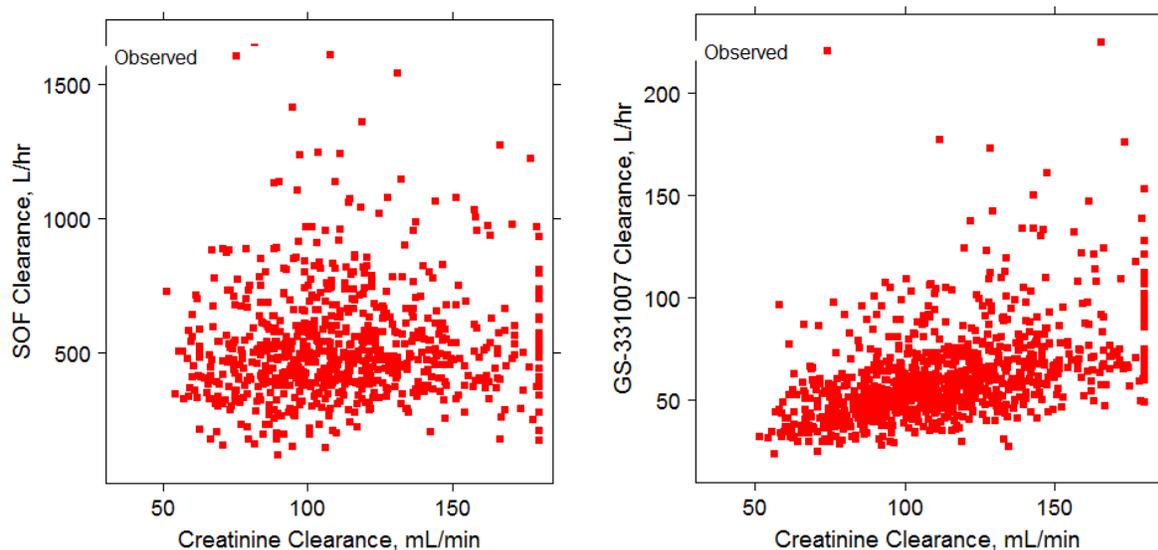
2.3 Intrinsic Factors

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

The impact of intrinsic factors was evaluated as covariates in the population PK analyses of GS-331007 and SOF based on data from adult subjects with HCV infection in the Phase 3 studies. The final population PK models for GS-331007 (18 studies) and SOF (14 studies) were based on final datasets that included observations from a total of 2089 and 1374 subjects, respectively.

The assessment of the potential effects of intrinsic factors on the PK of GS-331007 and SOF included creatinine clearance (CrCL, calculated by the Cockcroft-Gault equation), age, gender, BMI, race, and cirrhosis. Baseline creatinine clearance (calculated by the Cockcroft-Gault equation) was identified as the only significant intrinsic covariate that affected the CL/F of the renally excreted GS-331007 metabolite, but not for SOF CL/F (Figure 5). However, no subjects with $CrCL < 50$ mL/min were enrolled in the Phase III studies.

Figure 5: Apparent clearance of GS-331007, but not SOF, is associated with creatinine clearance



Renal Impairment:

The impact of renal impairment and hepatic impairment was studied in Studies P7977-0915 and P2938-0515, respectively. As shown in [Figure 6](#), plasma exposures of SOF, GS-566500 and GS-331007 were moderately higher in subjects with mild and moderate renal impairment compared with subjects with normal renal function. Relative to subjects with normal renal function, SOF AUC_{inf} was 61% and 107% higher in subjects with mild and moderate renal impairment, GS-566500 AUC_{inf} was 61% and 135% higher, and GS-331007 AUC_{inf} was 55% and 88% higher, respectively. An increase in GS-331007 exposure with decreasing renal function was expected as GS-331007 is primarily renally eliminated. The sponsor indicated that for SOF and GS-566500, the increase in exposure was less likely a result of decrease in renal clearance (CL_r), as renal excretion of SOF or GS-566500 is a minor pathway for its elimination. However, although SOF and GS-566500 account for only <4% of the excreted dose, the renal clearance (CL_r) value for SOF and GS-566500 in subjects with normal renal function are comparable to GS-331007. Because absolute bioavailability was not determined, it is difficult to estimate the percentage of the total clearance (CL_t) that is due to CL_r. If SOF is substantially converted to metabolites in epithelial cells in the GI tract and during first pass, then CL_r of SOF could account for a significant portion of its CL_t, since a large majority of the parent drug would not be bioavailable and thus not subject to renal excretion. Therefore, it is still possible that the increase in exposure of SOF and GS-566500 was less likely a result of decrease in renal clearance (CL_r).

Although no subjects with CrCL < 50 mL/min were enrolled in the Phase III studies, safety margins calculated from results of toxicology studies using AUCs are 7.3-, and 5.4-fold for SOF; 3.6-, and 2.4-fold for GS-566500; and 3.1-, and 2.0-fold for GS-331007 in subjects with mild, and moderate renal impairment. The impact of mild or moderate renal impairment on SOF and GS-566500 were less than the effect observed with the cyclosporine DDI study (see the discussion in Section 2.4.2.4.). Because RBV reduced GS-331007 exposure by about 50%, the combination of SOF with RBV in subjects with moderate renal impairment is expected to result in similar GS-331007 exposures as compared to when SOF is administered to HCV-infected subjects without RBV. Therefore, dose adjustment of SOF is not warranted in patients with mild to moderate renal impairment.

Unlike subjects with mild or moderate renal impairment, markedly higher exposures were observed for the renally eliminated GS-331007 in subjects with severe renal impairment or ESRD. Relative to subjects with normal renal function, SOF AUC_{inf} was 171% higher in severe renal impairment; GS-566500 AUC_{inf} was 244% higher, while the GS-331007 AUC_{inf} was 451% higher. In subjects with ESRD, SOF, GS-566500 and GS-331007 AUC_{inf} was 28%, 87% and 1280% higher when SOF was dosed 1 hour before hemodialysis compared with 60%, 259% and 2070% higher when SOF was dosed 1 hour after hemodialysis. Hemodialysis is required for the elimination of GS-331007 in subjects with ESRD, with a 4 hour hemodialysis removing approximately 18% of administered dose. There is no safety margin (≤ 0.6 based on GS-331007) for subjects with severe or ESRD. Due to the complicated intracellular metabolic pathway of SOF, it is difficult to propose a dose reduction in these populations. In addition, the efficacy of a substantially reduced dose of SOF in this population has not been established at this time. Therefore, SOF is not recommended in subjects with severe renal impairment or ESRD.

Hepatic Impairment:

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

GS-331007 plasma exposure was comparable (PK comparisons as GLSM ratios) in subjects with moderate or severe hepatic impairment and historical control subjects from Study P2938-0212 (NUCLEAR) with normal hepatic function.

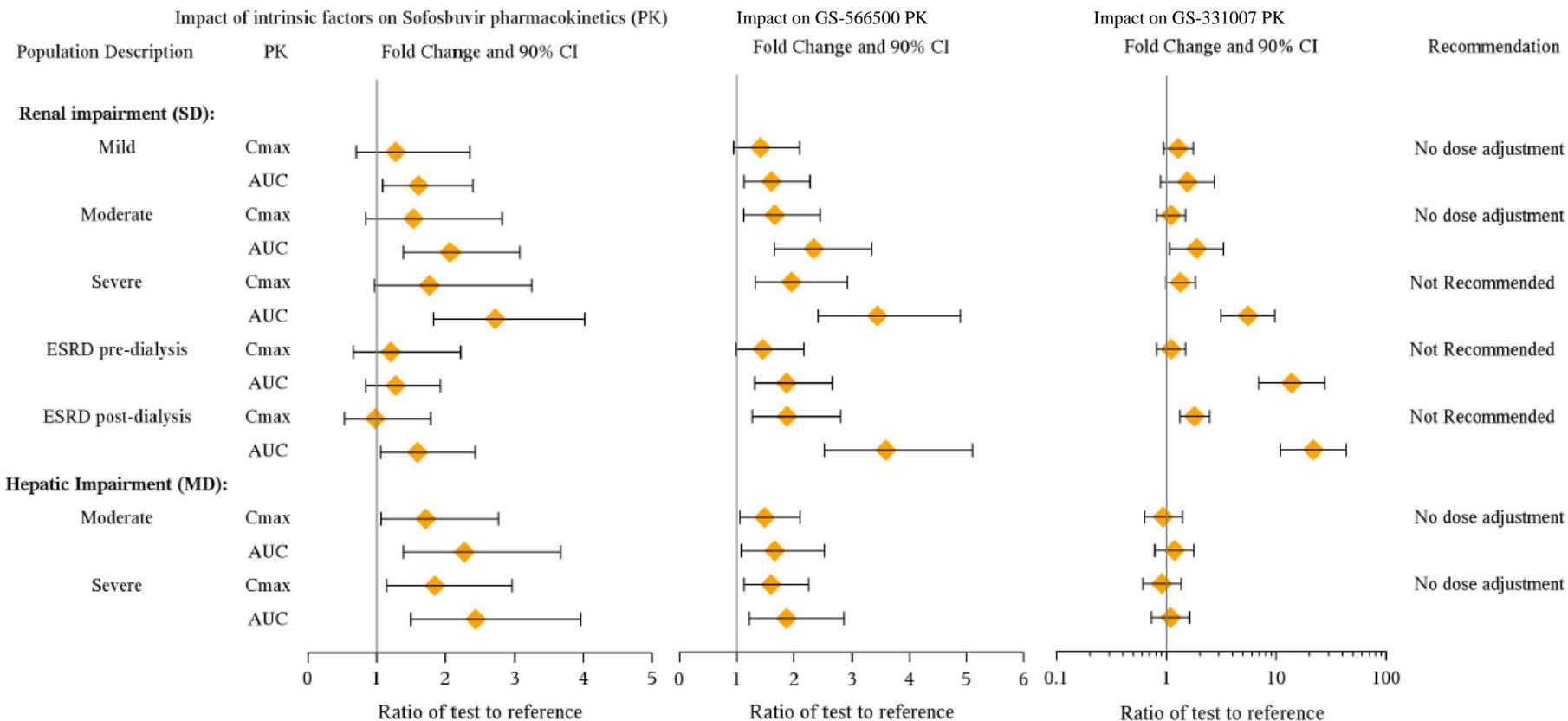
SOF mean plasma exposure parameters (AUCtau and Cmax) were similar in subjects with moderate or severe hepatic impairment (CPT Classifications B and C, respectively) and were modestly higher (AUCtau: 126–143%↑; Cmax: 72–85%↑) than those achieved in subjects with normal hepatic function. Safety margin calculated from results of toxicology studies are 6.4- and 5.8-fold for SOF AUCtau in moderate and severe hepatically impaired subjects, respectively.

GS-566500 mean plasma exposure parameters (AUCtau and Cmax) were also similar in subjects with moderate or severe hepatic impairment (CPT Classifications B and C, respectively) and were modestly higher (AUCtau: 66–87%↑; Cmax: 49–60%↑) than those achieved in subjects with normal hepatic function. Safety margin calculated from results of toxicology studies are 4-fold and 3.7-fold for GS-566500 AUCtau in moderate and severe hepatically impaired subjects, respectively.

In Study P2938-0515, HCV RNA decline in HCV-infected subjects with varying degrees of hepatic impairment was assessed after 7 days of dosing SOF. SOF provided potent antiviral activity in subjects with hepatic impairment as evidenced by > 3.5 log₁₀ declines in HCV RNA. A greater mean decrease and faster decline from baseline in HCV RNA were observed in the control subjects compared with subjects who had moderate or severe hepatic impairment. These differences in reductions of HCV RNA with short-term SOF monotherapy were not considered clinically meaningful.

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

Figure 6: The impact of intrinsic factors on Pharmacokinetics of sofosbuvir and its metabolites



2.3.2 What influence does IFNL3 genotype have on efficacy based on HCV genotype?

The applicant evaluated the role of *IFNL3* genotype on SVR12 in subjects infected with HCV (Table 12). *IFNL3* genotype was associated with modestly lower SVR12 rates in treatment naïve subjects with genotype 1, 4, 5, or 6 infection after 12-weeks of combination treatment with SOF/PEG/RBV. However, even among non-CC's, SVR rates were similar to those previously observed with triple drug regimens containing telaprevir or boceprevir.

Based on recent meta-analyses of published literature, the effect of *IFNL3* genotype on treatment response in HCV genotypes 2 and 3 is less pronounced than in genotype 1. In all of the treatment arms for genotype 2 and 3 patients (FISSION, POSITRON, and FUSION), no consistent correlation was found between *IFNL3* genotype and SVR12 rate, although treatment naïve, non-CC subjects tended to have lower SVR rates.

Among genotype 3 subjects, those who were treatment-naïve with the CC genotype tended to have higher SVR response rates with the 24-week peginterferon/ribavirin regimen compared to the 12-week SOF/RBV regimen. This observation suggests that 12-weeks of sofosbuvir/ribavirin may not be the most optimal regimen in this population. Further supporting longer treatment durations in this population, higher SVR rates were observed in treatment-experienced subjects after 16-weeks compared to 12-weeks.

Among genotype 2 subjects, those who were treatment-experienced tended to have better responses with 16-weeks of SOF/RBV as compared with 12 weeks. As with genotype 3, longer treatments in all genotype 2 subjects may produce a higher SVR rate, although this has not been directly studied. Given the overall high response in genotype 2 subjects, it does not appear that *IFNL3* genotype alone would be sufficient to identify patients that would benefit from longer treatment durations.

Table 12. SVR12 by HCV and *IFNL3* (*IL28B*) genotypes.

<i>IFNL3</i> genotype, N (%)	FISSION		POSITRON		FUSION		NEUTRINO
	Treatment Naïve		Interferon Ineligible, Intolerant, Unwilling		Treatment Experienced		Treatment Naïve
	SOF+RBV 12 weeks	PEG+RBV 24 weeks	SOF+RBV 12 weeks	Placebo 12 weeks	SOF+RBV 12 weeks	SOF+RBV 16 weeks	SOF+PEG+RBV 12 weeks
HCV Genotype 2	N = 70	N = 67	N = 109	N = 34	N = 36	N = 32	-
CC	31/31 (100%)	28/34 (82.4%)	40/45 (88.9%)	0/17 (0.0%)	6/7 (85.7%)	9/11 (81.8%)	-
Non-CC	37/39 (94.9%)	24/33 (72.7%)	61/64 (95.3%)	0/17 ^a (0.0%)	25/29 (86.2%)	21/21 (100.0%)	-
HCV Genotype 3	N = 183	N = 176	N = 98	N = 37	N = 64	N = 63	-
CC	43/75 (57.3%)	54/72 (75.0%)	34/52 (65.4%)	0/12 ^a (0.0%)	9/23 (39.1%)	10/16 (62.5%)	-
Non-CC	59/106 (55.7%)	55/103 (53.4%)	26/46 (56.5%)	0/25 (0.0%)	10/41 (24.4%)	29/47 (61.7%)	-
HCV Genotype 1,4,5,6	-	-	-	-	-	-	N = 327 ^b
CC	-	-	-	-	-	-	93/95 (97.9%)
Non-CC	-	-	-	-	-	-	202/232 (87.1%)

Prop Diff = difference in proportions, SOF = sofosbuvir, RBV = ribavirin, PEG = peginterferon.

^a = one subject discontinued treatment and was imputed by reviewer as a treatment failure in accordance with the sponsor's treatment of subjects with missing SVR12 data in this study.

^b = HCV genotype 1 (N = 292), HCV genotype 4 (N = 28), HCV genotype 5 (N = 1), and HCV genotype 6 (N = 6).

Data Source Tables: m5.3.5.3, ISE, Table 3; m5.3.5.1, P7977-1231 (FISSION), Section 15.1, Table 8.5, GS-US-334-0107 (POSITRON), Section 15.1, Ad Hoc Table 8.2.1; GS-US-334-0108 (FUSION), Section 15.1, Ad Hoc Tables 38.1 and 38.2; m5.3.5.3, ISE, Table 2; m5.3.5.1, GS-US-334-0110 (NEUTRINO), Section 15.1, Table 7.2. POSITRON placebo and prop diff calculated by reviewer using data from the 'adeffout' file

2.4 Extrinsic Factors

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

Only drug-drug interactions have been assessed. The differences in exposure of SOF and its metabolites are believed to have a minimal effect on response. See section 2.4.2.

2.4.2. Drug-Drug Interactions

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

Yes. *In vitro* studies suggest that SOF is a substrate for P-gp and BCRP. Drugs that are potent P-gp inducers in the intestine, although not studied in vivo, may decrease SOF plasma concentration leading to reduced therapeutic effect and thus should not be used

with SOF. The drug-drug interaction between SOF and cyclosporine, a P-gp/BCRP inhibitor, was assessed in vivo. See sections 2.4.2.4 and 2.4.2.8 for details.

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

Pathways involving CYP isozymes are not likely to be important considerations in the disposition of SOF, its metabolites, GS-566500, GS-606965, and GS-331007 based on in vitro microsome assay results.

When SOF was incubated in microsomes in the presence of 1-aminobenzotriazole (a nonspecific CYP inhibitor), an apparent decrease in the disappearance of SOF was observed compared to the control (incubation without 1-aminobenzotriazole). This suggests potential involvement of CYP isoforms in the metabolism of SOF. However, the following observations suggest that CYP isoforms do not play a clinically relevant role in SOF metabolism; in vitro and clinical studies have shown that SOF is rapidly metabolized to GS566500 by high capacity esterases (Cat A and CES1). No other metabolite directly derived from SOF was detected in vitro or in vivo. In vitro drug interaction studies with ritonavir and ketoconazole indicated no clinically relevant changes of those drugs on the metabolism of SOF. Based on these observations, the sponsor did not additionally characterize the roles of individual CYP isoforms on the metabolism of SOF using purified CYP isozymes. GS-566500, GS-606965, and GS-331007 were stable in microsome mixtures for an hour, indicating that these metabolites are not further metabolized by CYP isoforms. In vivo drug-drug interaction studies with efavirenz (in Atripla®) and darunavir/ritonavir (up to 45% and 34% increase on SOF C_{max} and AUC, respectively, could be due to the P-gp inhibition of ritonavir) indicated no clinically relevant changes of those drugs on the metabolism of SOF and its metabolites.

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

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

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

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

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

In agreement with in vitro data, Study P7977-1819 (Section 2.2.8), a DDI study with the potent P-gp and BCRP inhibitor CsA (administered at a high dose of 600 mg), resulted in an increase (approximately 4-fold) in SOF exposure, and Study GS-US-334-0131

(Section 2.2.1) revealed an increase of < 2-fold in SOF exposure with the less potent P-gp inhibitor, RTV-boosted DRV. The exposure of GS-331007 was unchanged in the presence of P-gp and/or BCRP inhibitors. The mass balance study shows that at least 76% of administered drug was recovered from urine, indicating that at least 76% of administered drug is absorbed from the GI tract. The result suggests P-gp/BCRP inhibition should not increase SOF concentrations by more than 31.5% (up to 100% absorption). In addition, based on the results from Study P7977-0613 (thorough QT study), exposure for GS-331007 should be increased near proportionally with increase of SOF. In Study P7977-0613, exposures for both SOF and GS-331007 increased near dose proportionally when SOF dose increased from 400 mg to 1200 mg. At SOF 1200 mg, SOF exposure was comparable to that of SOF at 400 mg when coadministered with CsA. Therefore, the lack of an increase in GS-331007 exposure when SOF was coadministered with CsA is not likely to be explained solely by P-gp/BCRP inhibition. Limited safety data from an ongoing post-transplant study (GS-US-334-0126) indicate that the safety of SOF+RBV is similar between subjects not taking CsA (n=30) and subjects taking CsA (n=10). Furthermore, safety margins for SOF, GS-566500 and GS-331007 were 2.3-, 1.9- and 3.5-fold, respectively, after coadministration with CsA, compared with exposures obtained in toxicology studies. These safety margins were considered adequate. Therefore, dose modification of SOF is not warranted when coadministered with CsA.

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

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

Telaprevir (a relatively nonspecific protease inhibitor) and boceprevir have been reported to inhibit SOF activation in vitro via inhibition of CatA. The sponsor indicated because CatA is a low affinity and high capacity hydrolase, boceprevir and telaprevir are not expected to be involved in a clinically relevant DDI with SOF. More data may be needed to support this conclusion. However, at this time, SOF is not likely to be coadministered with telaprevir or boceprevir.

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

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

Yes. SOF is indicated for the treatment of chronic HCV in combination with Peg-IFN and RBV in treatment-naïve patients with genotype 1, or 4; and in combination with RBV in patients with genotype 2 or 3. No drug-drug interaction study has been formally conducted between SOF and Peg-IFN or RBV. Most of the studies in HCV-infected subjects were evaluated in Phase III studies where SOF was combination with RBV± peginterferon except Study P2938-0212 (Phase I), where 8 HCV-infected subjects were administered with SOF 400 mg alone once daily for 7 days. In addition, Study P7977-0523 shows that GS-331007 exposures were higher in monotherapy as compared to when SOF is coadministered with PEG/RBV or RBV (Table 13). GS-331007 exposure is similar when SOF is coadministered with PEG/RBV or RBV. Drug interaction between GS-331007 and RBV is possible because both of them are mainly renal eliminated.

Table 13 Study P7977-0523: Comparison of Exposures for Sofosbuvir and GS-331007 in HCV-infected subjects with Sofosbuvir alone and in combination with PEG/RBV or RBV

GS-331007 PK Parameter Mean (%CV)	Treatment-Naïve Genotype 2/3						Null-Responders Genotype 1
	SOF+RBV 12 weeks (Group 1) (N = 10)	SOF+PEG+RBV 4 weeks/8 weeks (Group 2) (N = 9)	SOF+PEG+RBV 8 weeks/4 weeks (Group 3) (N = 10)	SOF+PEG+RBV 12 weeks (Group 4) (N = 11)	SOF 12 weeks (Group 5) (N = 10)	SOF+PEG+RBV 8 weeks (Group 6) (N = 10)	SOF+RBV 12 weeks (Group 7) (N = 10)
AUC _{tau} (h·ng/mL)	7035.0 (19.1)	7135.0 (25.3)	6562.7 (30.0)	7163.2 (30.5)	13,588.4 (30.0)	8977.5 (35.4)	8630.1 (39.1)
C _{max} (ng/mL)	676.3 (36.1)	664.8 (39.7)	702.0 (40.9)	723.3 (38.8)	1431.2 (31.0)	929.5 (35.5)	789.2 (31.2)
C _{tau} (ng/mL)	153.0 (13.3)	141.3 (20.4)	116.6 (32.3)	143.5 (27.7)	267.4 (33.3)	161.1 (48.4)	175.8 (45.0)
T _{max} (h) ^a	4.00 (4.00, 4.00)	4.00 (4.00, 4.00)	4.00 (4.00, 4.00)	4.00 (3.98, 4.00)	4.00 (4.00, 4.00)	4.00 (4.00, 4.00)	4.00 (4.00, 4.00)
T _{1/2} (h) ^a	15.90 (9.93, 20.00)	11.35 (9.94, 15.04)	10.16 (9.80, 13.11)	12.30 (11.01, 13.29)	12.88 (12.20, 16.50)	11.68 (8.68, 14.73)	13.55 (10.86, 16.01)

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

Other medications that are likely to be co-administered in HCV-infected patients include antiretroviral agents for the treatment of HIV, analeptics, anticonvulsant, antimycobacterials, methadone therapy for the treatment of opioid addiction, cyclosporine and tacrolimus in the prevention of organ rejection following liver transplant, antidepressants and other mood-stability medications, combined oral contraceptives in women to prevent pregnancy, and some herbal supplements. Drug interaction studies have been conducted with SOF in combination with representative antiretrovirals, methadone, cyclosporine and tacrolimus.

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

Drug interaction studies have been conducted with SOF in combination with representative antiretrovirals, methadone, cyclosporine and tacrolimus.

SOF as Victim: As shown in the [Figure 7](#), cyclosporine has the greatest effect on SOF and its metabolites. This is consistent with the *in vitro* data showing that SOF is a substrate of P-gp and BCRP, but not a substrate for CYP enzymes. The *in vivo* data show coadministration of CsA and SOF, resulted in an approximately 4-fold increase in SOF AUC, but had no effect on GS-331007. As discussed in Section 2.4.2.4, the effect of CsA on SOF and its metabolites is not considered clinically significant and no dose adjustment is required. Antiretrovirals and tacrolimus have much less of an effect on the PK of SOF and its metabolites, thus dose adjustment is not needed for SOF when coadministered with antiretrovirals and tacrolimus.

SOF as Perpetrator: The effects of SOF on PK of other drugs are shown in [Figure 8](#).

Tenofovir: Tenofovir-SOF drug-drug interaction has been evaluated in Study GS-US-334-0131 and Study P7977-1910. Study GS-US-334-0131 evaluated the effect of single dose of SOF on the PK of steady-state tenofovir in presence of efavirenz and emtricitabine (Atripla®) in healthy subjects (n =16). Study P7977-1910 evaluated the effect of steady-state SOF on the PK of steady-state tenofovir in Atripla® (ATR, n=8), or Truvada (TVD) when combined with atazanavir/ritonavir (ATV/r, n=8), darunavir/ritonavir (DRV/r, n =5), or raltegravir (RAL, n =5) in Human Immunodeficiency Virus and Hepatitis C Virus (HIV/HCV) Co-infected Patients.

SOF increased tenofovir C_{max} by 25% to 40% in 3 cohorts including the single dose study, but decreased by 17% in the cohort of TVD+DRV/r, and no change in the cohort of TVD and RAL. The small decrease of tenofovir C_{max} in the TVD+DRV/r cohort could be due to high variability associated with a small sample size (90% confidence intervals of the PK ratios cross 1). There are some safety data available for tenofovir in HCV/HIV coinfecting patients. Therefore, no dose adjustment is needed for tenofovir.

Emtricitabine: Emtricitabine (FTC) -SOF drug-drug interaction has been evaluated in Study GS-US-334-0131 and Study P7977-1910. Study GS-US-334-0131 evaluated the effect of single dose of SOF on the PK of steady-state FTC in presence of efavirenz and tenofovir (Atripla®) in healthy subjects (n =16). Study P7977-1910 evaluated the effect of steady-state SOF on the PK of steady-state FTC in Atripla® (ATR, n=8), or Truvada (TVD) when combined with atazanavir/ritonavir (ATV/r, n=8), darunavir/ritonavir (DRV/r, n =5), or raltegravir (RAL, n =5) in Human Immunodeficiency Virus and Hepatitis C Virus (HIV/HCV) Co-infected Patients. The results were consistent among these 5 cohorts and show no significant effect of SOF on the PK of FTC.

Lamivudine: Lamivudine (3TC)-SOF drug-drug interaction has been evaluated in Study P7977-1910 (n =4). The results show that coadministration of SOF with EFV+ZDV/3TC resulted in 11–20% lower 3TC AUC_{tau}, C_{max}, and C_{tau}, and the upper limits of 90% CIs were all below 1. These magnitudes of decreases in 3TC exposure parameters are not considered clinically significant; and the results suggest that SOF may be coadministered with EFV+ZDV/3TC.

Zidovudine: Zidovudine (ZDV)-SOF drug-drug interaction has been evaluated in Study P7977-1910 (n = 4). The result show that coadministration of SOF with EFV+ZDV/3TC resulted in 16% and 37% reduction of ZDV AUC and Ctau, respectively, and 27% increase on ZDV Cmax. However, the PK parameters had wide 90% confidence intervals (CIs) due to the small sample size.

Efavirenz: Efavirenz (EFV)-SOF drug-drug interaction has been evaluated in Study GS-US-334-0131 and Study P7977-1910. Study GS-US-334-0131 evaluated the effect of single dose of SOF on the PK of steady-state EFV in Atripla® in healthy subjects (n =16). Study P7977-1910 evaluated the effect of steady-state SOF on the PK of steady-state EFV in Atripla® (ATR, n=8), or when EFV was coadministered with ZDV/3TC (n=4) in Human Immunodeficiency Virus and Hepatitis C Virus (HIV/HCV) Co-infected Patients. The results were consistent among these 3 cohorts and show no significant effect of SOF on the PK of EFV.

Atazanavir: Atazanavir (ATV) -SOF drug-drug interaction has been evaluated in Study P7977-1910 (n = 8). The result show that the mean AUCtau and Cmax of ATV are comparable following administration of ATV/r +TVD alone and in combination with SOF; while Ctau was increased by 22% when ATV/r +TVD was coadministered with SOF. The effect is not considered clinically significant.

Darunavir: Darunavir (DRV) -SOF drug-drug interaction has been evaluated in Study GS-US-334-0131 (n=18) and Study P7977-1910 (n =5). Study GS-US-334-0131 shows that single dose of SOF had no effect on DRV AUC and Cmax, but reduced DRV Ctau by 14%. Study P7977-1910 shows that darunavir mean AUCtau and Ctau were comparable following administration of DRV/r+TVD alone or in combination with SOF, with wide 90%CI due to the small sample size. DRV Cmax was reduced by 20% when DRV/r+TVD were administered in combination with SOF. These effects are not considered clinically significant.

Ritonavir: The effect of SOF on the PK of ritonavir (RTV) was evaluated when RTV was used as a component of DRV/r or ATV/r in Study GS-US-334-0131 (n=18 in DRV/r cohort) and Study P7977-1910 (n =8 in ATV/r +TVD cohort, n=5 in DRV/r +TVD cohort). Study GS-US-334-0131 shows that single dose of SOF had no effect on ritonavir PK. However Study P7977-1910 shows that SOF reduced RTV AUC and Cmax by 21%-33% and 32%-55% and had no effect on RTV Ctau. These effects are considered clinically insignificant because RTV is used as a booster.

Raltegravir: Raltegravir (RAL) -SOF drug-drug interaction has been evaluated in Study GS-US-334-0131 (n=19) and Study P7977-1910 (n =4). Study GS-US-334-0131 shows that single dose of SOF reduced raltegravir Cmax and AUC by 43% and 27%, respectively; which is similar to the effects of EFV (36%↓ AUC and Cmax) and rifampin (38% ↓ for AUC and 40% ↓Cmax and 61% ↓ Cmin) on raltegravir. Single dose of SOF had no effect on raltegravir Ctau. No dose adjustment is recommended for EFV because efficacy data from the combination use of EFV and RAL support the combination use without dose adjustment, but it is recommended (in the Isentress label) to double RAL doses when RAL is combined with rifampin.

In direct contrast, Study P7977-1910 shows that multiple doses of SOF *increased* raltegravir Cmax, AUC, Ctau by 65%, 65%, and 87% respectively. The result from Study P7977-1910 is considered inconclusive due to the small sample size (N=4) resulting in wide 90% CIs.

The dose of RAL may not need to be adjusted when coadministered with SOF because raltegravir has highly variable PK and RAL has a wide therapeutic window. In addition, preliminary results from the ongoing Phase 3 study in HIV/HCV co-infected subjects (GS-US-334-0123) show that 45 of the 46 subjects on a SOF plus raltegravir-containing ARV regimen have HIV virologic suppression. A single subject (Subject 4262-8725; HCV genotype 3) receiving ARV treatment with raltegravir and emtricitabine/tenofovir DF had HIV-1 virologic rebound during the study. For this subject, HIV-1 RNA was not detected from baseline through Week 8, but was detected at Week 12 (no Week 10 assessment). Per the investigator (data on file), this subject had poor adherence to HIV medications at the time of HIV virologic rebound. In addition, this subject had HCV virologic relapse and may not have adhered to study drug, as evidenced by a lack of decline in hemoglobin concentration and a lack of increase in reticulocyte count during the treatment period.

Rilpivirine: Rilpivirine (RPV)-SOF drug-drug interaction has been evaluated in Study GS-US-334-0131 (n=17). The result indicated that a single dose of SOF had no effect on the PK of RPV.

Methadone: Study P7977-0814 shows that steady-state SOF had no effect on the PK of R-methadone or S-methadone.

Cyclosporine: Study P7977-0819 shows that a single dose of SOF had no effect on the PK of cyclosporine.

Tacrolimus: Study P7977-0819 shows that a single dose of SOF reduced tacrolimus C_{max} by 27%, but had no effect on tacrolimus AUC. No dose adjustment is necessary for tacrolimus.

Figure 7 Drug-Drug Interactions when SOF was a victim

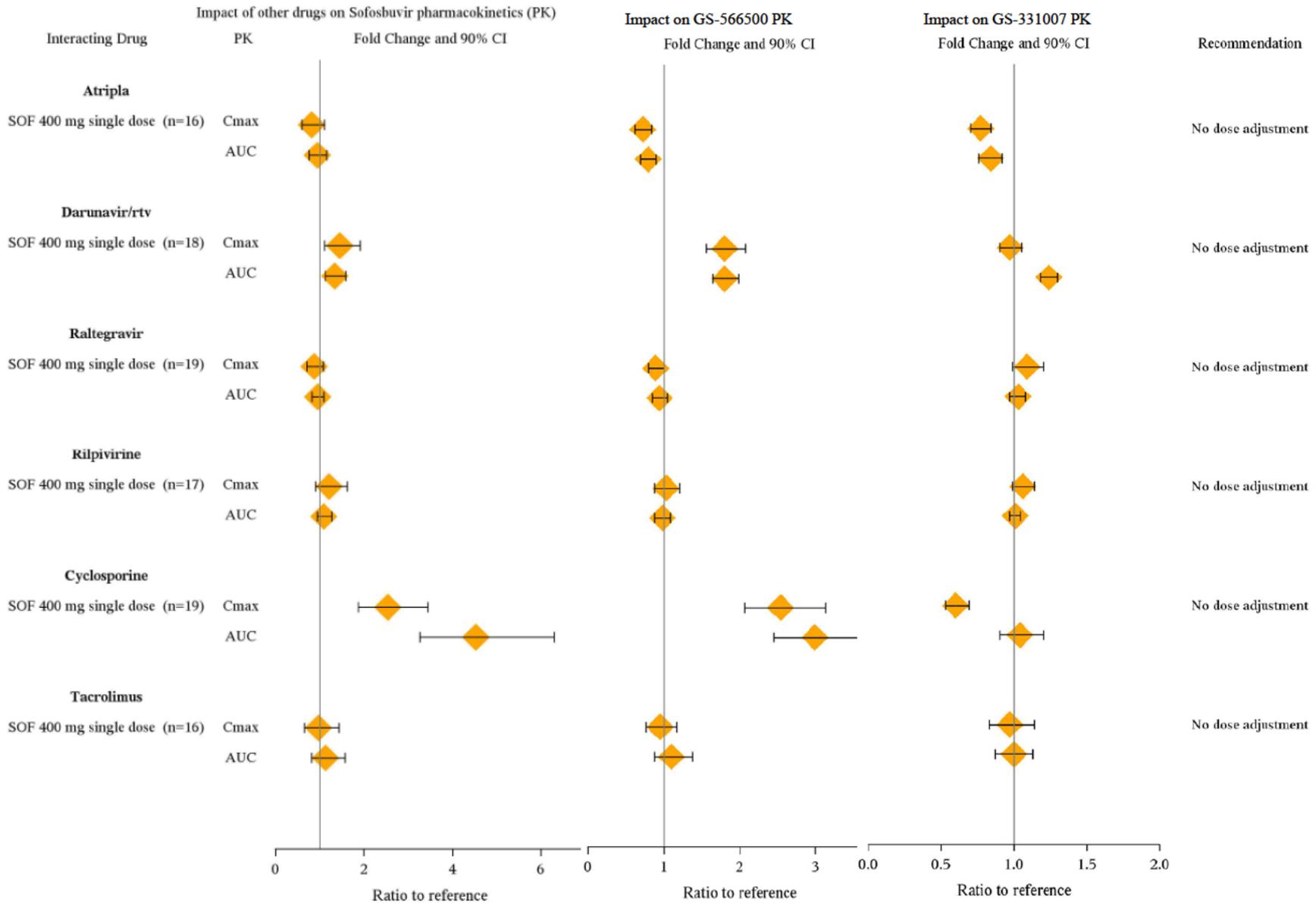
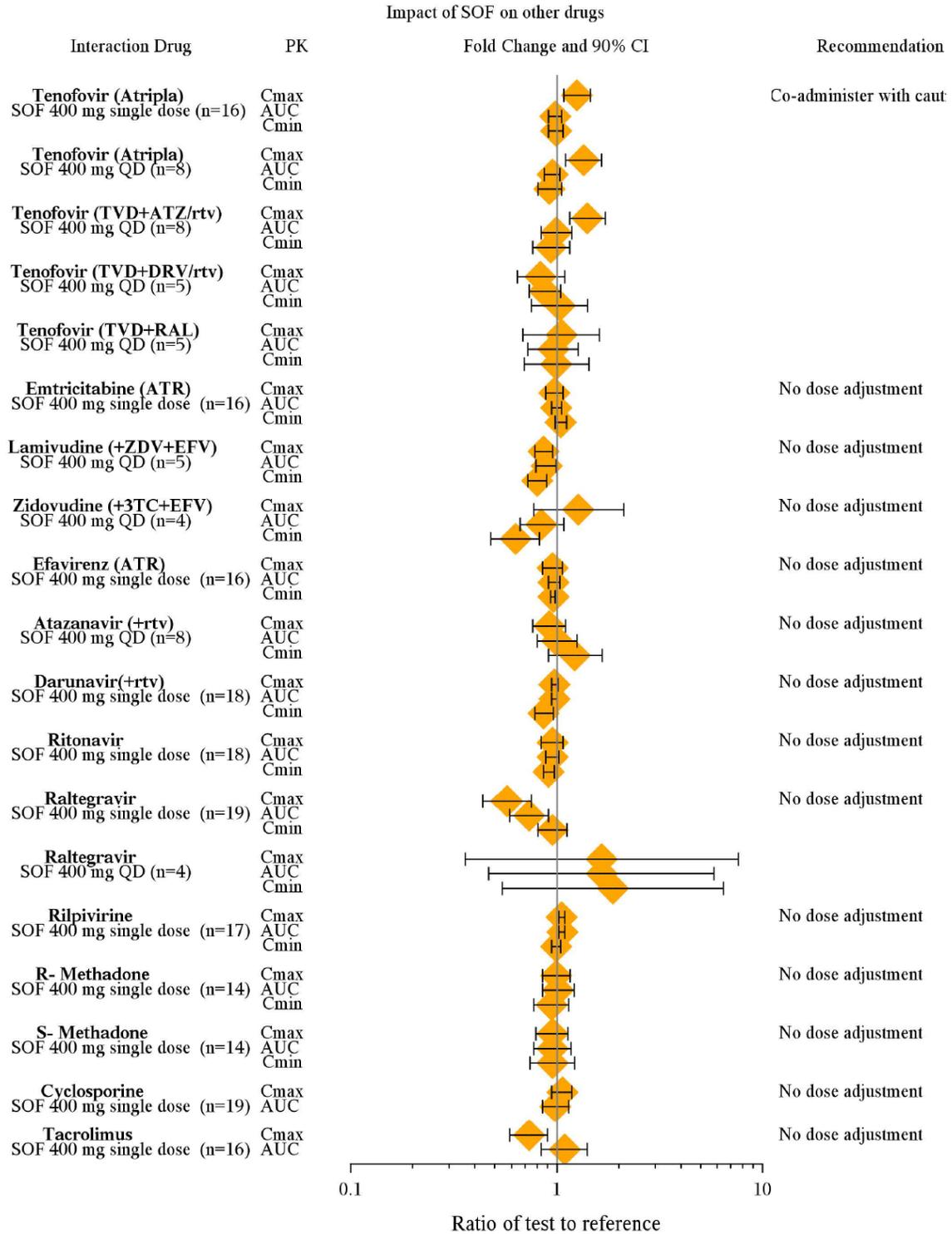


Figure 8 Drug-Drug Interactions when SOF was a Perpetrator



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

There is no known mechanistic basis for pharmacodynamics drug-drug interactions. No significant efficacy or safety based exposure-response relationship has been identified. Pharmacodynamic drug-drug interactions have not been assessed.

2.5 General Biopharmaceutics

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

The sponsor claimed that SOF is a high-solubility, low-permeability (BCS 3) compound. Based on the FDA Guidance for Industry “Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System”, “A drug substance is considered *highly soluble* when the highest dose strength is soluble in 250 ml or less of aqueous media over the pH range of 1-7.5.” Because SOF’s solubility is about 2 mg/mL for pH 2-7.7 (Table 14), 400 mg of SOF should be able to dissolve in 250 mL of aqueous media over the pH range of 1-7.5. Therefore, SOF is considered highly soluble based on the BCS definition.

Table 14 Solubility of SOF in Aqueous Media at 37 °C

pH (Media)	Solubility (mg/mL)	USP Solubility Description
2 (HCl)	2.0	Slightly soluble
4.5 (Acetate buffer)	2.1	Slightly soluble
6.8 (Phosphate buffer)	1.9	Slightly soluble
7.7 (Unbuffered)	2.2	Slightly soluble

The reviewer considers that it is reasonable to classify SOF as a lowly permeable compound based on 3 pieces of evidence:

1. <90% of the administered drug was recovered in urine in the mass balance study and the majority (77.7%) of the dose recovered in the urine was as GS-331007 ;
2. in vitro Caco-2 study indicates SOF is a low permeability compound;
3. SOF plasma concentrations were significantly increased by P-gp inhibitor cyclosporine.

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

During early stages of development, SOF drug substance was (b) (4) Early Phase 2 studies used (b) (4) tablets (b) (4) that were manufactured (b) (4) Based on the efficacy and safety results from Phase 2 studies, the SOF dose selected for further development was 400 mg once daily, and initial Phase 3 studies (P7977-1231 and GS-US-334-0107) used SOF tablets (b) (4) Bioequivalence of the (b) (4) was established in Study P7977-1318. The (b) (4) of SOF drug substance was

determined to have superior physicochemical properties compared with (b) (4) SOF 400-mg tablets containing the (b) (4) were used in the subsequent Phase 3 Studies GS-US-334-0110 (NEUTRINO) and GS-US-334-0108 (FUSION), and will be the to-be-marketed formulation. [Table 15](#) lists the SOF (b) (4) used in the pivotal Phase 3 studies.

Table 15 SOF (b) (4) Used in Pivotal Phase 3 Studies

(b) (4)	Tablet Strength (mg)	Phase 3 Study	Study Population
(b) (4)	(b) (4)	P7977-1231 (FISSION)	treatment-naïve with genotype 2 or 3
		GS-US-334-0107 (POSITRON)	IFN intolerant, IFN ineligible, or unwilling to take IFN with genotype 2 or 3
(b) (4)	400	GS-US-334-0110 (NEUTRINO)	treatment-naïve with genotype 1, 4, 5, or 6
		GS-US-334-0108 (FUSION)	Treatment-experienced with genotype 2 or 3

In Study GS-US-334-0131 (bioavailability study comparing Forms 1 and 2), the geometric least-squares mean (GLSM) ratios of GS-331007 (a primary circulating metabolite) AUC_{last}, AUC_{inf}, and C_{max} were between 92% and 99%, and the 90% CIs of the GLSM ratios were generally within ± 20%. However, 90% CIs of the GLSM ratios of SOF AUC_{last}, AUC_{inf}, and C_{max} and GS-566500 C_{max} were outside ± 20%, but within ± 30% ([Table 16](#)). Although pharmacokinetic equivalence of the Form I and II (b) (4) was not fully established, the to-be-marketed formulation is used in all genotypes in Phase III trials, and the efficacy and safety results were similar among three genotype 2 or 3 studies. Therefore, Study P7977-1318 is not considered pivotal and no inspection is necessary.

Table 16 Statistical Comparisons of SOF, GS-566500 Plasma, GS-331007 Pharmacokinetic Parameters Following Administration of (b) (4) SOF in Cohorts 1 and 3 and (b) (4) SOF in Cohort 5 at fasting condition

(b) (4)

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

Results from the food effect study P7977-1318 (400 mg SOF single dose) demonstrated that a high-fat meal resulted in a 24% decrease in GS-331007 C_{max}, while AUC values (AUC_{0-last} and AUC_{inf}) were unchanged. A high-fat meal also increased SOF and GS-566500 AUC_{inf}'s by 81% and 56%, respectively. A 200 mg SOF single dose food effect study (P7977-0111) showed similar results as Study P7977-1318. Food effect is not considered clinically significant. Dosing of SOF in Phase 2 and 3 clinical studies was without regard to food. SOF is recommended to be administered without regard to food.

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

Not applicable.

2.6 Analytical Section

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

The active metabolite (GS-461203) is converted from prodrug SOF intracellularly and is not detectable in plasma. Nonclinical characterization of the disposition of SOF across species revealed that SOF was extensively metabolized by hydrolase activity that led to low systemic exposure of SOF and predominant systemic exposure to 2 major metabolites in humans: GS-566500 and the primary circulating metabolite GS-331007, but not GS-461203. These findings were confirmed in a mass balance study such that SOF, GS-566500, and GS-331007 accounted for approximately 4%, approximately 7%, and > 90% of drug-related material respectively. GS-331007 was considered to be the primary analyte of interest in clinical pharmacology studies for purposes of PK analyses and interpretation of results, and was characterized in all clinical pharmacology studies and used for exposure-response analysis. GS-566500 was assessed in some of the early Phase I studies.

Liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) with positive ionization was utilized to determine the concentration of SOF and its metabolites in plasma, urine and dialysate. Calibration curves for SOF ranged from 5 (LLOQ) to 5000 ng/mL. Calibration curves for GS-566500 and GS-331007 ranged from 10 (LLOQ) to 5000 ng/mL. Standards, quality control solutions, blank matrix, and study samples (as applicable) were prepared according to the validated methods. All samples were analyzed within the time frame supported by long-term storage stability data. The standard curve and QC data indicated that the plasma and urine assay methods for SOF, GS-566500, and GS-331007 were precise and accurate. Details of the analytical methods for each study were reviewed in the individual study reviews.

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

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

3. DETAILED LABELING RECOMMENDATIONS

At the time of this review, labeling negotiations were still underway and labeling recommendations have not been agreed upon with the applicant.

4. APPENDICES

4.1 Individual Study Review

4.2.1 Biopharmaceutic

4.2.1.1 GS-US-334-0131: A Phase 1, Open-label, Pharmacokinetic Drug-Drug Interaction Study Between GS-7977 and Antiretrovirals Efavirenz/Emtricitabine/Tenofovir Disoproxil Fumarate (EFV/FTC/TDF), a Boosted Protease Inhibitor, Darunavir/Ritonavir (DRV/r), an Integrase Inhibitor, Raltegravir (RAL), and Non-Nucleoside Reverse Transcriptase Inhibitor, Rilpivirine (RPV) (BE portion: SOF Form II vs. Form I)

Objectives: To evaluate the relative bioavailability of sofosbuvir Form II vs. Form I.

Note: In this review, the PK in Cohort 5 was compared to PK in Cohort 1 and 3, because study drugs were all administered under fasting conditions in these 3 cohorts. Refer to Section 4.2.4.1 for detailed reviews of Cohort 1 to 4.

Study Design: Subjects were enrolled in 1 of the following 5 cohorts:

Cohort 1: Subjects received a single dose (400 mg) of sofosbuvir (Day 1; Treatment A) followed by a 3-day washout period (Days 2–4), ATR (EFV 600 mg/FTC 200 mg/TDF 300 mg once daily) for 14 days (Days 5–18; Treatment B), and a single dose of sofosbuvir+ATR (Day 19; Treatment C).

Cohort 2: Subjects received a single dose (400 mg) of sofosbuvir (Day 1; Treatment D) followed by a 3-day washout period (Days 2–4), DRV/r (800 mg/100 mg once daily) for 10 days (Days 5–14; Treatment E), and a single dose of sofosbuvir+DRV/r (Day 15; Treatment F).

Cohort 3: Subjects received a single dose (400 mg) of sofosbuvir (Day 1; Treatment G) followed by a 3-day washout period (Days 2–4), RAL (400 mg twice daily) for 10 days (Days 5–14; Treatment H), and a single dose of sofosbuvir+RAL (Day 15; Treatment I).

Cohort 4: Subjects received a single dose (400 mg) of sofosbuvir (Day 1; Treatment J) followed by a 3-day washout period (Days 2–4), RPV (25 mg once daily) for 10 days (Days 5–14; Treatment K), and a single dose of sofosbuvir+RPV (Day 15; Treatment L).

Cohort 5: Subjects received a single dose (400 mg) of Form II sofosbuvir (Day 1; Treatment M) followed by a 4-day washout period (Days 2–5).

Formulation: Sofosbuvir was administered orally as 400 mg (1 x 400 mg tablets, (b) (4) for Cohort 1 and 3, (b) (4) for Cohort 5.). The lot numbers of sofosbuvir and (b) (4) sofosbuvir evaluated as test product were 11J111-P1 and DC1203 B1, respectively. (u) (4) is the to-be-marketed formulation.

PK Sampling: Serial blood samples were collected relative to dosing at the following time points in Cohort 5: 0 (predose) and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 4.5, 5, 6, 8, 10, 12, 24, 36, 48, 72, and 96 hours postdose on Day 1.

Analytical Methods: Refer to Section 4.2.4.1.

Pharmacokinetic Results:

Sofosbuvir: Figure 1 shows mean (SD) plasma concentration-time profiles for sofosbuvir (Cohorts 1 and 3) and (b) (4) sofosbuvir (Cohort 5) following single doses administered under

fasting conditions. The plasma concentration-time profiles for sofosbuvir and (b) (4) sofosbuvir were similar.

(b) (4)

(b) (4)

(b) (4)

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Conclusion: Sofosbuvir and (b) (4) sofosbuvir resulted in similar plasma exposures of sofosbuvir metabolites GS-566500 and GS-331007, but not sofosbuvir.

4.4.1.2 P7977-1318: A Single-Dose, Randomized, 3-Period, Crossover Study to Evaluate the Bioequivalence of a (b) (4) PSI-7977 Tablet formulation to a 400 mg PSI-7977 Tablet Formulation and the Effect of Food on the Bioavailability of the 400 mg Tablet

Objectives:

- To compare the rate and extent of absorption of sofosbuvir (GS-7977; formerly PSI-7977) (b) (4) tablets to sofosbuvir 400-mg tablets as reflected in GS-331007 (formerly PSI-6206) exposure when administered as a single 400-mg dose following an overnight fast in healthy subjects
- To estimate the effect of a high-fat meal upon the PK of sofosbuvir and metabolites following single-dose administration of sofosbuvir 400-mg tablet to healthy subjects

Study Design: This is a Phase 1, single-dose, randomized, 3-way crossover study. Forty subjects were randomized to receive a single dose of each of the following treatments, with a washout period of at least 7 days between each dose:

- Sofosbuvir 400 mg administered as (b) (4) tablets in the fasted state (Treatment A)
- Sofosbuvir 400 mg administered as 1 × 400-mg tablets in the fasted state (Treatment B)
- Sofosbuvir 400 mg administered as 1 × 400-mg tablet with a high-fat meal (Treatment C)

Formulation: Sofosbuvir (b) (4) 400-mg tablets and (b) (4) tablets. The lot numbers of sofosbuvir were 11D050-P1 and 11D034-P1, respectively.

PK Sampling: Serial blood samples were collected relative to dosing at the following time points on Day 1 of each treatment period: (predose) and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 16, 24, 48, and 72hours postdose.

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

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

Table 1 Summary of Quality Control (QC) Results –Study P7977-1910

Analyte	Linear range (ng/mL)	Between Run Precision (%CV)	Between Run Bias (% Deviation)	QC samples (ng/mL)	Validation sample for stability and conditions
SOF	5 – 5000 R ² > 0.993	≤ 8.5	-2.2 to 2.4	5, 15, 30, 500 and 4000	Stable for 99 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
GS-566500	10 – 5000 R ² > 0.992	≤ 9.6	-1.1 to 1.9	10, 30, 500 and 4000	Stable for 125 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
GS-331007	10 – 5000 R ² > 0.994	≤ 8.4	-1.3 to 1.2	10, 30, 500 and 4000	Stable for 184 days at -70°C and ≥ 5 freeze/thaw cycles in plasma

Pharmacokinetic Results:

Sofosbuvir: Figure 1 shows a semi-logarithmic plot of the mean (SD) sofosbuvir plasma concentration-time profiles after oral administration of single doses of sofosbuvir (b) (4) tablets fasted or sofosbuvir 1 × 400-mg tablet fasted or with a high-fat meal. In the fasted condition, mean sofosbuvir plasma concentrations-time profiles in the sofosbuvir 1 × 400-mg tablet and sofosbuvir (b) (4) tablets were comparable. Peak concentrations were achieved within 0.5 to 1.5 hours following dosing. Concentrations of sofosbuvir declined in a monoexponential manner and were generally not detectable beyond 6 hours postdose, regardless of treatment. Administration of sofosbuvir with a high-fat meal resulted in plasma concentrations that were lower and peak achieved later in comparison to those observed in the fasted state.

Figure 1. P7977-1318: Mean (SD) Plasma Concentration-Time Profile of Sofosbuvir (b) (4) Tablet) or Sofosbuvir (1 × 400-mg Tablet) Tablet Formulations

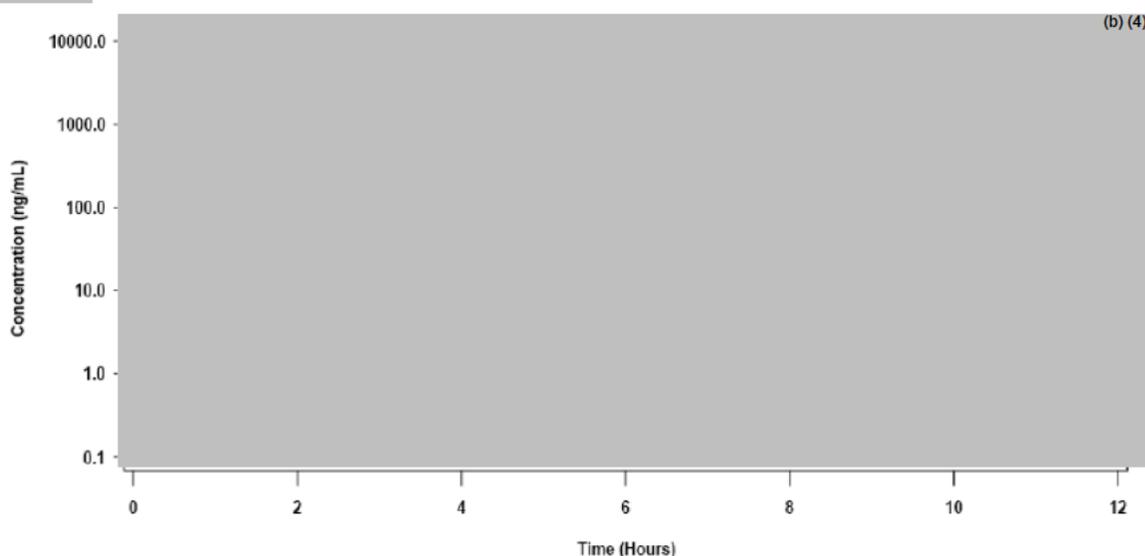


Table 2 presents single-dose PK parameters of sofosbuvir after oral administration of sofosbuvir (b) (4) tablets fasted and sofosbuvir 1 × 400-mg tablet fasted or with a high-fat meal. Sofosbuvir exhibits low and transient systemic exposure. Upon administration with a high-fat

meal, high variability was observed in sofosbuvir PK. Tmax was prolonged (1.50 hours versus 0.50 hours) and increases in other exposure parameters were also observed.

Table 2. P7977-1318: Sofosbuvir Pharmacokinetic Parameters Following Administration of Sofosbuvir ((b) (4) Tablet) or Sofosbuvir (1 × 400-mg Tablet) Tablet Formulations

SOF PK Parameter	(b) (4)	Mean (%CV)	
		SOF 400 mg (1 × 400-mg tablet) Fasted (N = 39) ^b	SOF 400 mg (1 × 400-mg tablet) High-fat meal (N = 38) ^{b, c}
AUC _{0-t} (ng·h/mL)		630.33 (41.51)	1110.79 (56.54)
AUC _{0-inf} (ng·h/mL)		646.08 (40.23) ^d	1232.38 (49.06) ^e
AUC _{exp} (%)		1.27 (66.46) ^d	5.40 (159.50) ^e
C _{max} (ng/mL)		675.03 (46.85)	764.01 (95.22)
C _{last} (ng/mL)		12.05 (62.88)	27.33 (91.21)
T _{max} (hour) ^d		0.50 (0.50, 1.00)	1.50 (1.00, 2.00)
t _{1/2} (hour) ^a		0.44 (0.39, 0.47) ^d	0.79 (0.48, 1.66) ^e

a Median (Q1, Q3)

b Subject 020 was not eligible for the PK analysis set and was excluded from the PK summary statistics.

c Subject 034 did not receive SOF 400 mg (1 × 400-mg tablet) with high-fat meal and was excluded from the PK summary statistics.

d Subject 028, AUC_{0-inf}, t_{1/2}, and associated PK parameters could not be estimated (N = 38).

e Subjects 011, 023, 036, and 038, AUC_{0-inf}, t_{1/2}, and associated PK parameters could not be estimated (N = 34).

(b) (4)

(b) (4)

Table 4 presents statistical comparisons of the primary sofosbuvir PK parameters following administration of single doses of sofosbuvir 1 × 400-mg tablets fasted and with a high-fat meal. A high-fat meal increased AUC_{inf} by ~80%. However, the effect of a high-fat meal on sofosbuvir PK was not considered clinically significant, because sofosbuvir is rapidly converted to metabolites with a short half-life.

Table 4: Summary of Statistical Analysis of Food Effect for Sofosbuvir

GS-7977 PK Parameter	Geometric LS Means		Ratio (%)	90% Confidence Intervals
	Treatment C	Treatment B		
C _{max} (ng/mL)	501.94	595.41	84.3	(67.2, 105.7)
AUC (0-t) (ng*hr/mL)	954.45	587.98	162.3	(143.0, 184.3)
AUC (0-inf) (ng*hr/mL)	1075.81	598.75	179.7	(160.1, 201.6)

(b) (4)

Table 5 presents single-dose PK parameters of GS-566500 after oral administration of sofosbuvir (b) (4) fasted and sofosbuvir (1 × 400-mg tablet) fasted or with a high-fat meal. Similar to the parent prodrug, GS-566500 PK parameters were comparable between the sofosbuvir (b) (4) and sofosbuvir (1 ×400-mg tablet) tablet formulations under fasted conditions

The effect of food on the systemic exposure of sofosbuvir was reflected in the PK profiles of GS-566500. GS-566500 T_{max} was prolonged (high-fat meal [3.00 hours] versus fasted [1.50 hours]) and increases in other exposure parameters were noted.

Table 5. P7977-1318: GS-566500 Pharmacokinetic Parameters Following Administration of Sofosbuvir (b) (4) or Sofosbuvir (1 × 400-mg Tablet) Tablet Formulations

GS-566500 PK Parameter	Mean (%CV)	
	SOF 400 mg (1 × 400-mg tablet) Fasted (N = 39) ^b	SOF 400 mg (1 × 400-mg tablet) High-fat meal (N = 38) ^{b, c}
AUC _{0-t} (ng·h/mL)	888.16 (31.53)	1404.32 (30.35)
AUC _{0-inf} (ng·h/mL)	943.38 (29.32)	1478.67 (28.91) ^d
AUC _{exp} (%)	6.46 (50.14)	4.31 (41.39) ^d
C _{max} (ng/mL)	245.52 (34.67)	293.61 (43.64)
C _{last} (ng/mL)	17.29 (28.67)	17.15 (36.36)
T _{max} (hour) ^a	1.50 (1.00, 1.50)	3.00 (2.00, 4.00)
t _½ (hour) ^a	2.20 (2.00, 2.42)	2.36 (2.20, 2.64) ^d

a Median (Q1, Q3)

b Subject 020 was not eligible for the PK analysis set and was excluded from the PK summary statistics.

c Subject 034 did not receive SOF 400 mg (1 × 400-mg tablet) with high-fat meal and was excluded from the PK summary statistics.

d Subject 011, AUC_{0-inf}, t_½, and associated PK parameters could not be estimated (N = 37).

(b) (4)

Table 7 presents statistical comparisons of the primary GS-566500 PK parameters following administration of single doses of sofosbuvir 1 × 400-mg tablets fasted and with a high-fat meal. A high-fat meal increased AUC_{inf} by 57%. However, the effect of a high-fat meal on GS-566500 PK was not considered clinically significant.

Table 7: Summary of Statistical Analysis of Food Effect for GS-566500

GS-566500 PK Parameter	Geometric LS Means			90% Confidence Intervals
	Treatment C	Treatment B	Ratio (%)	
C _{max} (ng/mL)	264.94	232.43	114.0	(100.7, 129.0)
AUC (0-t) (ng·hr/mL)	1335.40	849.52	157.2	(142.7, 173.1)
AUC (0-inf) (ng·hr/mL)	1424.06	908.02	156.8	(143.7, 171.2)

Table 8 presents single-dose PK parameters of GS-331007 after oral administration of sofosbuvir (b) (4) fasted and sofosbuvir (1 × 400-mg tablet) fasted or with a high-fat meal. Under fasted conditions, GS-331007 PK parameters were comparable between the sofosbuvir (b) (4) and sofosbuvir (1 × 400-mg tablet) tablet formulations. A slightly prolonged Tmax (4.00 hours versus 3.00 hours) and a lower Cmax (high-fat meal [909.42 ng/mL] versus fasted [1233.57 ng/mL]) of GS-331007 were observed with the high-fat meal.

Table 8. P7977-1318: GS-331007 Pharmacokinetic Parameters Following Administration of Sofosbuvir (b) (4) or Sofosbuvir (1 × 400-mg Tablet) Tablet Formulations

GS-331007 PK Parameter	(b) (4)	Mean (%CV)	
		SOF 400 mg (1 × 400-mg tablet) Fasted (N = 39) ^b	SOF 400 mg (1 × 400-mg tablet) High-fat meal (N = 38) ^{b, c}
AUC _{0-t} (ng·h/mL)	(b) (4)	12,374.01 (28.09)	12,185.20 (24.15)
AUC _{0-inf} (ng·h/mL)	(b) (4)	13,487.83 (28.41)	13,789.00 (23.59)
AUC _{exp} (%)	(b) (4)	7.94 (55.76)	11.37 (16.20)
C _{max} (ng/mL)	(b) (4)	1233.57 (32.05)	909.42 (23.48)
C _{last} (ng/mL)	(b) (4)	27.38 (43.67)	38.29 (38.23)
T _{max} (hour) ^a	(b) (4)	3.00(2.00, 4.00)	4.00(4.00, 6.00)
t _{1/2} (hour) ^a	(b) (4)	24.14 (20.36, 30.49)	26.89 (22.41, 31.29)

a Median (Q1, Q3)

b Subject 020 was not eligible for the PK analysis set and was excluded from the PK summary statistics.

c Subject 034 did not receive SOF 400 mg (1 × 400-mg tablet) with high-fat meal and was excluded from the PK summary statistics.



Conclusion:

- As assessed by GS-566500 and GS-331007, the new sofosbuvir (1 × 400-mg tablet) tablet formulation was bioequivalent to the existing sofosbuvir (b) (4) tablet formulation under fasted conditions. Although bioequivalence for the parent drug, sofosbuvir, was not fully met with the 2 formulations, the differences in exposure would not be anticipated to have a clinically significant effect.
- A high-fat meal reduced GS-331007 C_{max} by 24%, but had no effect on GS-331007 AUC. In contrast, a high-fat meal increased sofosbuvir and GS-56500 AUC_{inf} by 80% and 56%, respectively. The food effect was not considered clinically significant because it was administered without regard to food in all Phase III trials.

4.2.1.3 P7977-0111: A Single Dose, Randomized, 3-Period, Crossover Study to Evaluate the Relative Bioavailability of a PSI-7851 (b) (4) Formulation to a PSI-7977 Tablet Formulation and Food Effect**Objectives:**

- To compare the rate and extent of absorption of GS-9851 (formerly PSI-7851) 100-mg (b) (4) to sofosbuvir (GS-7977; formerly PSI-7977) 100-mg tablets when administered as a single 200-mg dose following an overnight fast in healthy subjects
- To estimate the effect of a high-fat meal upon the PK of sofosbuvir and metabolites following a single 200-mg dose administration of sofosbuvir 100-mg tablets to healthy subjects

Study Design: This was a Phase 1, single-dose, randomized, 3-way crossover study. Twenty-four healthy subjects were enrolled in the study. Each subject received a single dose of each of the following treatments, with a washout period of at least 7 days between each dose:

Treatment A: GS-9851 200 mg administered as 2 × 100 mg under fasting conditions

Treatment B: sofosbuvir 200 mg administered as 2 × 100 mg tablet under fasting conditions

Treatment C: sofosbuvir 200 mg administered as 2 × 100 mg tablet with a high-fat meal

Formulation:

GS-9851: 100-mg (b) (4) with lot # 9J075-P1

Sofosbuvir: 100-mg tablets with lot # 9J074-P2

PK Sampling: Serial blood samples for GS-9851, sofosbuvir, GS-566500, and GS-331007 plasma concentrations (if applicable) were collected at predose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 48, and 72 hours postdose in each treatment period. Urine samples for GS-9851, sofosbuvir, GS-566500, and GS-331007 urine concentrations (if applicable) were collected at predose baseline (empty bladder before dosing and retain sample) and 0- to 6-, 6- to 12-, 12- to 24-, 24- to 48- and 48- to 72-hour collection intervals.

Analytical Methods: Concentrations of GS-9851, sofosbuvir, GS-566500, and GS-331007 in plasma and urine samples were determined using fully validated high-performance liquid chromatography-tandem mass spectroscopy bioanalytical methods. All samples were analyzed in the timeframe supported by frozen stability storage data. The assays for GS-9851, sofosbuvir, GS-566500, and GS-331007 were all performed and validated by (b) (4)

The standard curve and QC data indicated that the plasma assay method for GS-9851, SOF, GS-566500, and GS-331007 were precise and accurate as shown in the following table.

Table1 Summary of Quality Control (QC) Results

Analyte	Linear range (ng/mL)	Between Run Precision (%CV)	Between Run Bias (% Deviation)	QC samples (ng/mL)	Validation sample for stability and conditions
GS-9851 (plasma)	5 – 5000 R ² > 0.991	≤ 8.8	-4.4 to 9.0	15, 30, 500 and 4000	Stable for 184 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
SOF (plasma)	5 – 5000 R ² > 0.993	≤ 10.4	-5.0 to 6.3	15, 30, 500 and 4000	Stable for 99 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
GS-566500 (plasma)	10 – 5000 R ² > 0.993	≤ 7.0 for GS-9851 ≤7.7 for SOF	3.3 to 7.7 for GS-9851 0.4 to 6.4 for SOF	30, 500 and 4000	Stable for 125 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
GS-331007 (plasma)	10 – 5000 R ² > 0.993	≤ 5.7 for GS-9851 ≤9.3 for SOF	3.8 to 5.0 for GS-9851 0.6 to 6.2 for SOF	30, 500 and 4000	Stable for 184 days at -70°C and ≥ 5 freeze/thaw cycles in plasma

GS-9851 (urine)	10 – 10000 R ² > 0.989	≤ 8.5	1.0 to 10.8	30, 800 and 8000	Stable for 125 days at -70°C and ≥ 5 freeze/thaw cycles in urine
SOF (urine)	10 – 10000 R ² = 0.994	≤ 4.6	-6.5 to 1.9	30, 800 and 8000	Stable for 178 days at -70°C and ≥ 5 freeze/thaw cycles in urine
GS-566500 (urine)	10 – 10000 R ² > 0.993	≤ 6.3 for GS-9851 ≤ 10.5 for SOF	1.4 to 4.2 for GS-9851 1.0 to 1.8 for SOF	30, 800 and 8000	Stable for 133 days at -70°C and ≥ 5 freeze/thaw cycles in urine
GS-331007 (urine)	10 – 10000 R ² > 0.993	≤ 10.7 for GS-9851 ≤ 7.1 for SOF	0.2 to 4.3 for GS-9851 1.8 to 5.4 for SOF	30, 500 and 4000	Stable for 133 days at -70°C and ≥ 5 freeze/thaw cycles in urine

Pharmacokinetic Results:

GS-331007

Figure 1 shows a semi-logarithmic plot of the mean (SD) GS-331007 plasma concentration-time profiles following administration of GS-9851 (b) (4) fasted, sofosbuvir (tablet) fasted, and sofosbuvir (tablet) with a high-fat meal.

Under fasted conditions, mean GS-331007 plasma concentrations were higher and occurred earlier following administration of sofosbuvir compared with GS-9851 administration. When sofosbuvir was administered with a high-fat meal, GS-331007 plasma concentrations were lower and occurred later compared with those concentrations observed under fasting conditions, but comparable to those concentrations achieved following administration of GS-9851 under fasting conditions. Concentrations of GS-331007 generally decreased in a biexponential manner and were detectable in most subjects through the last sampling point (72 hours) in each treatment.

Figure 1. P7977-0111: Mean (SD) GS-331007 Plasma Concentration-Time Profile by Treatment

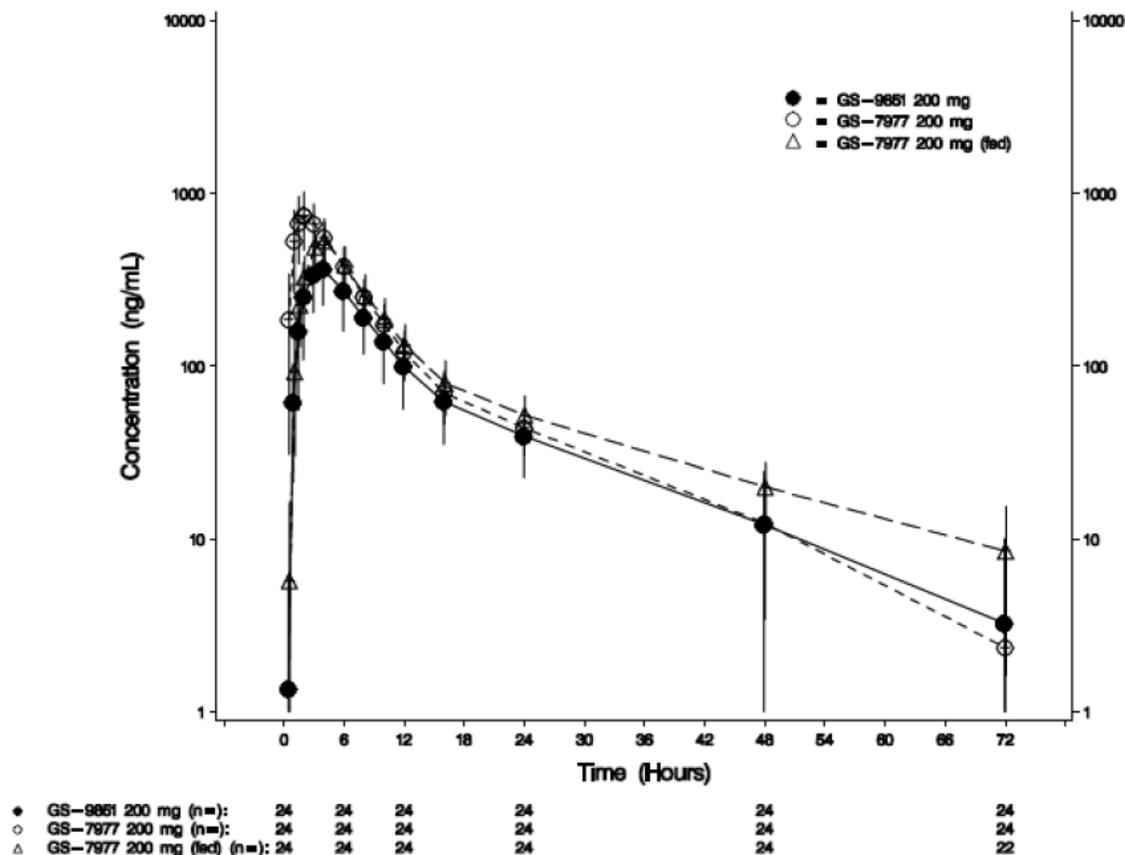


Table 2 presents the single-dose PK parameters of GS-331007 following administration of GS-9851 (b) (4) fasted, sofosbuvir (tablet) fasted, and sofosbuvir (tablet) with a high-fat meal. The GS-331007 C_{max} concentrations were achieved more rapidly for the sofosbuvir tablet formulation compared with the GS-9851 (b) (4) formulation under fasting conditions (median T_{max} of 2.0 vs. 4.0 h). Administration of the sofosbuvir tablet formulation under fasting conditions resulted in a 2.0-fold higher GS-331007 C_{max} and 1.4-fold higher AUC_{inf} compared with the GS-9851 (b) (4) formulation. GS-331007 elimination appeared to be unchanged between administration of the GS-9851 (b) (4) or sofosbuvir tablet formulations, with similar t_{1/2} and CL_r values. These data suggest an increase in rate and extent of absorption (increased bioavailability) with the sofosbuvir tablet formulation compared with GS-9851 (b) (4) formulation under fasting conditions. In support of this observation, a larger fraction of the dose was excreted in urine as GS-331007 after administration of sofosbuvir (54.3%) compared with administration of GS-9851 (36.7%). These data are consistent with the observations for the prodrug supporting increased bioavailability from the sofosbuvir tablet formulation compared with the GS-9851 (b) (4) formulation.

GS-331007 T_{max} was delayed when sofosbuvir tablet formulation was administered with a high-fat meal compared with fasting conditions (median T_{max} of 4.0 vs. 2.0 h). Mean GS-331007 C_{max} and AUC_{inf} values were approximately 30% and 6% lower, respectively, after administration of sofosbuvir tablet formulation with a high-fat meal compared with fasting conditions. Mean GS-331007 t_{1/2} values were approximately 50% longer after administration of sofosbuvir tablet formulation with a high-fat meal compared with fasting conditions; however,

CLr was similar between both sofosbuvir treatments. The fraction of the dose recovered in the urine as GS-331007 was unaffected following administration of sofosbuvir tablet formulation under fasting versus fed conditions.

Table 2. P7977-0111: GS-331007 Pharmacokinetic Parameters Following Administration of a Single Dose of GS-9851 (b) (4) Fasted, Sofosbuvir (Tablet) Fasted, or Sofosbuvir (Tablet) with a High-Fat Meal

GS-331007 PK Parameter	Mean (%CV)		
	GS-9851 200 mg (b) (4) (N = 24)	SOF 200 mg (Tablet) (N = 24)	SOF 200 mg (Tablet) With a High-fat Meal (N = 24)
AUC _{0-last} (h·ng/mL)	3710.12 (33.9)	5870.11 (26.3)	5393.41 (22.8)
AUC _{inf} (h·ng/mL)	4325.71 (31.9)	6261.32 (25.3)	5865.35 (21.8)
AUC _{exp} (%)	14.29 (61.1)	6.47 (39.4)	8.21 (34.5)
C _{max} (ng/mL)	387.72 (38.3)	780.46 (32.8)	541.31 (26.1)
T _{max} (h) ^a	4.0 (3.0, 4.0)	2.0 (2.0, 3.0)	4.0 (3.0, 4.0)
t _{1/2} (h) ^a	14.77 (9.34, 24.40)	14.62 (10.99, 17.15)	21.15 (15.88, 25.55)
CL _{renal} (L/h)	9.3 (28.1)	9.1 (21.1)	9.6 (25.9)
% Recovery in Urine	36.7 (34.5)	54.3 (16.4)	51.6 (15.4)

a median (Q1, Q3)

Table 3 presents statistical comparisons of GS-331007 primary PK parameters (AUC_{inf}, AUC_{0-last}, and C_{max}) following administration of GS-9851 (b) (4) fasted, sofosbuvir (tablet) fasted, and sofosbuvir (tablet) with a high-fat meal. The geometric least squares mean (GLSM) ratios and associated 90% CI were not within the equivalence bounds of 80% to 125% indicating an increase in GS-331007 exposure following administration of sofosbuvir tablet formulation compared with GS-9851 (b) (4) formulation under fasting conditions.

A high-fat meal slowed the rate of absorption of sofosbuvir, as evidenced as lower GS-331007 C_{max} value (30%) following administration of sofosbuvir tablet formulation with a high-fat meal compared with fasting conditions, but did not alter the overall exposure assessed as AUC_{inf} and AUC_{0-last}. The GLSM ratio and associated 90% CI for C_{max} of GS-331007 were outside the bounds of 80% to 125%; therefore, the PK equivalence criterion for C_{max} was not met. However, as the decrease in C_{max} was modest and the AUC parameters met the more strict criteria for bioequivalence (GLSM ratio and 90% CI were within the bounds of 80%-125%), the effect of a high-fat meal on GS-331007 PK was not considered clinically relevant.

Table 3. P7977-0111: Statistical Evaluations of GS-331007 Following Administration of a Single Dose of GS-9851 ^{(b) (4)} Fasted, Sofosbuvir (Tablet) Fasted, or Sofosbuvir (Tablet) With a High-Fat Meal

GS-331007 PK Parameter	%GLSM Ratio SOF (Tablet)/ GS-9851 ^{(b) (4)}	90% CI	%GLSM Ratio SOF (Tablet) With a High-fat Meal/ SOF (Tablet) Fasted	90% CI
C _{max}	207.38	(185.05, 232.40)	70.40	(62.8, 78.90)
AUC _{0-last} (h·ng/mL)	162.25	(148.13, 177.72)	92.69	(84.62, 101.53)
AUC _{inf} (h·ng/mL)	147.88	(136.50, 160.21)	94.46	(87.19, 102.33)

GS-9851/Sofosbuvir:

Figure 2 shows the semi-logarithmic plot of the mean (SD) GS-9851/sofosbuvir plasma concentration-time profiles following administration of GS-9851 ^{(b) (4)} fasted, sofosbuvir (tablet) fasted, and sofosbuvir (tablet) with a high-fat meal. Under fasting conditions, the mean GS-9851/sofosbuvir plasma concentrations were higher and were achieved earlier following administration of the sofosbuvir tablet formulation compared with the GS-9851 ^{(b) (4)} formulation. Dosing of sofosbuvir (tablet) with a high-fat meal resulted in sofosbuvir plasma concentrations that were slightly lower and increased at a slower rate compared with those concentrations observed under fasting conditions. Concentrations of GS-9851/sofosbuvir decreased rapidly in a monoexponential manner and generally were not detectable in most subjects after 6 hours postdose.

Figure 2. P7977-0111: Mean (SD) GS-9851/7977 Plasma Concentration-Time Profile by Treatment

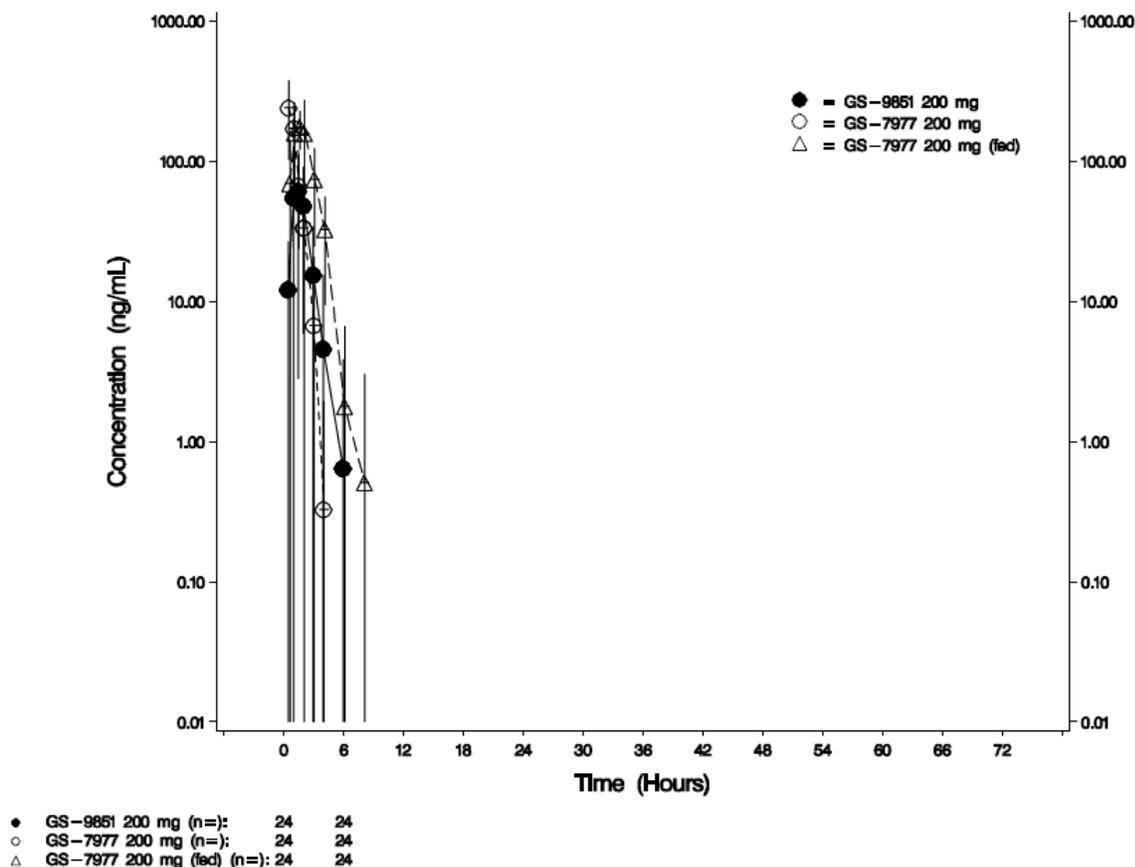


Table 4 presents the single-dose PK parameters of GS-9851/sofosbuvir following administration of GS-9851 (b) (4) fasted, sofosbuvir (tablet) fasted, and sofosbuvir (tablet) with a high-fat meal. A single dose of sofosbuvir tablet formulation was absorbed more rapidly compared with a single dose of the GS-9851 (b) (4) formulation under fasting conditions (median Tmax of 0.5 vs. 1.3 h). A single dose of sofosbuvir tablet formulation also resulted in a 3.3-fold higher Cmax and 2.0-fold higher AUCinf and approximately 6-fold lower Vz/F compared with the GS-9851 (b) (4) formulation under fasting conditions. Renal clearance (CLr) was comparable for both treatments under fasting conditions. The fraction of the dose excreted in urine as GS-9851/sofosbuvir was low for both treatments, with an approximately 3-fold higher excretion following administration of sofosbuvir tablet formulation (1.3%) compared with the GS-9851 (b) (4) formulation (0.4%). These data support increased rate and extent of absorption of sofosbuvir tablet formulation compared with GS-9851 (b) (4) formulation. In addition, intersubject variability in Cmax, AUCinf, and AUC0-last was substantially reduced with sofosbuvir tablet formulation compared with the GS-9851 (b) (4) formulation.

Sofosbuvir exhibited low and transient systemic exposure. Tmax was prolonged (1.5 vs. 0.5 h) and modest increases in other exposure parameters were observed following administration of the sofosbuvir tablet formulation with a high-fat meal compared with fasting conditions.

Table 4 P7977-0111: GS-9851/Sofosbuvir Pharmacokinetic Parameters Following Administration of a Single Dose of GS-9851 (b) (4) Fasted, Sofosbuvir (Tablet) Fasted, or Sofosbuvir (Tablet) With a High-Fat Meal

GS-9851/SOF PK Parameter	Mean (%CV)		
	GS-9851 200 mg (b) (4) (N = 24)	SOF 200 mg (Tablet) (N = 24)	SOF 200 mg (Tablet) With a High-fat Meal (N = 24)
AUC _{0-last} (h·ng/mL)	111.07 (89.8)	252.30 (41.9)	411.02 (41.4)
AUC _{inf} (h·ng/mL)	133.72 (79.0) ^a	262.92 (41.3)	440.19 (38.6)
C _{max} (ng/mL)	81.0 (92.1)	267.18 (47.7)	228.07 (49.5)
T _{max} (h) ^b	1.3 (1.0, 1.5)	0.5 (0.5, 1.0)	1.5 (1.0, 1.5)
t _{1/2} (h) ^b	0.70 (0.53, 1.16) ^a	0.37 (0.31, 0.45)	0.77 (0.61, 0.92)
CL _{renal} (L/h)	7.3 (36.8) ^a	10.1 (21.8)	10.7 (30.5)
% Recovery Urine	0.4 (76.0)	1.3 (40.4)	2.2 (29.4)
V _{z/F} (L)	3149.62 (108.1) ^a	501.60 (45.7)	632.61 (50.5)
CL/F (L/h)	2533.52 (84.1) ^a	904.26 (44.0)	509.34 (33.7)

a n = 23

b median (Q1, Q3)

GS-566500

Figure 3 shows a semi-logarithmic plot of the mean (SD) GS-566500 plasma concentration-time profiles following administration of GS-9851 (b) (4) fasted, sofosbuvir (tablet) fasted, and sofosbuvir (tablet) with a high-fat meal. The concentration-time profile of GS-566500 was similar to the parent prodrug, sofosbuvir, under the fasting and fed conditions. Under fasting conditions, the mean GS-566500 plasma concentrations were higher and were achieved earlier following administration of sofosbuvir tablet formulation compared with the GS-9851 (b) (4) formulation. A single dose of sofosbuvir with a high-fat meal resulted in GS-566500 plasma concentrations that were comparable and increased at a slower rate than those observed following a single dose of sofosbuvir under fasting conditions. Concentrations of GS-566500 decreased rapidly in a monoexponential manner and generally were not detectable in most subjects after 12 hours postdose.

Figure 3. P7977-0111: Mean GS-566500 Plasma Concentration-Time Profile by Treatment

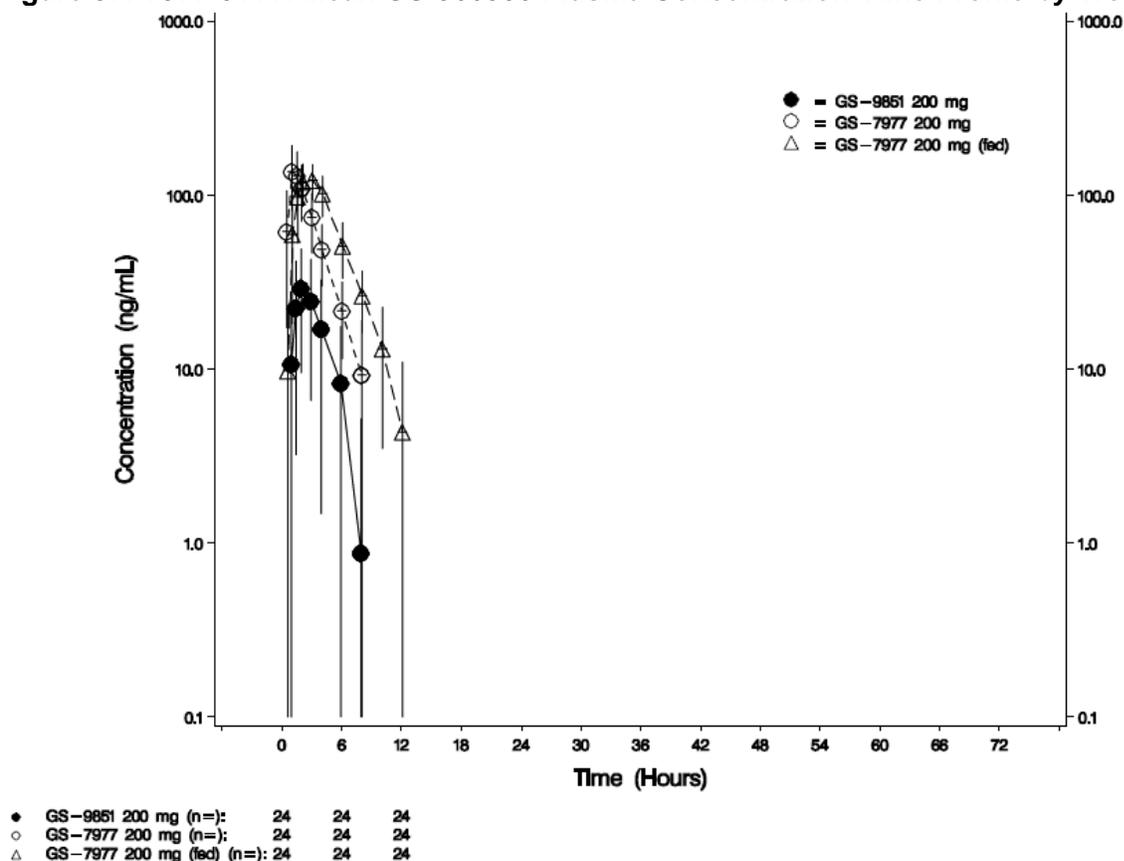


Table 5 presents the single-dose GS-566500 PK parameter following administration of GS-9851 (b) (4) fasted, sofosbuvir (tablet) fasted, and sofosbuvir (tablet) with a high-fat meal. Similar to the parent prodrug, GS-566500 was absorbed more rapidly and to a greater extent (median Tmax of 1.0 vs. 2.0 h) and resulted in a 4.3-fold higher Cmax and 2.5-fold higher mean AUCinf when administered as the sofosbuvir tablet formulation compared with GS-9851 (b) (4) formulation. The renal clearance (CLr) was comparable for both treatments. The fraction of the dose excreted in urine as GS-566500 was low, with an approximately 3-fold higher excretion following administration of sofosbuvir tablet formulation (2.1%) compared with GS-9851 (b) (4) formulation (0.7%). In addition, intersubject variability in Cmax and AUC of GS-566500 was substantially decreased following administration of the sofosbuvir tablet formulation compared with the GS-9851 (b) (4) formulation.

The effect of food on the systemic exposure of sofosbuvir was reflected in the PK profiles of its metabolite. GS-566500 Tmax was prolonged (2.0 vs. 1.0 h) and modest increases in other exposure parameters were observed following administration of the sofosbuvir tablet formulation with a high-fat meal compared with fasting conditions.

Table 5. P7977-0111: GS-566500 Pharmacokinetic Parameters Following Administration of a Single Dose of GS-9851 (b) (4) Fasted, Sofosbuvir (Tablet) Fasted, or Sofosbuvir (Tablet) With a High-Fat Meal

GS-566500 PK Parameter	Mean (%CV)		
	GS-9851 200 mg (b) (4) (N = 24)	SOF 200 mg (Tablet) (N = 24)	SOF 200 mg (Tablet) With a High-fat Meal (N = 24)
	n = 23	n = 24	n = 24
AUC _{0-last} (h·ng/mL)	97.93 (77.6)	437.66 (35.6)	615.67 (23.4)
C _{max} (ng/mL)	33.72 (54.1)	146.22 (37.2)	135.24 (22.0)
T _{max} (h) ^a	2.0 (1.5, 3.0)	1.0 (1.0, 1.5)	2.0 (1.8, 3.0)
	n = 19	n = 24	n = 24
AUC _{inf} (h·ng/mL)	193.26 (50.0)	480.29 (32.3)	658.93 (22.2)
AUC _{exp} (%)	39.2 (60.9)	9.90 (56.2)	6.88 (34.8)
t _{1/2} (h)	2.30 (1.83, 4.25)	1.80 (1.63, 1.90)	2.14 (1.98, 2.31)
CL _{renal} (L/h)	6.7 (38.5)	6.8 (24.4)	8.3 (31.0)
% Recovery in Urine	0.7 (56.4) ^b	2.1 (37.9)	3.4 (24.5)

a median (Q1, Q3)

b n = 24

Discussion: GS-9851 is a 1:1 mixture of sofosbuvir and GS-491241. In vitro studies indicated that GS-491241 is mainly converted to GS-566500 by carboxylesterase 1 (CES1), while sofosbuvir is mainly converted to GS-566500 by cathepsin A (CatA). As shown in the table below, the GLSM ratio of sofosbuvir exposure parameters (SOF tablets:GS-9851 (b) (4)) were similar for sofosbuvir and GS-56650, indicating the degree of conversion of sofosbuvir to GS-56650 is similar irrespective of the formulation administered (SOF tablets or GS-9851 (b) (4)).

Study P7977-0111	PK Parameter	GLSM		GLSM ratio
		GS-9851 (1x 200 mg (b) (4))	SOF (2 x 100 mg tablets)	SOF/GS-9851
SOF or GS-9851	AUCinf (ng.h/mL)	103.64	241.59	2.33
	Cmax(ng/mL)	58.68	241.57	4.12
GS-566500	AUCinf (ng.h/mL)	174.13	453.82	2.61
	Cmax (ng/mL)	28.81	136.01	4.72
GS-331007	AUCinf (ng.h/mL)	4099.53	6062.32	1.48
	Cmax (ng/mL)	358.23	742.89	2.07

The food effect on SOF and its metabolites at SOF dose of 200 mg were similar to observe at SOF dose of 400 mg in Study P7977-1318.

Conclusion:

- GS-9851 (b) (4) and sofosbuvir tablet formulations did not meet the prespecified bioequivalence criteria with respect to sofosbuvir, GS-566500, and GS-331007 exposures.
- Sofosbuvir tablet formulation administered with a high-fat meal produced approximately 30% lower GS-331007 C_{max} values compared with fasted administration.

4.2.2 General Pharmacokinetics/Pharmacodynamics

4.2.2.1 P7977-0312: An Open Label, Non-Randomized, Single Dose, Mass Balance Study to Investigate the Pharmacokinetics, Excretion and Recovery of [14C]PSI-7977 Administered as a Single Oral Dose to Healthy Adult Subjects

Objectives: To explore the routes and rates of elimination of [14C]-sofosbuvir

Study Design: This is a Phase 1, open-label, nonrandomized, single-dose, mass balance study. Seven eligible healthy subjects were enrolled into a single cohort and received a single oral dose of sofosbuvir 400 mg containing a mixture of [12C]-sofosbuvir and [14C]-sofosbuvir.

Formulation: Sofosbuvir 400-mg (b) (4) containing a mixture of [12C]-sofosbuvir powder and [14C]-sofosbuvir solution
[12C]-sofosbuvir powder lot number: 40409002
[14C]-sofosbuvir solution lot number: 20100414

PK Sampling: Whole blood, plasma, urine, expired air, and feces were collected for the assessment of plasma PK and radioanalysis. Emesis was collected if a subject spontaneously vomited.

Whole blood (for radiocarbon concentration) and plasma (for radiocarbon, sofosbuvir, GS-566500, and GS-331007 concentrations) were collected predose, and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 48, 72, 96, 120, 144, and 168 hours postdose and each subsequent 24-hour interval for a maximum of 312 hours.

Expired air (for radiocarbon concentration) was collected predose, and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, and 48 hours postdose.

Urine (for radiocarbon, sofosbuvir, GS-566500, and GS-331007 concentrations) was collected 24 hours before dosing, and thereafter over the following intervals: 0 to 6, 6 to 12, 12 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, 144 to 168 hours postdose and each subsequent 24 hour interval for up to 312 hours.

Feces (for radiocarbon concentration) were collected predose (if available), and thereafter over the following intervals 0 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, 144 to 168 hours and each subsequent 24 hour interval for up to 312 hours.

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

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

Table 1 Summary of Quality Control (QC) Results –Study P7977-1910

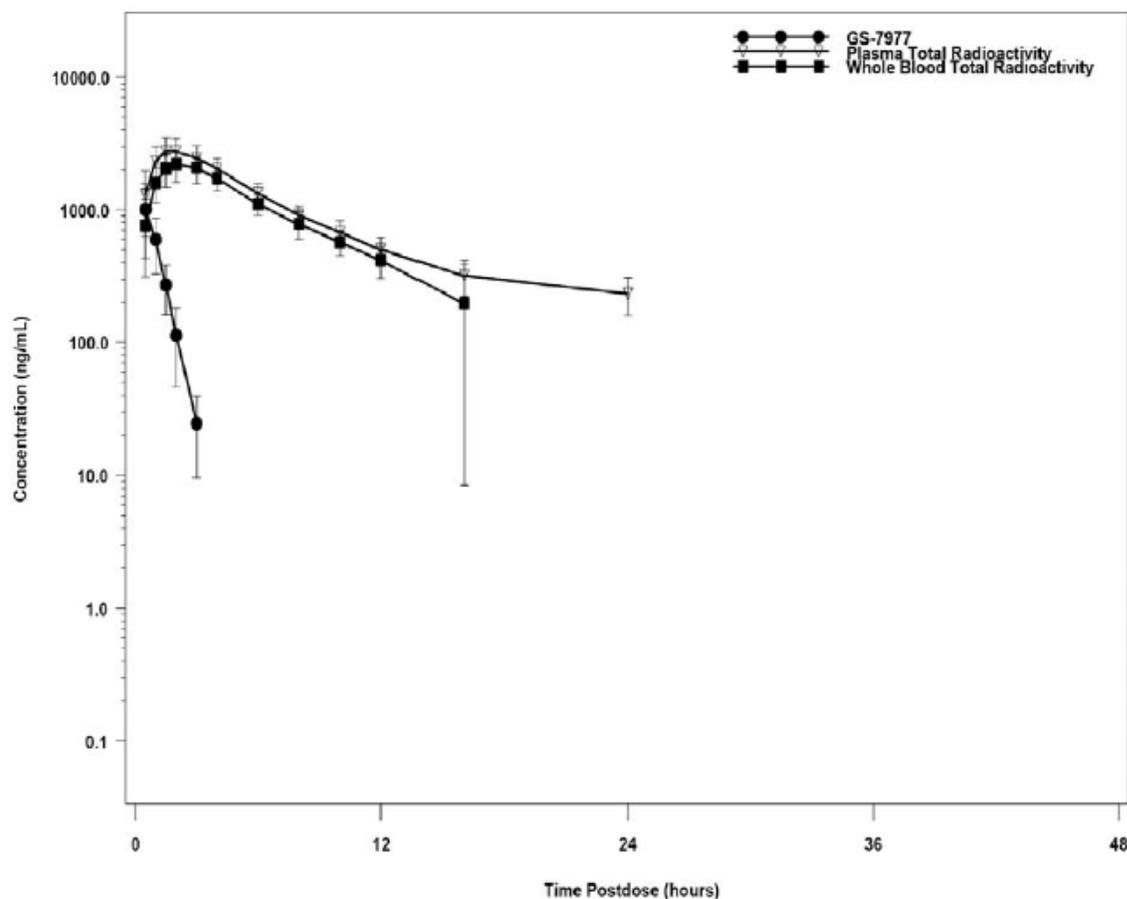
Analyte	Linear range (ng/mL)	Between Run Precision (%CV)	Between Run Bias (% Deviation)	QC samples (ng/mL)	Validation sample for stability and conditions
SOF (plasma)	5 – 5000 R ² > 0.990	≤ 7.3	2.2 to 9.5	15, 30, 500 and 4000	Stable for 99 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
GS-566500 (plasma)	10 – 5000 R ² > 0.993	≤ 4.6	-4.8 to -0.1	30, 500 and 4000	Stable for 125 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
GS-331007 (plasma)	10 – 5000 R ² > 0.991	≤ 7.7	-1.4 to 0.7	30, 500 and 4000	Stable for 184 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
SOF (urine)	10 – 10000 R ² = 0.993	≤ 9.1	2.0 to 9.3	10, 30, 800 and 8000	Stable for 178 days at -70°C and ≥ 6 freeze/thaw cycles in urine
GS-566500 (urine)	10 – 5000 R ² > 0.993	≤ 10.9	0.9 to 5.0	10, 30, 800 and 8000	Stable for 133 days at -70°C and ≥ 5 freeze/thaw cycles in urine
GS-331007 (urine)	10 – 5000 R ² > 0.996	≤ 8.3	1.2 to 11.7	30, 500 and 4000	Stable for 133 days at -70°C and ≥ 5 freeze/thaw cycles in urine

Total radiocarbon in all samples of plasma and urine were determined directly by liquid scintillation counting (LSC). Whole blood and fecal samples were combusted, with the resulting CO₂ trapped before LSC. Liquid scintillation counting for ¹⁴C radioactivity was performed on the CO₂ in each subject's expired air. This analysis was not within the scope of the Food and Drug Administration Good Laboratory Practice Regulations (GLPs), 21 CFR 58, but was conducted in accordance with generally recognized good laboratory practices as defined by the GLP and Good Clinical Practice (GCP) regulations.

Pharmacokinetic Results:

PK profiles: The mean whole blood and plasma concentration-time profiles for ¹⁴C radioactivity using LSC in comparison with sofosbuvir plasma concentration-time profiles using LC/MS/MS are presented in Figure 1. When assessed as ¹⁴C radioactivity (LSC measurements), the mean whole blood and plasma concentration-time profiles reached a maximum concentration at approximately 2 hours. In comparison, the plasma concentration-time profile of sofosbuvir measured by LC/MS/MS rapidly reached a maximum concentration after approximately 0.5 hours and exhibited a very short half-life as compared to total ¹⁴C radioactivity.

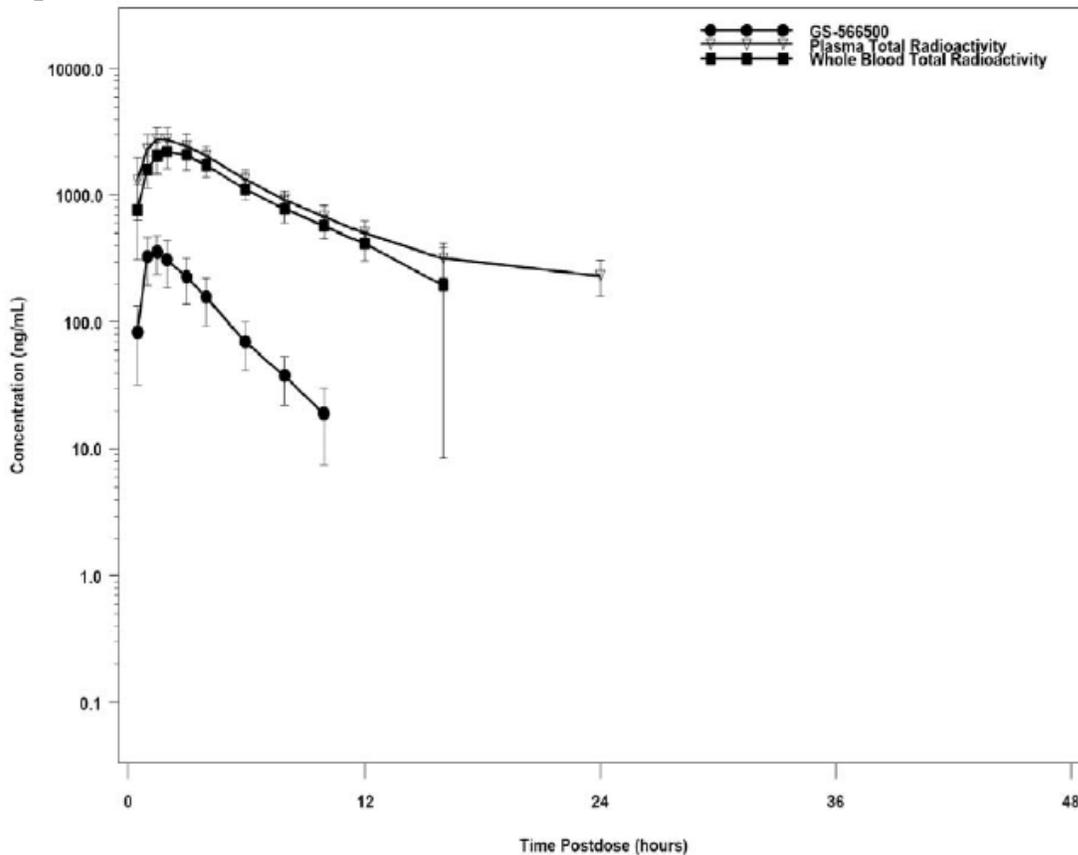
Figure 1. P7977-0312: Mean (SD) Whole Blood and Plasma Concentrations of Total ¹⁴C Radioactivity versus Time Determined Using LSC and Sofosbuvir Plasma Concentrations Using LC/MS/MS



Note: Plasma or whole blood concentration below the lower limit of quantitation were treated as 0 for summary statistics and as missing for log-normalized data. Plasma concentrations where there was no sample or the sample was not available were treated as missing for summary statistics. For GS-7977, y-axis units are ng/mL and for total radioactivity y-axis units are ng eq/mL.

The mean whole blood and plasma concentration-time profiles for ¹⁴C radioactivity using LSC in comparison with GS-566500 plasma concentration-time profiles using LC/MS/MS are presented in Figure 2. The plasma concentration of GS-566500 reached a maximum after 1 hour and exhibited a short median half-life as compared to total ¹⁴C radioactivity.

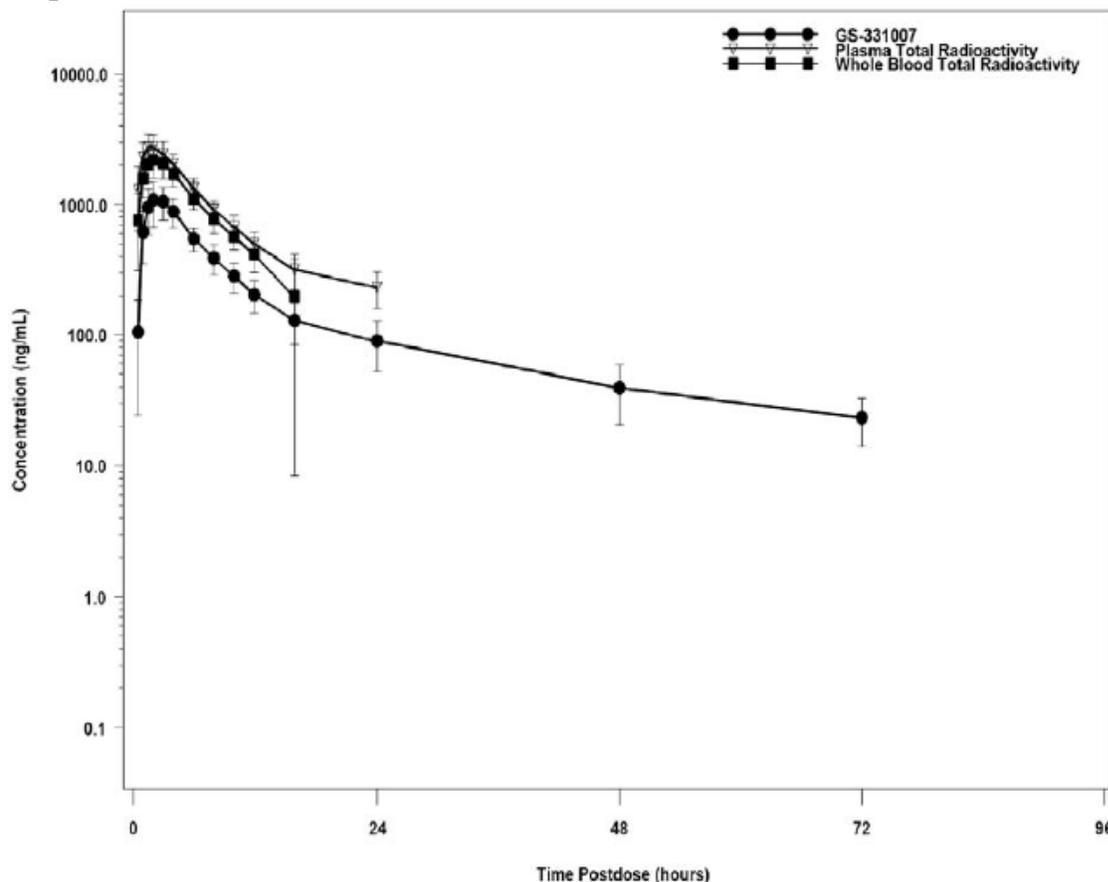
Figure 2. P7977-0312: Mean (SD) Whole Blood and Plasma Concentrations of Total ¹⁴C Radioactivity versus Time Determined Using LSC and GS-566500 Plasma Concentrations Using LC/MS/MS



Note: Plasma concentration below the lower limit of quantitation were treated as 0 for summary statistics and as missing for log-normalized data. Plasma concentrations where there was no sample or the sample was not available were treated as missing for summary statistics. For GS-566500, y-axis units are ng/mL and for total radioactivity y-axis units are ng eq/g.

The mean whole blood and plasma concentration-time profiles for ¹⁴C radioactivity using LSC in comparison with GS-331007 plasma concentration-time profiles using LC/MS/MS are presented in Figure 3. The plasma concentration-time profile of GS-331007 reached a maximum concentration after 2 hours (comparable with total radioactivity), exhibited comparable decline in concentration to the total circulating radioactivity profiles over the timeframe in which total radioactivity was detectable, and showed a longer half-life terminal phase as compared to sofosbuvir and GS-56500. GS-331007 accounted for a substantial proportion of total circulating radioactivity.

Figure 3. P7977-0312: Mean (SD) Whole Blood and Plasma Concentrations of Total ¹⁴C Radioactivity versus Time Determined Using LSC and GS-331007 Plasma Concentrations Using LC/MS/MS



Note: Plasma concentration below the lower limit of quantitation were treated as 0 for summary statistics and as missing for log-normalized data. Plasma concentrations where there was no sample or the sample was not available were treated as missing for summary statistics. For GS-331007, y-axis units are ng/mL and for total radioactivity y-axis units are ng eq/g.

Whole Blood and Plasma PK parameters: Table 10-1 summarizes the whole blood and plasma PK parameters for total ¹⁴C radioactivity measured using LSC. Using LSC, mean peak levels of ¹⁴C radioactivity were observed 2.0 hours postdose, with an observed median half-life of 4.65 and 8.57 hours for whole blood and plasma, respectively. The difference in half-life between whole blood and plasma was a probably result of whole blood samples falling below the limit of quantitation earlier than plasma samples. The mean ratio of radioactivity AUC_{0-inf} in whole blood to that in plasma was approximately 0.708 indicating that total radioactivity was not highly associated with red blood cells.

Table 2. P7977-0312: Pharmacokinetic Parameters for Total ¹⁴C Radioactivity in Whole Blood and Plasma Using LSC

PK Parameter	Total ¹⁴ C radioactivity Mean (%CV)	
	Whole Blood	Plasma
C _{max} (ng eq/g)	2266 (24.6)	2883 (24.2)
T _{max} (h) ^a	2.00 (2.00, 3.00)	2.00 (1.00, 3.00)
AUC _{0-t} (ng eq·h/g)	15,413 (23.5)	21,673 (23.4)
AUC _{0-inf} (ng eq·h/g)	17,684 (24.8)	24,979 (27.6)
AUC _{exp} (%)	13.2 (26.7)	12.0 (35.5)
t _{1/2} (h) ^a	4.65 (3.60, 5.99)	8.57 (8.10, 9.93)

a Median (Q1, Q3)

Table 3 summarizes the plasma PK parameters for sofosbuvir, GS-566500, and GS-331007 measured using LC/MS/MS.

Table 3. P7977-0312: Pharmacokinetic Parameters for Sofosbuvir, GS-566500, and GS-331007 in Plasma Using LC/MS/MS

PK Parameter	LC/MS/MS Analysis Mean (%CV)		
	SOF	GS-566500	GS-331007
C _{max} (ng/mL)	1102 (39.3)	379 (31.2)	1167 (34.2)
T _{max} (h) ^a	0.50 (0.50, 0.50)	1.00 (1.00, 1.50)	2.00 (2.00, 3.00)
AUC _{0-t} (ng·h/mL)	990 (32.7)	1315 (37.5)	10,716 (22.8)
AUC _{0-inf} (ng·h/mL)	999 (32.5)	1364 (36.6)	11,225 (21.5)
AUC _{exp} (%)	0.985 (38.4)	3.91 (35.3)	4.70 (24.5)
AUC _{0-inf} (ng eq. SOF·h/mL)	—	1755 (36.6)	22,838 (21.5)
t _{1/2} (h) ^a	0.426 (0.36, 0.48)	2.21 (1.87, 2.39)	26.6 (21.1, 28.7)
CL/F (L/hr)	439 (32.7)	—	—
V _z /F (L)	288 (48.1)	—	—

a Median (Q1, Q3)

Based on mean molecular weight-adjusted plasma AUC_{0-inf} of GS-566500 and GS-331007 (1755 and 22,838 ng eq sofosbuvir h/mL, respectively), 7.0% and 91% of the mean circulating plasma total radioactivity (24,979 ng eq·h/g) were accounted for by GS-566500 and GS-331007, respectively. These results indicate GS-331007 is the major circulating metabolite of sofosbuvir.

Urine: The mean cumulative urinary recovery of total ¹⁴C radioactivity determined by LSC is presented in Figure 10-4. The mean (SD) cumulative urinary recovery of the administered radioactive dose was 76.1% (8.61%). As expected, renal excretion of ¹⁴C radioactivity was a major pathway for elimination of the dose.

Figure 4. P7977-0312: Mean (SD) Cumulative Urinary Recovery of Total ¹⁴C Radioactivity (Total Cumulative % Radioactive Dose) Versus Time Using LSC

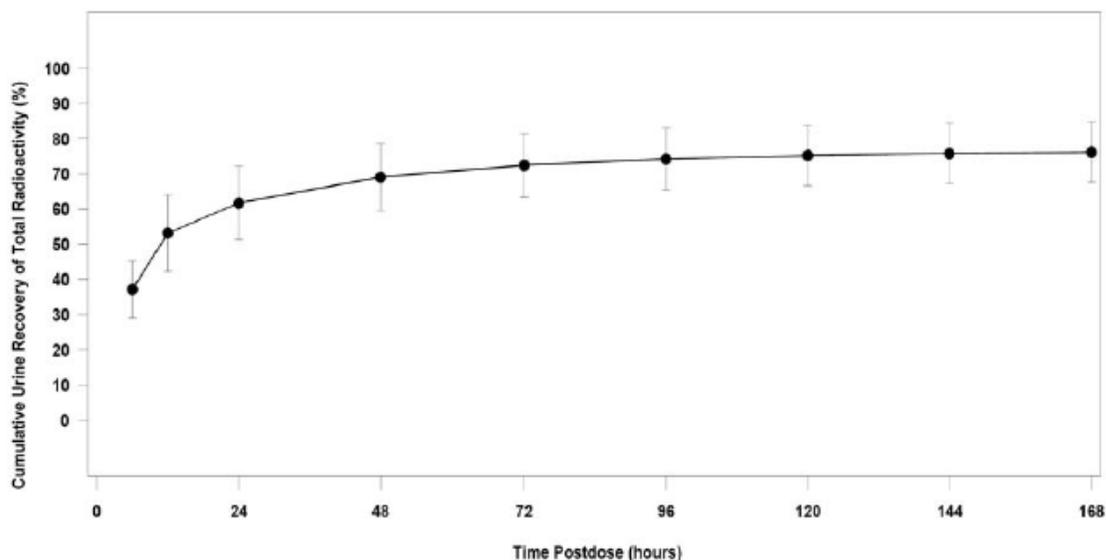


Table 4 summarizes urine PK parameters for sofosbuvir, GS-566500, and GS-331007 measured using LC/MS/MS. The majority of the sofosbuvir dose (determined by LC/MS/MS analysis) was recovered in the urine as GS-331007 (77.7%) with sofosbuvir (3.47%) and GS-566500 (3.67%) contributing minimally to the overall urine recovery. The sponsor indicated that renal clearance is a minor pathway for elimination of sofosbuvir and GS-566500.

Reviewer's note: Although sofosbuvir and GS-566500 accounts for only <4% of dose excreted, the CL_r value for SOF is comparable to GS-331007. Because it is not clear what bioavailability is for SOF, it is hard to determine how much CL_r accounts for in the total clearance (CL_t). If SOF is substantially converted to metabolites during first pass, CL_r could count for a significant portion of CL_t. In renal impairment study, SOF and GS-566500 both increased with increased renal impairment, which implied that renal pathway is still an important pathway for elimination of sofosbuvir and GS-566500.

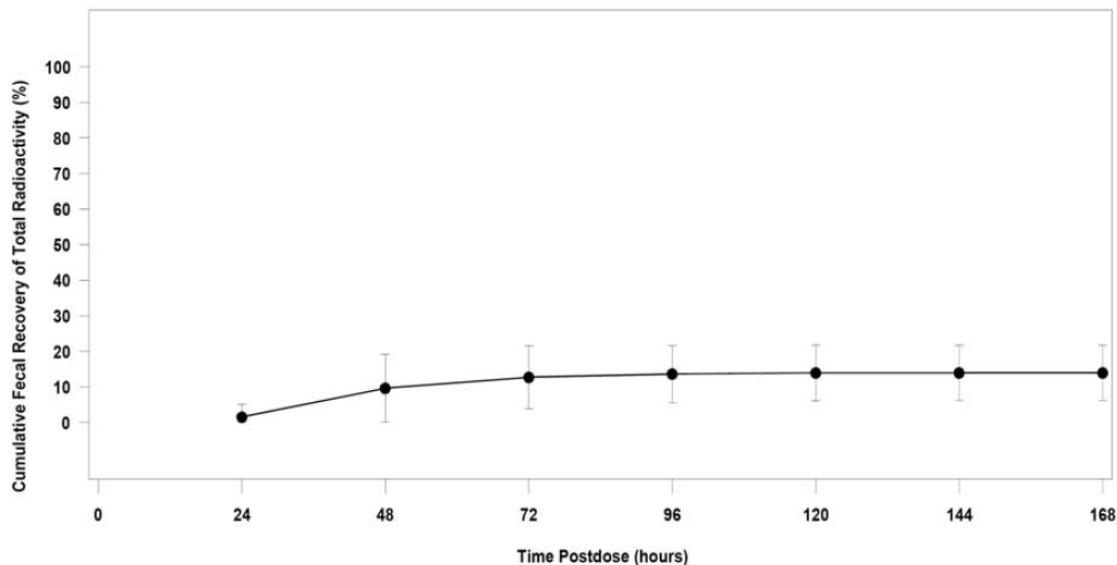
Table 4. P7977-0312: Pharmacokinetic Parameters for Sofosbuvir, GS-566500, and GS-331007 in Urine Using LC/MS/MS

PK Parameter	LC/MS/MS Analysis Mean (%CV)		
	SOF	GS-566500	GS-331007
Ae _{u(0-∞)} (mg)	13.9 (36.7)	11.4 (29.4)	153 (19.4)
%Dose _{excreted} (%)	3.47 (36.7)	3.67 (29.4)	77.7 (19.4)
CL _r (L/h)	14.3 (25.0)	8.95 (22.0)	14.5 (25.4)

Renal clearance for Sofosbuvir, GS-566500, and GS-331007 were higher than glomerular filtration rate (7.2 L/h); suggesting a role of active secretion in the renal elimination of Sofosbuvir, GS-566500, and GS-331007.

Fecal: The mean cumulative fecal recovery of total ^{14}C radioactivity determined by LSC is presented in Figure 10-5. Mean (SD) cumulative fecal recovery of the administered radioactive dose was 14.0% (7.77%), indicating that the radioactive dose was modestly eliminated in the feces.

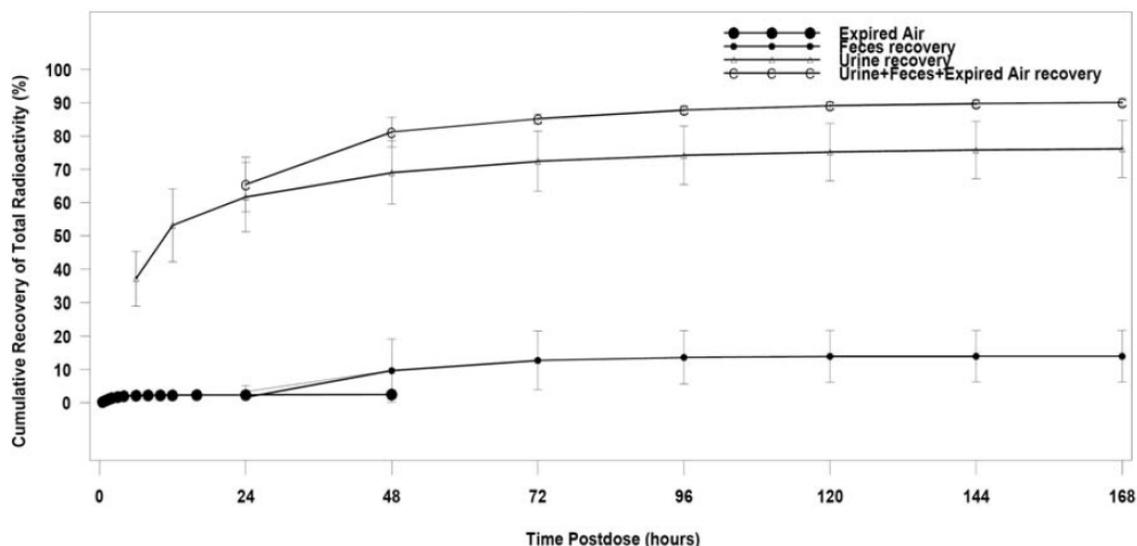
Figure 5. P7977-0312: Mean (SD) Cumulative Fecal Recovery of Total ^{14}C Radioactivity (Total Cumulative % Radioactive Dose) Versus Time



Total ^{14}C Radioactivity

Mean (SD) percent of total radioactive dose recovered in expired air through 48 hours postdose was 2.50% (0.39%). The mean cumulative urinary, fecal, and expired air recovery of total ^{14}C radioactivity is presented in Figure 10-6. The mean (SD) cumulative urinary, fecal, and expired air recovery of ^{14}C radioactivity was 92.6% (1.23%), accounting for nearly the entire administered radioactive dose.

Figure 6. P7977-0312: Mean (SD) Cumulative Recovery of Total Radioactivity (%) Versus Time for Urine, Feces and Expired Air



Conclusion:

- Sofosbuvir was rapidly absorbed and primarily eliminated in the urine as the nucleoside metabolite, GS-331007. The majority of circulating total radioactivity in plasma pertained to sofosbuvir metabolites, with GS-331007 as the major circulating species.
- Mean total recovery of the radioactive dose was 92.6%, consisting of 76.1, 14.0, and 2.50% recovered in urine, feces, and expired air, respectively.

4.2.2.2 P7977-0613: A Single Dose, Randomized, Blinded, Placebo and Positive Controlled, Four Period Cross Over Study to Investigate the Effect of PSI 7977 at a Projected Therapeutic and Supratherapeutic Dose on the QT/QTc Interval in Healthy Volunteers

This study was reviewed by Interdisciplinary Review Team for QT Studies Consultation, dated 11/26/2012. This review only summarizes the PK data from this study to aid the discussion of PK results from other studies.

Summary: This was a randomized, blinded, placebo and positive controlled, four period cross over study. Sixty (60) subjects received sofosbuvir 400 mg, sofosbuvir 1200 mg, placebo, and moxifloxacin 400 mg.

A summary of sofosbuvir, GS-566500 and GS-331007 PK following single dose administration of sofosbuvir 400 mg or sofosbuvir 1200 mg are presented in the table below.

Analyte	PK Parameter (units) ^a	Sofosbuvir 400 mg (N=59)	Sofosbuvir 1200 mg (N=59)
Sofosbuvir	AUC _{0-inf} (ng*hr/ml)	628.73 (44.85)	2482.31 (42.49)
	C _{max} (ng/mL)	621.76 (56.13)	2252.32 (47.47)
	T _{max} (hr)	0.50 (0.50, 1.00)	0.50 (0.50, 1.00)
	t _{1/2} (hr)	0.45 (0.39, 0.54)	0.49 (0.42, 0.57)
GS-566500	AUC _{0-inf} (ng*hr/ml)	855.60 (30.96)	3091.50 (34.26)
	C _{max} (ng/mL)	232.31 (30.91)	747.09 (35.34)
	T _{max} (hr)	1.50 (1.00, 1.50)	1.50 (1.00, 2.00)
	t _{1/2} (hr)	1.99 (1.87, 2.10)	2.12 (2.01, 2.26)
GS-331007	AUC _{0-inf} (ng*hr/ml)	11097.29 (22.61)	27634.59 (24.14)
	C _{max} (ng/mL)	1113.34 (28.04)	2098.93 (28.53)
	T _{max} (hr)	2.50 (2.00, 3.50)	3.00 (2.50, 3.50)
	t _{1/2} (hr)	25.07 (20.35, 29.14)	27.69 (23.52, 30.28)

a PK parameters are presented as mean (%CV [percentage coefficient of variation]) except for T_{max} and t_{1/2} which are presented as median (Q1, Q3 [first quartile; third quartile])

Approximately dose-proportional increases in sofosbuvir and metabolite exposures (AUC and C_{max}) were observed with an increase in sofosbuvir dose. The exposures achieved in this study are deemed adequate to cover the therapeutic exposure (400 mg) or supratherapeutic exposure (1200 mg) of sofosbuvir in the case of an overdose or a potential drug-drug interaction (i.e., drug interaction with cyclosporine). At these concentrations there is no detectable prolongation of the QT interval.

4.2.2.3 P2938-0212: A Two-Part, Double-Blind, Parallel, Randomized, Placebo-Controlled Study to Assess the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Multiple Ascending Doses of PSI-352938 and the Combination of PSI-352938 and PSI-7977 in Patients with Genotype 1 Chronic Hepatitis C Infection (only the PK of PSI-7977 (sofosbuvir) is reviewed here*)

(b) (4)

Objective(s) reviewed here: To evaluate the pharmacokinetics of sofosbuvir (SOF) following SOF 400 mg administered once daily for 7 days in patients with Genotype 1 Chronic Hepatitis C (CHC) infection

Study Design: In Part 2, first part of Cohort 3, SOF 400 mg administered once daily for 7 days (Days 1 to 7).

Formulation: SOF 200-mg tablets, lot# 0G069-P1

PK Sampling: Blood samples for SOF and its metabolites were collected predose and 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, and 16 hours postdose on Day 7. Urine samples were collected on Day 7

at predose (bladder was emptied before dosing and collected sample for baseline), 0 to 6 hour, 6 to 12 hour, and 12 to 24 hour collection intervals.

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

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

Table1 Summary of Quality Control (QC) Results

Analyte	Linear range (ng/mL)	Between Run Precision (%CV)	Between Run Bias (% Deviation)	QC samples (ng/mL)	Validation sample for stability and conditions
SOF (plasma)	5 – 5000 R ² > 0.994	≤ 6.7	2.3 to 8.4	15, 30, 500 and 4000	Stable for 99 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
GS-566500 (plasma)	10 – 5000 R ² > 0.994	≤ 9.2	-0.5 to 3.7	30, 500 and 4000	Stable for 125 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
GS-331007 (plasma)	10 – 5000 R ² > 0.994	≤ 6.1	-1.2 to 3.8	30, 500 and 4000	Stable for 184 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
SOF (urine)	10 – 10000 R ² = 0.993	≤ 5.7	-2.6 to 2.0	30, 800 and 8000	Stable for 178 days at -70°C and ≥ 6 freeze/thaw cycles in urine
GS-566500 (urine)	10 – 10000 R ² > 0.995	≤ 6.2	0.8 to 3.6	30, 800 and 8000	Stable for 133 days at -70°C and ≥ 6 freeze/thaw cycles in urine
GS-331007 (urine)	10 – 10000 R ² > 0.996	≤ 12.5	-1.5 to 5.1	30, 500 and 4000	Stable for 133 days at -70°C and ≥ 6 freeze/thaw cycles in urine

Pharmacokinetic Results: Table 2 presents SOF, GS-566500, and GS-331007 plasma and urine PK parameters upon multiple dose administration of sofosbuvir 400 mg once daily alone. The results show that the exposure for SOF and its metabolites after 7-day administration of 400 mg SOF in HCV-infected subjects are similar to that in healthy volunteers.

Table 2. P2938-0212: Mean (SD) SOF, GS-566500 and GS-331007 Plasma and Urine Pharmacokinetic Parameters Following Administration of Sofosbuvir Alone (Day 7)

PK Parameter Mean (%CV)	SOF (Cohort 3, Day 7, n=8))	GS-566500 (Cohort 3, Day 7, n=8))	GS-331007 (Cohort 3, Day 7, n=8))
AUC _{0-tau} (h·ng/mL)	538.12 (38.97)	853.13 (45.69)	9638.94 (18.72)
C _{max} (ng/mL)	602.59 (47.15)	235.16 (38.43)	1378.33 (19.16)
C _{tau} (ng/mL)	0.00 ^a	0.00 ^a	98.15 (23.44)
T _{max} (h) ^b	0.50 (0.50, 0.77)	1.50 (1.00, 1.75)	2.00 (1.75, 3.00)
CL/F (L/h)	871.64 (46.00)	-	-
%Excreted in Urine (%)	1.28 (27.44)	3.43 (97.84) ^c	72.60 (17.37) ^d
CL _r (L/min)	0.173 (32.15)	0.199 (54.15)	0.252 (22.37) ^e
t _{1/2} (h) ^b	0.48 (0.44, 0.52)	2.14 (1.99, 2.24) ^e	9.42 (8.84, 12.24)
V _z /F (L)	654.72 (52.52)	-	-

^a All C_{tau} measurements were BLQ

^b Median (Q1, Q3)

^c Corrected for molecular weight of GS-566500

^d Corrected for molecular weight of GS-331007

^e N = 7

Conclusion: The exposure to SOF and its metabolites after 7-day administration of 400 mg SOF in HCV-infected subjects are similar to that in healthy volunteers.

4.2.2.4 P2938-0221: A Multi-center, Double-Blind, Parallel Group, Randomized, Placebo-Controlled, Dose Ranging Study to Investigate the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics following Oral Administration of PSI-7977 in Combination with Standard of Care (Pegylated Interferon and Ribavirin) in Treatment-Naive Patients with Chronic HCV Infection Genotype 1*

* Only the PK of PSI-7977 (sofosbuvir) is reviewed here.

Objective(s) reviewed: To characterize the steady-state plasma pharmacokinetics (PK) of sofosbuvir and metabolites.

Study Design: This Phase 2a randomized, double-blind study primarily evaluated the safety of sofosbuvir in combination with PEG+RBV in treatment-naive genotype 1 HCV-infected subjects. Sixty-four subjects were randomized equally to parallel treatment groups to receive 1 of 3 sofosbuvir doses (100 mg, 200 mg, or 400 mg) or matching placebo, once daily based upon stratification for IL28B status (CC or CT/TT). Subjects received sofosbuvir or placebo on Days 0 to 27. Subjects also received treatment with PEG+RBV starting on Day 0 of the study which continued for 48 weeks. Subjects were then followed for 24 weeks after the end of dosing to assess sustained virologic response at 12 and 24 weeks (SVR12 and SVR24).

Formulation:

Sofosbuvir: 100-mg tablets (lot# 9J074-P1)

Pegylated interferon alfa-2a: marketed Pegasys®

Ribavirin: marketed Copegus®

PK Sampling: Blood samples were collected relative to the dosing of sofosbuvir or placebo at the following time points: Day 0 (predose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours postdose),

predose on Days 1, 3, 7, 14, and 21, Day 27 (predose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours post morning dose), and Day 28 (24 hours after study drug administration on Day 27). Urine samples were collected on Day 0 (predose, 0-6, 6-12, and 12-24 hour collection intervals), and Day 27 (0-6, 6-12, 12-24, and 24-48 hours [postdose] hour collection intervals).

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

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

Table 1 Summary of Quality Control (QC) Results

Analyte	Linear range (ng/mL)	Between Run Precision (%CV)	Between Run Bias (% Deviation)	QC samples (ng/mL)	Validation sample for stability and conditions
SOF (plasma)	5 – 5000 R ² ≥ 0.989	≤ 12.8	-1.8 to 5.9	15, 30, 500 and 4000	Stable for 99 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
GS-566500 (plasma)	10 – 5000 R ² > 0.992	≤ 10.8	-1.2 to 0.2	30, 500 and 4000	Stable for 125 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
GS-331007 (plasma)	10 – 5000 R ² ≥ 0.990	≤ 11.8	0.3 to 4.0	30, 500 and 4000	Stable for 184 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
SOF (urine)	10 – 10000 R ² = 0.991	≤ 13.6	-3.8 to 1.2	30, 800 and 8000	Stable for 71 days at -70°C and ≥ 6 freeze/thaw cycles in urine
GS-566500 (urine)	10 – 10000 R ² > 0.992	≤ 6.6	-2.5 to 7.8	30, 800 and 8000	Stable for 133 days at -70°C and ≥ 5 freeze/thaw cycles in urine
GS-331007 (urine)	10 – 10000 R ² > 0.992	≤ 14.0	0.5 to 5.8	30, 500 and 4000	Stable for 133 days at -70°C and ≥ 5 freeze/thaw cycles in urine

Pharmacokinetic Results:

Sofosbuvir: Table 2 presents the mean single dose (first dose: Day 0) and steady-state plasma PK parameters of sofosbuvir following oral administration of increasing doses of sofosbuvir. Sofosbuvir exhibited linear PK. The mean sofosbuvir exposure as measured by AUC (AUC_{0-last}, AUC_{inf} or AUC_{tau}) and C_{max} increased proportionally with increasing doses. Median time at which maximum plasma sofosbuvir concentrations were achieved (T_{max}) were similar (median [range]: 1 [0.5 – 3.0]) following single or multiple dosing and across the different doses. As expected, sofosbuvir exhibits a short plasma half-life; median plasma half-life ranged between 0.54 to 0.75 hours. Renal excretion is unaltered over increasing doses.

Table 2. P7977-0221: Summary of Sofosbuvir Pharmacokinetic Parameters with Increasing Doses of Sofosbuvir

sofosbuvir Plasma PK Parameters ^a	sofosbuvir 100 mg (N = 16)	sofosbuvir 200 mg (N = 17) ^b	sofosbuvir 400 mg (N = 15)
Day 0 (Single Dose)			
C _{max} (ng/mL)	218.08 (83.25)	332.26 (64.87)	1257.50 (58.56)
AUC ₀₋₂₄ (ng·h/mL)	280.20 (71.21)	585.48 (58.51)	1864.84 (51.35)
AUC _{0-last} (ng·h/mL)	268.18 (74.82)	562.44 (59.62)	1850.07 (51.67)
%AUC _{exp} (%)	5.68 (107.40)	3.83 (119.89)	1.03 (51.73)
AUC _{inf} (ng·h/mL)	280.19 (71.22)	585.56 (58.50)	1867.24 (51.38)
C _{last} (ng/mL)	11.68 (61.72)	15.27 (82.52)	16.43 (76.36)
T _{max} (h)	1.00 (0.5, 1.31)	1.00 (0.5, 1.58)	1.00 (0.5, 1.03)
t _{1/2} (h)	0.54 (0.40, 0.73)	0.73 (0.52, 1.08)	0.75 (0.46, 1.10)
T _{last} (h)	3.08 (3.00, 4.00)	4.25 (3.02, 6.00)	6.00 (4.00, 10.00)
CL/F (mL/h)	9.46 (82.06)	8.15 (69.49)	4.87 (64.07)
CL _r (L/min)	0.081 (46.8)	0.083 (43.5)	0.082 (40.1)
Day 27 (Steady State)			
C _{max} (ng/mL)	253.54 (67.49)	475.14 (75.53)	1355.65 (62.98)
AUC _{tau} (ng·h/mL)	375.70 (54.07)	732.27 (40.58)	2011.23 (49.14)
C _{tau} (ng/mL)	BLQ	0.56 (400)	BLQ
T _{max} (h)	0.53 (0.50, 1.01)	1.00 (0.63, 1.27)	1.00 (0.50, 1.50)
t _{1/2} (h)	0.63 (0.49, 0.98)	0.74 (0.47, 1.03)	0.68 (0.53, 1.00)
T _{last} (h)	3.99 (2.99, 4.00)	4.00 (3.05, 6.00)	8.00 (3.97, 8.17)
CL _{ss} /F (mL/h)	6.71 (78.66)	5.33 (42.60)	4.42 (64.71)
CL _r (L/min)	0.104 (36.0)	0.085 (43.2)	0.072 (47.8)

a All parameters are reported as Mean (%CV) except T_{max}, T_{1/2} and T_{last} which are reported as Median (Q1, Q3)

b Subject 1005-1043 was excluded from the PK analysis set due to an inadequate PK profile. Subject 1005-1085 was excluded from Day 27 summary statistics as this subject only received 15 days sofosbuvir+PEG+RBV before being lost to follow-up

BLQ = below the limit of quantitation

GS-566500: Table 3 presents the mean single dose (first dose: Day 0) and steady-state plasma PK parameters of GS-566500 following oral administration of increasing doses of sofosbuvir. Increases in GS-566500 parallels that of the parent prodrug sofosbuvir. GS-566500 exhibited linear PK; mean exposure (AUC_{0-last}, AUC_{inf}, AUC_{tau}, or C_{max}) increased proportionally over increasing doses of sofosbuvir. Median time at which maximum plasma sofosbuvir concentrations were achieved (T_{max}) were comparable (range: 0.5 to 4 hrs) following single or multiple sofosbuvir doses and across the different dose levels. A marginally longer plasma half-life of GS-566500 was observed as compared to the prodrug, sofosbuvir (median t_{1/2}: 1.86 to 2.25 hrs). Similar to sofosbuvir, renal clearance for GS-566500 was not substantially altered over the dose range. Clearance of GS-566500 is slightly decreased with increasing doses at steady-state.

Table 3. P7977-0221: Summary of GS-566500 Pharmacokinetic Parameters with Increasing Doses of Sofosbuvir

GS-566500 Plasma PK Parameters ^a	sofosbuvir 100 mg (N = 16)	sofosbuvir 200 mg (N = 17) ^b	sofosbuvir 400 mg (N = 15)
Day 0 (Single Dose)			
C _{max} (ng/mL)	69.99 (51.41)	145.51 (21.95)	293.35 (31.62)
AUC ₀₋₂₄ (ng·h/mL)	270.37 (45.05)	644.51 (35.32)	1312.82 (46.96)
AUC _{0-last} (ng·h/mL)	218.71 (54.03)	598.37 (36.79)	1239.14 (46.23)
%AUC _{exp} (%)	19.35 (47.45)	7.85 (36.44)	5.77 (34.38)
AUC _{inf} (ng·h/mL)	266.22 (45.98)	645.95 (35.31)	1315.94 (47.04)
C _{last} (ng/mL)	15.76 (43.66)	15.58 (26.17)	21.63 (63.62)
T _{max} (h)	1.74 (1.01, 2.07)	1.50 (1.00, 2.03)	1.50 (1.00, 3.00)
T _{1/2} (h)	1.86 (1.63, 2.20)	2.01 (1.94, 2.18)	2.25 (2.09, 2.68)
T _{last} (h)	6.00 (5.05, 6.03)	8.15 (8.00, 10.00)	12.00 (9.98, 12.00)
CL _r (L/min)	0.127 (46.1)	0.123 (31.1)	0.148 (33.1)
Day 27 (Steady State)			
C _{max} (ng/mL)	64.75 (59.48)	132.72 (37.17)	237.46 (21.76)
AUC _{tau} (ng·h/mL)	262.0 (46.05)	571.95 (27.38)	1072.91(30.49)
C _{tau} (ng/mL)	BLQ	BLQ	BLQ
T _{max} (h)	1.50 (1.02, 2.00)	2.00 (1.26, 2.02)	1.97 (1.05,3.00)
T _{1/2} (h)	1.92 (1.65, 2.33)	1.87 (1.65, 2.11)	2.26 (1.90, 2.37)
T _{last} (h)	6.01 (4.01, 7.03),	8.00 (8.00, 10.00)	12.00 (10.00, 12.00)
CL _r (L/min)	0.166 (34.8)	0.151 (34.0)	0.144 (46.2)

a All parameters are reported as Mean (%CV) except T_{max}, t_{1/2} and T_{last} which are reported as Median (Q1, Q3).

b Subject 1005-1043 was excluded from the PK analysis set due to an inadequate PK profile. Subject 1005-1085 was excluded from Day 27 summary statistics as this subject only received 15 days sofosbuvir+PEG+RBV before being lost to follow-up

BLQ = below the limit of quantitation

GS-331007: Table 4 presents the mean single dose (first dose: Day 0) and steady-state plasma PK parameters of GS-331007 following oral administration of increasing doses of sofosbuvir. GS-331007 exhibited linear PK; mean exposure (AUC_{0-last}, AUC_{inf}, AUC_{tau}, or C_{max}) increased proportionally over increasing doses of sofosbuvir. GS-331007 is the predominant species circulating in plasma, and as seen in previous studies, exhibits a longer T_{max} (median 3.03-4.00) and plasma half-life (median t_{1/2}: 7.37–13.0) compared to sofosbuvir or GS-566500.

GS-331007 is primarily excreted renally by glomerular filtration (Study P7977-0312). As presented in Table 10-3, GS-331007 is slightly increased with increased sofosbuvir dose and relatively higher at steady-state as compared to the matching single dose. Of note, CL_r of GS-331007 slightly exceeds glomerular filtration rate (GFR), suggesting a possible role of secretion in renal clearance of GS-331007.

Table 4. P7977-0221: Summary of GS-331007 Pharmacokinetic Parameters with Increasing Doses of Sofosbuvir

GS-331007 Plasma PK Parameters ^a	sofosbuvir 100 mg (N = 16)	sofosbuvir 200 mg (N = 17) ^b	sofosbuvir 400 mg (N = 15)
Day 0 (Single Dose)			
C _{max} (ng/mL)	197.32 (44.94)	357.33 (27.95)	662.13 (32.43)
AUC ₀₋₂₄ (ng·h/mL)	1766.67 (39.17)	3338.48 (22.89)	6492.71 (39.37)
AUC _{0-last} (ng·h/mL)	1757.60 (40.14)	3346.58 (22.55)	6507.70 (39.40)
%AUC _{exp} (%)	18.99 (69.73)	15.42 (61.81)	13.13 (56.67)
AUC _{inf} (ng·h/mL)	2173.18 (39.22)	3984.88 (22.48)	7559.91 (40.55)
C _{last} (ng/mL)	25.62 (60.77)	42.65 (36.79)	84.70 (58.86)
T _{max} (h)	3.50 (3.00, 4.00)	4.00 (3.92, 4.25)	4.00 (4.00, 4.25)
t _{1/2} (h)	9.18 (6.30, 12.81)	7.68 (6.51, 12.99)	7.37 (6.26, 10.06)
T _{last} (h)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
CL _r (L/min)	0.128 (35.8)	0.153 (27.7)	0.175 (27.9)
Day 27 (Steady State)			
C _{max} (ng/mL)	233.79 (44.92)	357.38 (30.75)	717.23 (29.09)
AUC _{tau} (ng·h/mL)	2256.81 (43.53)	3389.23 (24.55)	7398.99 (35.60)
C _{tau} (ng/mL)	33.05 (53.31)	57.02 (32.90)	116.02 (42.78)
T _{max} (h)	3.03 (3.00, 4.00)	4.00 (2.55, 6.00)	4.00 (1.58, 6.00)
t _{1/2} (h)	9.93 (8.19, 12.70)	12.23 (10.70, 15.67)	13.00 (7.17, 16.34)
T _{last} (h)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
CL _r (L/min)	0.173 (33.4)	0.192 (22.9)	0.211 (29.7)

a All parameters are reported as Mean (%CV) except T_{max}, T_{1/2} and T_{last} which are reported as Median (Q1, Q3)

b Subject 1005-1043 was excluded from the PK analysis set due to an inadequate PK profile. Subject 1005-1085 was excluded from Day 27 summary statistics as this subject only received 15 days sofosbuvir+PEG+RBV before being lost to follow-up

Accumulation Indices: Accumulation indices were calculated by comparing the AUC₀₋₂₄ value following single-dose administration of sofosbuvir with the AUC_{tau} value following dosing of sofosbuvir for 28 days of dosing. As shown in Table 5, higher intersubject variability was observed in the accumulation indices estimates for sofosbuvir compared to the other analytes. No significant accumulation with time was noted for sofosbuvir, GS-566500, and GS-331007; accumulation indices ratio (GMR) for all three analytes approached 1. These results are consistent with the half-life estimates for sofosbuvir, GS-566500, and GS-331007 relative to the sofosbuvir dosing interval.

Table 5. P7977-0221: Summary of Sofosbuvir Accumulation Analysis

Analyte	Treatment	Geometric LS Means				Accumulation Ratio (%)	90% Confidence Intervals
		N	Day 0 AUC ₂₄ (hr*ng/mL)	N	Day 27 AUC _{tau} (hr*ng/mL)		
GS-331007	100 mg SOF	16	1635.09	16	2109.47	129.0	(109.6, 151.8)
	200 mg SOF	17	3256.88	16	3251.35	99.8	(92.6, 107.7)
	400 mg SOF	15	5968.61	15	7074.50	118.5	(100.8, 139.4)
GS-566500	100 mg SOF	16	244.51	16	233.62	95.5	(79.1, 115.5)
	200 mg SOF	17	610.13	16	553.44	90.7	(79.7, 103.2)
	400 mg SOF	15	1180.32	15	1028.41	87.1	(75.4, 100.7)
sofosbuvir	100 mg SOF	16	225.20	16	313.95	139.4	(104.7, 185.7)
	200 mg SOF	17	495.57	16	671.65	135.5	(112.2, 163.7)
	400 mg SOF	15	1616.26	15	1766.71	109.3	(92.7, 128.9)

Nonlinearity in PK as a function of time was explored in the time invariance analyses (comparison of AUC_{inf} value following single-dose administration of sofosbuvir with the AUC_{tau} value following dosing of sofosbuvir for 28 days). As shown in [Table 6](#), sofosbuvir and metabolites exhibit time independent linear pharmacokinetic characteristics because GLSM ratio approached 1. In addition, clearance (oral or renal) of sofosbuvir and metabolites was not significantly changed over the study duration. ([Tables 2 to 4](#)).

Table 6. P7977-0221: Summary of Time Invariance Analysis

Analyte	Treatment	Geometric LS Means				Accumulation Ratio (%)	90% Confidence Intervals
		N	Day 0 AUC _{inf} (hr*ng/mL)	N	Day 27 AUC _{tau} (hr*ng/mL)		
GS-331007	100 mg SOF	16	2024.68	16	2109.47	104.2	(88.3, 122.9)
	200 mg SOF	17	3887.46	16	3247.59	83.5	(78.4, 89.0)
	400 mg SOF	15	6908.48	15	7074.50	102.4	(86.5, 121.2)
GS-566500	100 mg SOF	16	240.52	16	233.62	97.1	(80.7, 116.9)
	200 mg SOF	17	611.52	16	553.40	90.5	(79.5, 103.0)
	400 mg SOF	15	1182.73	15	1028.41	87.0	(75.2, 100.5)
sofosbuvir	100 mg SOF	16	225.18	16	313.95	139.4	(104.7, 185.7)
	200 mg SOF	17	495.64	16	671.65	135.5	(112.2, 163.6)
	400 mg SOF	15	1616.26	15	1766.71	109.2	(92.5, 128.8)

Dose Proportionality

Dose proportionality (across the range of sofosbuvir doses evaluated in this study) was examined using a power model and ANOVA analyses. Consistent results were observed by both methodologies; results from ANOVA testing are presented in [Table 7](#).

Following single (Day 0) or multiple dose (Day 27) administration of the prodrug, sofosbuvir; higher intersubject variability was observed in the exposure parameters (AUC_{inf} and AUC_{tau}) for sofosbuvir compared to the other analytes. Across the doses evaluated, the GLSM ratio and the 90% CI for sofosbuvir on Day 0 and Day 27 were greater than 1; suggesting more than dose proportional increases. However, result be interpreted with caution, given the significant variability in the estimate.

For metabolites GS-331007 and GS-566500, increasing doses of sofosbuvir resulted in dose proportional increases in their exposure (AUC_{inf} and AUC_{tau}). The GLSM ratio for GS-331007 and GS-566500 on Day 0 and Day 27 approached 1.

Table 7. P7977-0221: Summary of ANOVA Analysis of Dose Proportionality

Analyte	PK Day	PK Parameter	sofosbuvir Dose Level (mg)	N	Geometric LS Mean Dose Normalized to 100 mg	Dose Comparison	Ratio (%)	90% Confidence Intervals
sofosbuvir	0	AUC (0-inf) (ng*hr/mL)	100	16	225.2			
			200	17	247.8	200 mg vs 100 mg	110.1	(75.7, 159.9)
			400	15	404.6	400 mg vs 100 mg	179.7	(122.2, 264.2)
	27	AUC _{tau} (ng*hr/mL)	100	16	314.0			
			200	16	338.8	200 mg vs 100 mg	107.9	(77.5, 150.3)
			400	15	441.7	400 mg vs 100 mg	140.7	(100.4, 197.0)
GS-566500	0	AUC (0-inf) (ng*hr/mL)	100	16	240.5			
			200	17	305.8	200 mg vs 100 mg	127.1	(98.4, 164.2)
			400	15	295.7	400 mg vs 100 mg	122.9	(94.4, 160.1)
	27	AUC _{tau} (ng*hr/mL)	100	16	233.6			
			200	16	276.2	200 mg vs 100 mg	118.2	(94.3, 148.2)
			400	15	257.1	400 mg vs 100 mg	110.1	(87.5, 138.5)
GS-331007	0	AUC (0-inf) (ng*hr/mL)	100	16	2024.7			
			200	17	1943.7	200 mg vs 100 mg	96.0	(77.3, 119.2)
			400	15	1727.1	400 mg vs 100 mg	85.3	(68.2, 106.6)
	27	AUC _{tau} (ng*hr/mL)	100	16	2109.5			
			200	16	1644.3	200 mg vs 100 mg	77.9	(65.0, 93.5)
			400	15	1768.6	400 mg vs 100 mg	83.8	(69.7, 100.9)

Efficacy Summary:

During the sofosbuvir treatment period (Days 0 to 28), mean log₁₀ HCV RNA levels rapidly declined with all doses of sofosbuvir+PEG+RBV compared with placebo+PEG+RBV. At Day 3 and Day 7, there was a 1 log difference in reduction of HCV RNA levels between the 400 mg PEG+RBV (-3.643 and -4.435 respectively) and 100 mg PEG+RBV (-2.754 and -3.488 respectively) groups. By Day 27, near maximal suppression of HCV RNA levels had been achieved in the sofosbuvir 100 mg PEG+RBV, 200 mg PEG+RBV, and 400 mg+PEG+RBV groups. Near maximal suppression of HCV RNA levels in the placebo+PEG+RBV group was not achieved until Week 24. The mean change from baseline in HCV RNA (log₁₀ IU/mL) at Day

28 was -5.323, -5.081, and -5.346 for the sofosbuvir 100 mg+PEG+RBV, 200 mg+PEG+RBV, and 400 mg +PEG+RBV groups, respectively, compared with -2.800 for placebo+PEG+RBV.

At Day 28, RVR rates achieved by all 3 sofosbuvir+PEG+RBV groups were substantially higher than the placebo+PEG+RBV group. The RVR rates were 87.5%, 94.4%, and 93.3% for the sofosbuvir 100 mg+PEG+RBV, 200 mg+PEG+RBV, and 400 mg +PEG+RBV groups, respectively, compared to 21.4% for the placebo+PEG+RBV group. Four subjects treated with sofosbuvir+PEG+RBV did not achieve RVR. One of the 4 subjects (1005-1085 receiving sofosbuvir 200 mg +PEG+RBV) was lost to follow-up after receiving 15 days of study treatment and had detectable HCV RNA at their last visit. The remaining 3 subjects (2 subjects [1001-1028, 1005-1082] receiving sofosbuvir 100 mg+PEG+RBV and 1 subject [1005-1070] receiving sofosbuvir 400 mg+PEG+RBV) all experienced substantial declines in HCV RNA levels by Day 27, however only Subject 1005-1070 had HCV RNA levels below LOD (15 IU/mL) at Week 6 and achieved SVR12 and SVR24. The other 2 subjects had increasing HCV RNA levels after discontinuing sofosbuvir 100 mg+PEG+RBV treatment.

SVR12 and SVR24 rates achieved by the sofosbuvir 200 mg+PEG+RBV and 400 mg+PEG+RBV groups were greater than those achieved by the sofosbuvir 100 mg+PEG+RBV or placebo+PEG+RBV groups.

Visit	sofosbuvir 100 mg +PEG+RBV (N = 16)	sofosbuvir 200 mg +PEG+RBV (N = 18)	sofosbuvir 400 mg +PEG+RBV (N = 15)	Placebo +PEG+RBV (N = 14)
RVR (HCV RNA < LOD [15 IU/mL] at Day 28)	14 (87.5%)	17 (94.4%)	14 (93.3%)	3 (21.4%)
SVR12 (HCV RNA < LOD [15 IU/mL] 12 weeks after end of treatment)	9 (56.3%)	13 (72.2%)	13 (86.7%)	7 (50.0%)
SVR24 (HCV RNA < LOD [15 IU/mL] 24 weeks after end of treatment)	9 (56.3%)	15 (83.3%)	12 (80.0%)	6 (42.9%)

Note: Subject 1005-1057 randomized to sofosbuvir 100 mg+PEG+RBV actually received 400 mg+PEG+RBV for the last weeks of the sofosbuvir treatment period. This subject achieved RVR, SVR12 and SVR24.

Safety Summary: Sofosbuvir in combination with PEG+RBV was generally well tolerated in treatment naive genotype 1 HCV-infected subjects. There were no toxicities identified in sofosbuvir +PEG+RBV treatment groups that were not observed in the placebo+PEG+RBV group. All AEs were of mild or moderate severity during the sofosbuvir treatment period and were typical of the known safety profile of PEG+RBV (such as flu-like symptoms). No SAEs or discontinuations of study drug due to AEs were reported in the sofosbuvir treatment period. Except for decline in serum ALT and AST levels, there were no sofosbuvir-related changes in laboratory parameters.

Conclusion:

- SVR12 and SVR24 rates were greatest in the sofosbuvir 200 mg+PEG+RBV (72.2% and 83.3%, respectively) and 400 mg+PEG+RBV (86.7% and 80.0%, respectively) groups versus the sofosbuvir 100 mg+PEG+RBV (56.3% and 56.3%, respectively) and placebo+PEG+RBV groups (50.0% and 42.9%, respectively). As SVR12 and SVR24 rates were lowest, and breakthrough and relapse rates were highest, in the sofosbuvir

100 mg+PEG+RBV group, sofosbuvir 200 mg+PEG+RBV and 400 mg+PEG+RBV were the therapeutic doses carried forward to be evaluated in Study P7977-0422 over a longer treatment duration.

- Sofosbuvir was absorbed quickly (C_{max} occurring within 1 hour) and exhibited time independent, linear PK across evaluated doses. The primary metabolites, GS-566500 and GS-331007, demonstrated dose-proportional increases in exposure over increasing sofosbuvir doses. No significant accumulation with time was observed for sofosbuvir, GS-331007, and GS-566500.

4.2.2.5 P7977-0523 (ELECTRON): A Multi-center, Open-Labeled Exploratory Study to Investigate the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics following Oral Administration of PSI-7977 400 mg and Ribavirin for 12 Weeks With and Without Pegylated Interferon in Treatment-Naïve Patients with Chronic HCV Infection Genotype 2 or Genotype 3 (non-IND study, interim Report)

Objectives:

- To assess the safety and tolerability of sofosbuvir 400 mg for 8 or 12 weeks, administered with and without ribavirin (RBV) and/or pegylated interferon alfa-2a (PEG) in subjects with hepatitis C virus (HCV) genotypes 1, 2, or 3.
- To characterize the steady-state plasma pharmacokinetics (PK) of the GS-331007 metabolite of sofosbuvir.
- To evaluate the efficacy of sofosbuvir different treatment regimens of sofosbuvir alone and in combination with RBV with and without PEG.

Study Design: This Phase 2a, multiple-dose, open label study was conducted to evaluate different treatment regimens of sofosbuvir alone and in combination with RBV with and without PEG. The sponsor submitted interim data for Parts 1, 2, and 3 (Groups 1–9).

During Part 1 of the study, approximately 40 treatment-naïve subjects with genotype 2 or 3 HCV infection were enrolled. Forty subjects were randomized at baseline into 1 of 4 treatment groups (10 subjects per group):

- **Sofosbuvir+RBV 12 weeks:** Sofosbuvir 400 mg once daily + RBV 1000 to 1200 mg/day for 12 weeks in treatment-naïve subjects with genotype 2 or 3 HCV infection (Group 1)
- **Sofosbuvir+PEG+RBV 4 weeks/sofosbuvir+RBV 8 weeks:** Sofosbuvir 400 mg once daily + PEG 180 µg weekly + RBV 1000 to 1200 mg/day for 4 weeks followed by sofosbuvir 400 mg once daily + RBV 1000 to 1200 mg/day for 8 weeks in treatment-naïve subjects with genotype 2 or 3 HCV infection (Group 2)
- **Sofosbuvir+PEG+RBV 8 weeks/sofosbuvir+RBV 4 weeks:** Sofosbuvir 400 mg once daily + PEG 180 µg weekly + RBV 1000 to 1200 mg/day for 8 weeks followed by sofosbuvir 400 mg once daily + RBV 1000 to 1200 mg/day for 4 weeks in treatment-naïve subjects with genotype 2 or 3 HCV infection (Group 3)
- **Sofosbuvir+PEG+RBV 12 weeks:** Sofosbuvir 400 mg once daily + PEG 180 µg weekly + RBV 1000 to 1200 mg/day for 12 weeks in treatment-naïve subjects with genotype 2 or 3 HCV infection (Group 4)

After enrollment of the first group of 40 subjects was complete, Part 2 of the study was enrolled with an additional 30 subjects. Twenty treatment-naïve subjects with genotype 2 or 3 HCV infection were enrolled to 1 of the following 2 treatment groups (10 subjects per group):

- **Sofosbuvir 12 weeks:** Sofosbuvir 400 mg once daily for 12 weeks in treatment-naïve subjects with genotype 2 or 3 HCV infection (Group 5)
- **Sofosbuvir+PEG+RBV 8 weeks:** Sofosbuvir 400 mg once daily + PEG 180 µg weekly + RBV 1000 or 1200 mg/day for 8 weeks in treatment-naïve subjects with genotype 2 or 3 HCV infection (Group 6)

In addition, 10 subjects with genotype 1 HCV infection who demonstrated null response (defined as $< 2\log_{10}$ IU/ml decrease from baseline in HCV RNA) following previous PEG+RBV therapy were enrolled into the following treatment group:

- **Sofosbuvir+RBV 12 weeks:** Sofosbuvir 400 mg once daily + RBV 1000 or 1200 mg/day for 12 weeks in null responders with genotype 1 HCV infection (Group 7)

For Part 3 of the study, 25 treatment-naïve subjects with genotype 1 HCV infection and 25 treatment-experienced subjects with genotype 2 or 3 HCV infection were enrolled into 1 of the 2 following treatment groups (25 subjects per group):

- **Sofosbuvir+RBV 12 weeks:** Sofosbuvir 400 mg once daily + RBV 1000 to 1200 mg/day for 12 weeks in treatment-naïve subjects with genotype 1 HCV infection (Group 8)
- **Sofosbuvir+RBV 12 weeks (treatment-experienced genotype 2 or 3):** Sofosbuvir 400 mg once daily + RBV 1000 to 1200 mg/day for 12 weeks in treatment-experienced subjects with genotype 2 or 3 HCV infection (Group 9)

Formulation:

- Sofosbuvir was administered orally as 400 mg (2 × 200-mg tablets). lot#: 0G069-P1, 1A005-P1, 11D034-P1, and 11J110-P1.
- Pegasys® (pegylated interferon alfa-2a): 180 µg was administered via the subcutaneous route, using sterile technique according to package insert dosing recommendations.
- Ribavirin (generic RBV or Copegus®) was administered as 1000 to 1200 mg/day oral capsules or tablets according to package insert weight based dosing recommendations.

PK Sampling: For Parts 1 and 2, except for Group 6, a blood sample was collected prior to study drug administration at Days 1, 2, and 3 and Weeks 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12. At Week 4, serial blood samples were also collected at 1, 2, 4, 8, and 12 hours postdose. For Group 6, a blood sample was collected prior to study drug administration at Days 1, 2, and 3 and Weeks 1, 2, 3, 4, 5, 6, 7, and 8. At Week 4, serial blood samples were also collected at 1, 2, 4, 8, and 12 hours postdose.

For Part 3, Groups 8 and 9, a blood sample was collected prior to study drug administration at Weeks 1, 4, 8, and 12.

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

(b) (4)

The standard curve and QC data indicated that the plasma assay method for GS-331007 was precise and accurate as shown in the following table.

Table 1 Summary of Quality Control (QC) Results

Analyte	Linear range (ng/mL)	Between Run Precision (%CV)	Between Run Bias (% Deviation)	QC samples (ng/mL)	Validation sample for stability and conditions
GS-331007	5 – 5000 R ² ≥ 0.994	≤ 7.1	-0.6 to 1.0	30, 500 and 4000	Stable for 308 days at -70°C and ≥ 5 freeze/thaw cycles in plasma

Pharmacokinetic Results: Table 2 presents the mean (%CV) plasma PK parameters for GS-331007. GS-331007 PK parameters for Groups 1 to 4 and 6 to 7 were comparable across groups, as well as comparable to exposures observed in previous Phase 2 studies (Studies P7977-0221 and P7977-0422) and the predicted results in HCV-infected subjects from population PK analysis. GS-331007 AUC_{tau}, C_{max} and C_{tau} for the sofosbuvir monotherapy group (Group 5) were higher (51%–129%) than those observed for Groups 1 to 4 and 6 to 7. In Study P2938-0212, where sofosbuvir monotherapy was administered for 7 days, the GS-331007 C_{max} (1378.3 ng/mL) was comparable to the GS-331007 C_{max} in Group 5 and AUC_{tau} was within ~40% of AUC_{tau} (9638.9 hr·ng/mL) observed in Group 5.

Table 2. P7977-0523: GS-331007 Plasma Pharmacokinetic Parameters Measured at Week 4 of Treatment in Parts 1 and 2

GS-331007 PK Parameter Mean (%CV)	Treatment-Naive Genotype 2/3						Null-Responders Genotype 1
	SOF+RBV 12 weeks (Group 1) (N = 10)	SOF+PEG +RBV 4 weeks/ SOF+RBV 8 weeks (Group 2) (N = 9)	SOF+PEG +RBV 8 weeks/ SOF+RBV 4 weeks (Group 3) (N = 10)	SOF+PEG +RBV 12 weeks (Group 4) (N = 11)	SOF 12 weeks (Group 5) (N = 10)	SOF+PEG +RBV 8 weeks (Group 6) (N = 10)	SOF+RBV 12 weeks (Group 7) (N = 10)
AUC _{tau} (h·ng/mL)	7035.0 (19.1)	7135.0 (25.3)	6562.7 (30.0)	7163.2 (30.5)	13,588.4 (30.0)	8977.5 (35.4)	8630.1 (39.1)
C _{max} (ng/mL)	676.3 (36.1)	664.8 (39.7)	702.0 (40.9)	723.3 (38.8)	1431.2 (31.0)	929.5 (35.5)	789.2 (31.2)
C _{tau} (ng/mL)	153.0 (13.3)	141.3 (20.4)	116.6 (32.3)	143.5 (27.7)	267.4 (33.3)	161.1 (48.4)	175.8 (45.0)
T _{max} (h) ^a	4.00 (4.00, 4.00)	4.00 (4.00, 4.00)	4.00 (4.00, 4.00)	4.00 (3.98, 4.00)	4.00 (4.00, 4.00)	4.00 (4.00, 4.00)	4.00 (4.00, 4.00)
T _{1/2} (h) ^a	15.90 (9.93, 20.00)	11.35 (9.94, 15.04)	10.16 (9.80, 13.11)	12.30 (11.01, 13.29)	12.88 (12.20, 16.50)	11.68 (8.68, 14.73)	13.55 (10.86, 16.01)

Efficacy Summary:

- In treatment-naive subjects with genotype 2 or 3 HCV infection, sofosbuvir in combination with RBV alone and PEG+RBV provided very high virologic response rates with SVR12 rates of 100% indicating that PEG is not required to provide durable SVR in this initial group of subjects.
- In treatment-experienced subjects with genotype 2 or 3 HCV infection, sofosbuvir in combination with RBV alone provided high virologic response rates; the SVR12 rate was 68% in this population which has limited treatment options.

- In treatment-naive subjects with genotype 1 HCV infection, sofosbuvir in combination with RBV alone provided high virologic response with an SVR12 rate of 84%.
- In null-responder subjects with genotype 1 HCV infection, sofosbuvir in combination with RBV alone was not sufficient and resulted in a relapse rate of 90%. These subjects may require longer treatment duration and/or the addition of another DAA to provide high rates of sustained virologic response.

Safety Summary: The sponsor indicated that sofosbuvir was generally well tolerated with no discontinuations of sofosbuvir. The adverse event and laboratory safety profile observed was consistent with that previously reported for PEG/RBV or RBV. There was no new safety signal or toxicity observed in this study.

Conclusion: Plasma exposure of GS-331007, the predominant circulating metabolite of sofosbuvir, was comparable to exposures observed in previous Phase 2 studies. GS-331007 exposures were higher in monotherapy as compared to when sofosbuvir is coadministered with PEG/RBV or RBV. GS-331007 exposure is similar when sofosbuvir is coadministered with PEG/RBV or RBV.

4.2.2.6 P7977-0422 (PROTON): A Multi-center, Placebo-Controlled, Dose Ranging Study to Investigate the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics following Oral Administration of PSI-7977 in Combination with Pegylated Interferon and Ribavirin in Treatment-Naive Patients with Chronic HCV Infection Genotype 1, and an Open Label Assessment of PSI-7977 in Patients with HCV Genotypes 2 or 3

Objectives:

- To assess the safety and tolerability of sofosbuvir (GS-7977) for 12 weeks, administered in combination with standard-of-care (pegylated interferon alfa-2a [PEG] + ribavirin [RBV]), in treatment-naive subjects with hepatitis C virus (HCV) genotypes 1, 2, or 3
- To characterize the steady-state plasma pharmacokinetics (PK) of the GS-331007 (formerly PSI-6206) metabolite of sofosbuvir
- To evaluate the pharmacodynamics of sofosbuvir following Oral Administration of sofosbuvir in Combination with Pegylated Interferon and Ribavirin in Treatment-Naive Patients with Chronic HCV Infection Genotypes 1, 2 and 3.

Study Design: This Phase 2b double-blind study primarily evaluated the safety of sofosbuvir in combination with PEG+RBV in treatment-naive genotypes 1, 2, and 3 HCV-infected subjects. Overall, 122 treatment-naive subjects with HCV genotype 1, stratified for IL28B status (C/C or any T allele) and baseline HCV RNA levels (< 800,000 IU/mL or ≥ 800,000 IU/mL) were randomized to receive sofosbuvir 200 mg once daily, 400 mg once daily, or matching placebo together with PEG+RBV for 12 weeks. Genotype 1 HCV-infected subjects were randomized in a 2:2:1 ratio. Genotype 1 HCV-infected subjects who achieved an eRVR (HCV RNA < lower LOD at Weeks 4 through 12 inclusive) received an additional 12 weeks of PEG+RBV. Genotype 1 HCV-infected subjects who did not achieve an eRVR received an additional 36 weeks of PEG+RBV. Genotype 1 HCV-infected subjects who received placebo and achieved an eRVR still received an additional 36 weeks of PEG+RBV.

In addition, 25 treatment-naive genotype 2 or genotype 3 HCV-infected subjects received open-label sofosbuvir 400 mg once daily together with PEG+RBV for 12 weeks, with no PEG+RBV follow up.

Formulation:

- **Sofosbuvir** was administered orally as 200 mg (2 × 100 mg tablets) or 400 mg (4 × 100 mg tablets). Lot#: 0A004-P1 and 0B019-P1
- **Placebo** tablets matching for sofosbuvir were also administered orally. Lot#: 0A003-P1
- **Pegasys® (pegylated interferon alfa-2a)** was administered via the subcutaneous route, using sterile technique according to package insert dosing recommendations. Subjects with HCV genotype-2 or genotype-3 received a total of 12 doses of Pegasys®. Subjects with HCV genotype-1 received either 24 or 48 doses of Pegasys®.
- **Copegus® (generic RBV)** was administered as oral tablets according to package insert weight based dosing recommendations (Genotype 1: < 75kg = 1000 mg and ≥ 75 kg = 1200 mg; Genotype 2/3: 800 mg in 5 subjects, 1000-1200 mg (weight-based dosing) for the rest of the subjects).

PK Sampling: Blood samples were collected relative to the dosing of sofosbuvir or placebo at the following time points: trough plasma sample: Days 1 (predose), 3, 8, 15, 22, 29, and Weeks 6, 8, 10, and 12. On 4 days (Days 1, 8, 15, and 29), additional samples were drawn at 1, 2, and 4 hours after the dose. On these 4 days, at selected sites, subjects were offered the opportunity to have additional samples drawn at 8 and 12 hours after the dose of sofosbuvir to provide additional PK data.

Analytical methods: Concentrations of GS-331007 in plasma were determined using fully validated high-performance LC/MS/MS bioanalytical methods. All samples were analyzed in the timeframe supported by frozen stability storage data. The assays for GS-331007 were all performed and validated by (b) (4)

The standard curve and QC data indicated that the plasma assay method for GS-331007 was precise and accurate as shown in the following table.

Table 1 Summary of Quality Control (QC) Results

Analyte	Linear range (ng/mL)	Between Run Precision (%CV)	Between Run Bias (% Deviation)	QC samples (ng/mL)	Validation sample for stability and conditions
GS-331007	10 – 5000 R ² ≥ 0.994	≤ 23.3	0.9 to 6.5	30, 500 and 4000	Stable for 184 days at -70°C and ≥ 5 freeze/thaw cycles in plasma

Pharmacokinetic Results: Table 2 to Table 4 present the mean steady state plasma PK parameters of GS-331007 following oral administration of sofosbuvir (200 mg or 400 mg). GS-331007 exhibited linear pharmacokinetics; mean exposure (AUC_{tau} or C_{max}) increased near proportionally over an increasing dose of sofosbuvir. Median T_{max} (median 2.00–4.01 hours) and plasma half-life (median: 9.53–11.79 hours in genotype 1 HCV infected subjects) were comparable to results from a previous Phase 2 study (P7977-0221). The pharmacokinetic parameter estimates observed on Day 8 were comparable to values observed at later timepoints (Day 15 or 29) suggesting that steady state was achieved by Day 8. In this small number of subjects, lower GS-331007 exposure (assessed as AUC_{tau}, C_{max}, and C_{tau}) and a longer plasma elimination half-life were observed in genotype 2 or 3 HCV-infected subjects compared with genotype 1 HCV-infected subjects. This difference was not observed in Study P7977-0523, where sample size was greater.

Table 2. P7977-0422: Summary of GS-331007 Plasma Pharmacokinetic Parameters (Pharmacokinetic Analysis Set) – Day 8

GS-331007 Plasma PK Parameters	SOF 200mg+PEG+RBV GT1 (N = 3) ^a	SOF 400mg+PEG+RBV GT1 (N = 3) ^a	SOF 400mg+PEG+RBV GT2/3 (N = 4) ^a
C _{max} (ng/mL) Mean (%CV)	331.48 (50.47)	750.78 (14.39)	518.18 (40.95)
T _{max} (h) Median (Q1, Q3)	4.00 (4.00, 4.00)	4.00 (4.00, 4.00)	3.00 (2.00, 4.00)
t _{1/2} (h) Median (Q1, Q3)	12.61 (5.96, 14.93)	11.79 (10.13, 12.20)	30.25 (14.65, 43.37)
T _{last} (h) Median (Q1, Q3)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
AUC _{tau} (ng•h/mL) Mean (%CV)	3020.30 (42.73)	8620.07 (19.92)	5738.61 (35.02)
C _{tau} (ng•h/mL) Mean (%CV)	44.90 (40.55)	159.50 (21.65)	168.44 (62.07)

a Data presented in this table are only from subjects with evaluable PK parameters

Table 3. P7977-0422: Summary of GS-331007 Plasma Pharmacokinetic Parameters (Pharmacokinetic Analysis Set) – Day 15

GS-331007 Plasma PK Parameters	SOF 200mg+PEG+RBV GT1 (N = 4) ^a	SOF 400mg+PEG+RBV GT1 (N = 1) ^a	SOF 400mg+PEG+RBV GT2/3 (N = 5) ^a
C _{max} (ng/mL) Mean (%CV)	269.93 (24.34)	1087.21 ^b	515.13 (34.67)
T _{max} (h) Median (Q1, Q3)	4.00 (3.00, 4.00)	2.00 (2.00, 2.00)	2.00 (2.00, 4.00)
t _{1/2} (h) Median (Q1, Q3)	10.65 (9.23, 12.41)	9.53 (9.53, 9.53)	16.25 (12.23, 23.58)
T _{last} (h) Median (Q1, Q3)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
AUC _{tau} (ng•h/mL) Mean (%CV)	3178.53 (37.11)	11245.43 ^b	5465.41 (19.59)
C _{tau} (ng•h/mL) Mean (%CV)	55.02 (32.86)	166.24 ^b	123.48 (15.21)

a Data presented in this table are only from subjects with evaluable PK parameters

b SD not available as n = 1

Table 4. P7977-0422: Summary of GS-331007 Plasma Pharmacokinetic Parameters (Pharmacokinetic Analysis Set) – Day 29

GS-331007 Plasma PK Parameters	SOF 200mg+PEG+RBV GT1 (N = 5) ^a	SOF 400mg+PEG+RBV GT1 (N = 2) ^a	SOF 400mg+PEG+RBV GT2/3 (N = 5) ^a
C _{max} (ng/mL) Mean (%CV)	285.77 (31.99)	897.41 (20.93)	574.13 (42.04)
T _{max} (h) Median (Q1, Q3)	4.00 (4.00, 4.00)	4.01 (4.00, 4.02)	4.00 (2.00, 4.00)
t _{1/2} (h) Median (Q1, Q3)	11.05 (8.40, 12.04)	9.68 (8.31, 11.05)	13.41 (10.58, 13.64)
T _{inst} (h) Median (Q1, Q3)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00,24.00)
AUC _{0-∞} (ng•h/mL) Mean (%CV)	2789.62 (33.39)	9191.86 (25.99)	5874.52 (26.14)
C _∞ (ng•h/mL) Mean (%CV)	42.78 (44.43)	161.06 (23.45)	108.25 (17.19)

a Data presented in this table are only from subjects with evaluable PK parameters

Efficacy Summary:

- Sofosbuvir 200 mg and 400 mg, in combination with PEG and RBV, provided high virologic response rates (i.e., SVR12 90–92%) in subjects infected with HCV genotypes 1, 2, and 3.
- Relapse among subjects who completed the full treatment course was uncommon in all sofosbuvir treatment groups (2 subjects) and occurred within the first 4 weeks after discontinuing all treatment.
- No clinically significant resistance mutations, including mutations at positions S282T and M289L, were detected following virological failure by population and deep sequencing, which suggests that sofosbuvir has a high barrier to resistance.
- In genotype 1 HCV-infected subjects, virologic breakthroughs during treatment with PEG+RBV following treatment with sofosbuvir+PEG+RBV were more common in the sofosbuvir 200 mg+PEG+RBV group compared with the sofosbuvir 400 mg+PEG+RBV group, suggesting that the sofosbuvir 400 mg dose may provide greater suppression of viral activity.

Safety Summary: Sofosbuvir was well tolerated with no discontinuations attributable to sofosbuvir. The AE and laboratory profile observed was consistent with that previously reported for PEG and RBV and no liver toxicity, i.e., severe ALT elevations or persistent direct bilirubin elevations, were observed with sofosbuvir. The duration of PEG+RBV exposure accounts for the majority of the differences between the genotype 1 and genotype 2 or 3 HCV-infected subjects.

Conclusion: The limited PK of GS-331007 were generally similar to other Phase 2 and 3 studies of SOF in combination of PEG/RBV, except the exposure of GS-331007 in Genotypes 2 and 3 were relatively lower than other studies. Steady-state of GS-331007 was achieved in 8 days.

4.2.2.7 P2938-0721 (QUANTUM): An International, Multi-center, Blinded, Randomized Study to Investigate Safety, Tolerability, Pharmacokinetics and Pharmacodynamics

following Administration of Regimens Containing PSI-352938 (GS-0938, PSI-7977, and Ribavirin in Patients with Chronic HCV Infection.

Objectives:

- To assess the efficacy of the study regimens (SOF+RBV for 12 weeks or 24 weeks) in treatment-naïve subjects with chronic HCV infection
- To assess the safety and PK of SOF+RBV for 12 weeks or 24 weeks in treatment-naïve subjects with chronic HCV infection

Study Design: Approximately 450 treatment-naïve subjects with chronic HCV infection were to be randomized in equal ratios, stratified by genotype (i.e., genotype 1a versus genotype 1b versus other), HCV RNA (< 6 log₁₀ IU/mL or ≥ 6 log₁₀ IU/mL), and cirrhosis (present or absent), to 1 of 9 initial treatment groups. At least 50% of the subjects were to be genotype 1a, at least 25% genotype 1b, and up to 10% of all subjects could be cirrhotic (Child-Turcotte-Pugh [CTP] A). Subjects were enrolled sequentially into 2 cohorts; approximately 25 subjects per group (225 total) were enrolled into Cohort 1 and after the last subject in Cohort 1 completed Week 12, an interim analysis (safety and efficacy) was conducted after which time enrollment initiated into the second cohort. Due to the safety signal associated with GS-0938, enrollment of the second cohort of 225 subjects did not occur.

Treatment groups were as follows:

- Group A: GS-0938 300 mg for 12 weeks
- Group B: GS-0938 300 mg and sofosbuvir 400 mg for 12 weeks
- Group C: Sofosbuvir 400 mg and RBV (1200 mg or 1000 mg) for 12 weeks
- Group D: GS-0938 300 mg and sofosbuvir 400 mg in combination with RBV (1200 mg or 1000 mg) for 12 weeks
- Group E: GS-0938 300 mg for 24 weeks
- Group F: GS-0938 300 mg and sofosbuvir 400 mg for 24 weeks
- Group G: Sofosbuvir 400 mg and RBV (1200 mg or 1000 mg) for 24 weeks
- Group H: GS-0938 300 mg and sofosbuvir 400 mg in combination with RBV (1200 mg or 1000 mg) for 24 weeks
- Group I: Deferred start group (placebo) for 24 weeks, then rerandomized to an active group

Only Groups C and G are reviewed, because the applicant has stopped the development of GS-0938.

Formulation and Regimen: Sofosbuvir (Lot# 11D034-P1) was administered orally once daily as 400-mg (2 × 200-mg) tablets. Ribavirin (Ribasphere® [generic Rebetol®], Lot # A67906Z) was administered as oral capsules in a divided daily dose according to weight-based dosing: 1200 mg per day if ≥ 75 kg or 1000 mg per day if < 75 kg.

PK Sampling: Plasma samples were collected for all subjects at Weeks 1, 4, 8, and 12. All subjects who had an early termination visit also had plasma samples drawn for PK analysis. At selected sites, subjects were asked to volunteer to participate in serial blood sampling at the Week 4 visit (i.e., a trough sample collected predose and then at 0.5, 1, 2, 3, 4, 8, and 12 hours postdose).

Analytical methods: Concentrations of GS-331007 in plasma were determined using fully validated high-performance (LC/MS/MS) bioanalytical methods. All samples were analyzed in

the timeframe supported by frozen stability storage data. The assay for GS-331007 was performed and validated by (b) (4)

The standard curve and QC data indicated that the plasma assay method for GS-331007 was precise and accurate as shown in the following table.

Table 1 Summary of Quality Control (QC) Results

Analyte	Linear range (ng/mL)	Between Run Precision (%CV)	Between Run Bias (% Deviation)	QC samples (ng/mL)	Validation sample for stability and conditions
GS-331007	10 – 5000 R ² > 0.992	≤ 7.6	-2.0 to 1.5	30, 500 and 4000	Stable for 308 days at -70°C and ≥ 5 freeze/thaw cycles in plasma

Pharmacokinetic Results: Only 5 subjects had intense PK data available for GS-331007, as shown in Table 2. The limited data indicates that GS-331007 concentrations following administration of SOF and RBV in HCV-infected patients in this study are within the range observed in other Phase 2 studies.

Table 2: GS-331007 Plasma Pharmacokinetic Parameters when Administered as SOF+RBV

Mean (%CV) GS-331007 PK Parameter	SOF+RBV (N = 5)
AUC _{tau} (h*ng/mL)	8257.9 (41.0)
C _{max} (ng/mL)	705.8 (36.8)
C _{tau} (ng/mL)	191.9 (64.0)
Median T _{max} (h) ^a	3.00 (2.00, 4.00)
Median t _{1/2} (h) ^a	12.53 (10.19, 15.43)

a T_{max} and t_{1/2} presented as median (Q1, Q3)

Efficacy Summary:

- Sofosbuvir 400 mg in combination with RBV caused a rapid suppression of HCV RNA with 100% of subjects achieving RVR.
- Sofosbuvir 400 mg in combination with RBV given for 12 weeks was as effective as 24 weeks of treatment in achieving SVR in subjects with genotypes 1 through 3 HCV (56% versus 52%, respectively).
- Virologic relapse occurred in a similar proportion of subjects who received either 12 or 24 weeks of sofosbuvir+RBV treatment (i.e., 39% and 44%, respectively).
- No S282T mutation, a previously identified sofosbuvir-associated resistance mutation in NS5B, was detected among subjects at baseline and virologic relapse or early discontinuation. No other amino acid change in NS5B was associated with resistance to sofosbuvir.

Safety Summary: Sofosbuvir in combination with RBV was well tolerated up to 24 weeks with a similar safety profile observed in the 12- and 24-week treatment groups. No significant safety signals were observed and only expected changes in laboratory parameters consistent with RBV treatment were observed.

Conclusion: The limited data indicates that GS-331007 concentrations following administration of SOF and RBV in HCV-infected patients in this study are within the range observed in other Phase 2 studies.

4.2.2.8 P7977-0724: The ATOMIC Study: A Multicenter, Open-label, Randomized, Duration Finding Study to Investigate the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics following Oral Administration of PSI-7977 in Combination with Pegylated Interferon and Ribavirin in Treatment-Naive Patients with Chronic HCV Infection Genotype 1, 4, 5, or 6.

Objectives:

- To assess the safety, tolerability, and efficacy of sofosbuvir administered in combination with PEG+RBV for 12 or 24 weeks in treatment-naive subjects with HCV genotypes 1, 4, 5, 6, or indeterminate genotype
- To assess PK of GS-331007 following administration of SOF+PEG+RBV

Study Design: This Phase 2b, multicenter, open-label randomized, duration-finding study evaluated the safety and efficacy of 2 treatment durations (12 and 24 weeks) of sofosbuvir 400 mg in combination with PEG+RBV in treatment-naive genotypes 1, 4, 5, and 6 HCV infected subjects.

Following screening, approximately 300 treatment-naive subjects with genotype 1 chronic HCV infection, stratified for IL28B gene (IL28B) status (CC or any T allele) and baseline HCV RNA levels (< 800,000 IU/mL or ≥ 800,000 IU/mL) were randomized in a 1:2:3 ratio into 1 of 3 open-label treatment groups (A, B or C) to receive sofosbuvir+PEG+RBV for at least 12 weeks.

- **Group A:** sofosbuvir 400 mg in combination with PEG+RBV for 12 weeks
- **Group B:** sofosbuvir 400 mg in combination with PEG+RBV for 24 weeks
- **Group C (total):** sofosbuvir 400 mg in combination with PEG+RBV for 12 weeks.

Subjects who did not discontinue prior to the end of this initial 12 week treatment period were then re-randomized to receive further treatment with either:

- **Group C1:** sofosbuvir 400 mg monotherapy for 12 weeks or
- **Group C2:** sofosbuvir 400 mg+RBV for 12 weeks

In addition, up to 25 eligible treatment-naive subjects with genotype 4, 5, 6 or indeterminate genotype chronic HCV-infection were enrolled into Group B to receive sofosbuvir 400 mg+PEG+RBV for 24 weeks.

Groups C1 and C2 were to investigate the therapeutic role of RBV in the absence of PEG.

All subjects who received at least 1 dose of sofosbuvir were followed for 24 weeks after discontinuation of therapy to determine if a SVR (SVR24) was achieved, as well as to determine the presence of any drug-resistant variants.

Formulation and Dose Regimens:

- **Sofosbuvir** was administered orally once daily as 400 mg (2 × 200-mg tablets).
- **PEGASYS®** (pegylated interferon alfa-2a, PEG) was administered via the subcutaneous route once a week, using sterile technique according to package inserts dosing recommendations. Subjects with HCV genotype 1 received a total of 12 or 24 doses of PEG. Subjects with genotype-4, -5, -6 or indeterminate genotype chronic HCV infection (enrolled into group B only) received 24 doses of PEG.
- **COPEGUS®** (RBV) was administered as oral capsules in a divided daily dose according to weight based dosing: 1200 mg per day if ≥ 75 kg or 1000 mg per day if < 75 kg.

The lot numbers of study drug (sofosbuvir) administered in this study were 0G069-P1 and 1A005-P1.

PK Sampling: Blood samples were collected relative to the dosing of sofosbuvir at the following time points: Days 8, 15, and 22, Weeks 4, 8, 10, and End of Treatment visit. Subjects in Group B and C had additional samples collected at Weeks 12, 14, 16, and 20. For the Day 22 samples, subjects were asked to take their study drug approximately 24 hours before the scheduled study visit and to withhold their dose on the day of the visit. A trough plasma sample was then collected before dosing and further samples taken at 1, 2, 3, and 4 hours after the dose, with selected sites drawing additional samples at 8 and 12 hours.

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

The standard curve and QC data indicated that the plasma assay method for GS-331007 was precise and accurate as shown in the following table.

Table 1 Summary of Quality Control (QC) Results

Analyte	Linear range (ng/mL)	Between Run Precision (%CV)	Between Run Bias (% Deviation)	QC samples (ng/mL)	Validation sample for stability and conditions
GS-331007	10 – 5000 R ² > 0.992	≤ 7.0	-0.8 to 0.4	30, 500 and 4000	Stable for 308 days at -70°C and ≥ 5 freeze/thaw cycles in plasma

PK Results: Mean (SD) plasma PK parameters of GS-331007 for subjects with intensive PK sampling are shown in Table 2. The data indicates that GS-331007 concentrations following administration of SOF+PEG+RBV in HCV-infected patients in this study are within the range observed in other Phase 2 studies.

Table 2 P7977-0724: Summary of GS-331007 Plasma Pharmacokinetic Parameters at Week 3

GS-331007 Mean (%CV) PK Parameter	SOF+PEG+RBV (n = 38)
AUC ₀₋₂₄ (h·ng/mL)	7177.8 (26.6)
C _{max} (ng/mL)	644.1 (39.6)
C _{min} (ng/mL)	159.5 (49.6)
T _{max} (h) ^a	3.95 (3.00, 4.00)
t _{1/2} (h) ^a	12.67 (9.43, 16.79)

a median (Q1, Q3)

Efficacy Summary:

- Sofosbuvir 400 mg once daily in combination with PEG+RBV for 12 weeks was effective in the treatment of genotype 1 HCV infected subjects as evidenced by SVR12 and SVR24 rates > 90%.
- The SVR24 rate in genotype 4 (n=11) and 6 (n=5) HCV infected subjects was similar to that achieved in genotype 1 HCV infected subjects.
- Twelve weeks of sofosbuvir 400 mg+PEG+RBV was as effective as 24 weeks sofosbuvir 400 mg+PEG+RBV for the treatment of genotype 1 HCV infected subjects as evidenced by SVR12 and SVR24 rates > 90%.
- No subject relapsed between SVR12 and SVR24, showing the durability of SVR12.
- No phenotypic or genotypic resistance was detected at virologic failure.

Safety Summary:

- Sofosbuvir was well tolerated, with an AE and laboratory profile consistent with that reported in previous studies and also for PEG+RBV. Adverse events considered related to study drug were consistent with those reported in the PROTON (P7977-0724) study and were consistent with the known safety profile of PEG+RBV.
- AEs leading to permanent discontinuation of study drug or dose modification of study drug were reported at the highest frequency in subjects treated with 24 weeks of sofosbuvir 400 mg+PEG+RBV in Group B. The duration of exposure to PEG+RBV may account for the observed higher reporting frequency of AEs leading to discontinuation or modification in Group B.
- No additional toxicities or increased toxicity frequency or severity were reported when sofosbuvir is used in combination with PEG+RBV which is consistent with that observed in previous sofosbuvir clinical studies. This contrasts to what has been reported in the labeling information for telaprevir or boceprevir in combination with PEG+RBV.
- Data from this study warrants larger studies investigating 12 weeks sofosbuvir 400 mg+PEG+RBV in Genotype 1, 4 or 6.

Conclusion: The data indicates that GS-331007 concentrations following administration of SOF+PEG+RBV in HCV-infected patients in this study are within the range observed in other Phase 2 studies.

4.2.3 Intrinsic Factors

4.2.3.1 P2938-0515: An Open-Label Study to Characterize the Pharmacokinetics and Pharmacodynamics of Multiple Oral Doses of PSI-7977 or PSI-352938 in HCV-infected Subjects with Varying Degrees of Hepatic Impairment

(b) (4)

Objectives: To characterize the PK of sofosbuvir (GS-7977; formerly PSI-7977) and metabolites over 7 days of dosing with sofosbuvir in HCV-infected subjects with varying degrees of hepatic impairment compared to historical PK data.

Study Design: Equal numbers of HCV-infected subjects (8 subjects per group below) with mild, moderate, or severe hepatic impairment received sofosbuvir 400 mg once daily for 7 days as follows:

- Mild hepatic impairment (Child-Pugh Class A [score 5 to 6]; Group A)
- Moderate hepatic impairment (Child-Pugh Class B [score 7 to 9]; Group B)
- Severe hepatic impairment (Child-Pugh Class C [score 10 to 15]; Group C)

Subjects with chronic HCV infection and normal liver function who participated in a multiple ascending dose study (Study P2938-0212, N=8) were used as an historical control group (same formulation was used in that study). Age and weight ranges were used to match the historical control subjects with subjects with hepatic insufficiency (Groups A, B, and C). If possible, a similar gender distribution among groups was maintained.

Formulation: Sofosbuvir was administered orally as 400 mg (two 200 mg tablets, Formulation I). The batch numbers for sofosbuvir were 0G069-P2 and 11D034-P1.

PK Sampling: Blood samples were collected relative to the dosing of sofosbuvir at the following time points on Days 1 to 11 as follows:

- Day 1: predose (time 0), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 9, and 12 hours postdose
- Day 2: 24 hours after Day 1 dose (before Day 2 dose)
- Day 3: 48 hours after Day 1 dose (before Day 3 dose)
- Day 5: 96 hours after Day 1 dose (before Day 5 dose)
- Day 7: predose (time 0), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 9, and 12 hours postdose
- Day 8: 24 hours after Day 7 dose
- Day 9: 48 hours after Day 7 dose
- Day 10: 72 hours after Day 7 dose
- Day 11: 96 hours after Day 7 dose

Urine samples were collected relative to sofosbuvir dosing at the following time points:

- Day 1: pre-morning dose (bladder emptied before dosing for baseline sample), 0 to 6 hour, 6 to 12 hour, 12 to 24 hour collection intervals
- Day 7: 0 to 6 hour, 6 to 12 hour, 12 to 24 hour, 24 to 48 hour, 48 to 72 hour, and 72 to 96 hour collection intervals

Analytical methods: Concentrations of GS-0938, sofosbuvir, GS-566500, and GS-331007 in plasma and urine samples were determined using fully validated high-performance liquid chromatography-tandem mass spectroscopy (LC-MS-MS) bioanalytical methods. All samples were analyzed in the timeframe supported by frozen stability storage data. The assays for GS-

0938, sofosbuvir, GS-566500, and GS-331007 were performed and validated by (b) (4)

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

Table 1 Summary of Quality Control (QC) Results

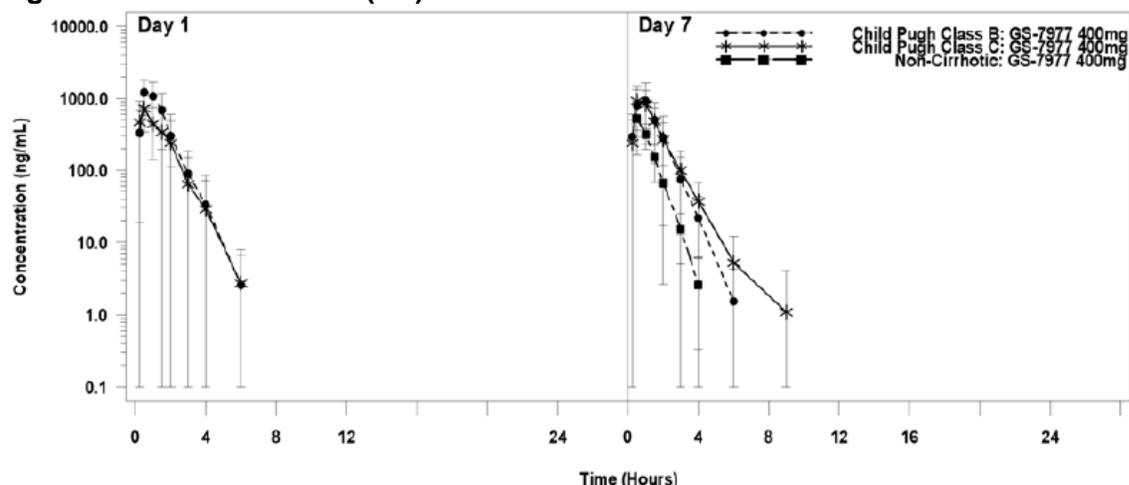
Analyte	Linear range (ng/mL)	Between Run Precision (%CV)	Between Run Bias (% Deviation)	QC samples (ng/mL)	Validation sample for stability and conditions
SOF (plasma)	5 – 5000 R ² > 0.991	≤ 7.4	-2.2 to 7.5	15, 30, 500 and 4000	Stable for 99 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
GS-566500 (plasma)	10 – 5000 R ² > 0.992	≤ 9.4	-1.4 to 0.7	30, 500 and 4000	Stable for 125 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
GS-331007 (plasma)	10 – 5000 R ² > 0.995	≤ 8.1	-3.9 to 0.8	30, 500 and 4000	Stable for 184 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
SOF (urine)	10 – 10000 R ² > 0.994	≤ 9.5	-1.5 to 4.3	30, 800 and 8000	Stable for 178 days at -70°C and ≥ 6 freeze/thaw cycles in plasma
GS-566500 (urine)	10 – 10000 R ² > 0.995	≤ 7.1	-0.8 to 2.3	30, 800 and 8000	Stable for 133 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
GS-331007 (urine)	10 – 10000 R ² > 0.994	≤ 7.7	-1.6 to 3.6	30, 800 and 8000	Stable for 133 days at -70°C and ≥ 5 freeze/thaw cycles in plasma

Pharmacokinetic Results:

Sofosbuvir:

Day 1 and Day 7 mean plasma concentration-time profiles of sofosbuvir following dosing of sofosbuvir 400 mg QD to subjects with HCV infection and moderate or severe hepatic impairment and to historical control subjects with HCV infection and normal hepatic function (Study P2938-0212, Day 7 only) are shown in semi-logarithmic plot in [Figure 1](#).

Figure 1 Sofosbuvir Mean (SD) Plasma Concentration-Time Profiles



Day 7 plasma and urine PK parameters of sofosbuvir following administration of sofosbuvir 400 mg QD to subjects with normal hepatic function, or moderate or severe hepatic impairment are shown in [Table 2](#).

Table 2 Sofosbuvir Day 7 Pharmacokinetic Parameters Following Administration of Sofosbuvir 400 mg QD to Subjects with Normal Hepatic Function or Subjects with Moderate or Severe Hepatic Impairment

SOF PK Parameter Mean (%CV) (N=8)	Normal Hepatic Function (Study P2938-0212) ^a	Moderate Hepatic Impairment	Severe Hepatic Impairment
Plasma PK Parameters			
AUC _{tau} (ng·hr/mL)	538.12 (38.97)	1349.71 (58.13)	1379.07 (52.00)
AUC _{last} (ng·hr/mL)	NA	1340.85 (58.68)	1367.40 (52.41)
C _{max} (ng/mL)	602.59 (47.15)	1127.25 (60.97)	1125.12 (49.43)
C _{last} (ng/mL)	8.63 (31.12)	10.61 (52.41)	10.53 (54.41)
T _{max} (hr) ^b	0.50 (0.50, 0.77)	0.50 (0.50, 1.00)	0.50 (0.50, 1.00)
t _{1/2} (hr) ^b	0.48 (0.44, 0.52)	0.59 (0.49, 0.61)	0.67 (0.64, 0.83)
T _{last} (hr) ^b	3.00 (3.00, 4.00)	4.00 (4.00, 5.00)	5.00 (4.00, 6.00)
CL _{ss} /F (L/hr)	871.64 (46.00)	332.40 (78.99)	280.93 (53.38)
V _z /F (L)	NA	308.89 (114.10)	300.77 (44.48)
Urine PK Parameters			
CL _r (L/min)	0.17 (32.2)	0.18 (62.2)	0.15 (73.4)
%Dose _{excreted}	1.28 (27.4)	2.74 (33.2)	2.34 (52.6)

NA = not available

a Historical control subjects with normal hepatic function from Cohort 3 (Part 2) of Study P2938-0212; subjects received sofosbuvir 400 mg for 7 days

b Median (Q1, Q3)

Statistical comparisons of sofosbuvir plasma primary exposure parameters AUC_{tau} and C_{max}, following 7 days of sofosbuvir 400 mg in subjects with moderate or severe hepatic impairment

or in subjects with normal hepatic function are presented in [Table 3](#). Day 7 sofosbuvir C_{max} and AUC_{tau} were 72% to 85% and 126% to 143% higher, respectively, in subjects with moderate or severe hepatic impairment than in control subjects with normal hepatic function. Sofosbuvir mean plasma exposure parameters (AUC and C_{max}) were similar in subjects with moderate and severe hepatic impairment.

Table 3 Statistical Comparisons of Sofosbuvir PK Parameters for Test versus Reference Treatments

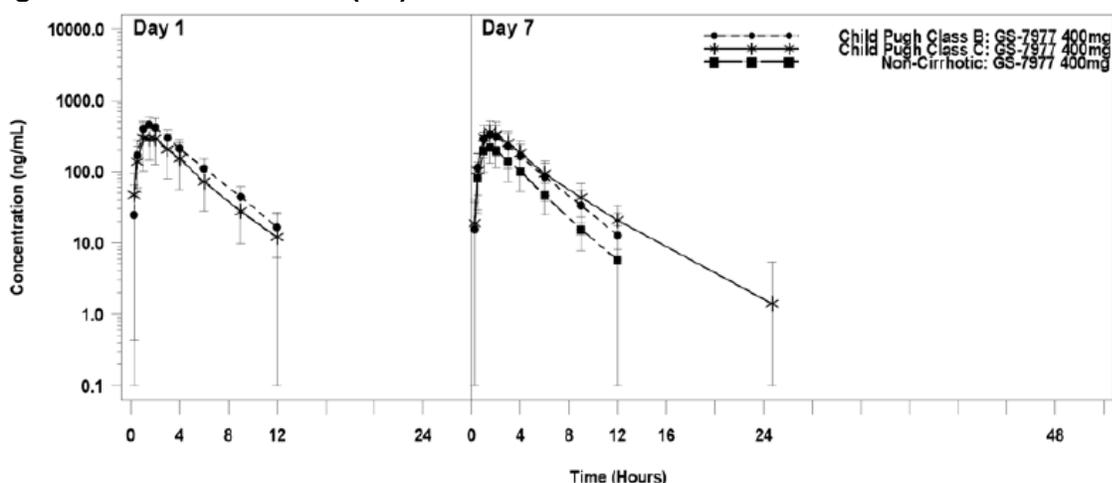
SOF PK Parameters (N = 8)	Geometric Least Squares Means		Geometric Least Squares Mean Ratio (90% CI)
	Reference Treatment: Normal Hepatic Function (Study P2938-0212) ^a	Test Treatment: Moderate Hepatic Impairment	Moderate Hepatic Impairment/Normal Hepatic Function
C _{max} (ng/mL)	548.5	943.3	172.0 (107.3, 275.5)
AUC _{tau} (ng·h/mL)	499.2	1127.0	225.8 (138.9, 367.0)
SOF PK Parameters (N = 8)	Reference Treatment: Normal Hepatic Function (Study P2938-0212) ^a	Test Treatment: Severe Hepatic Impairment	Severe Hepatic Impairment/Normal Hepatic Function
	C _{max} (ng/mL)	548.5	1012.3
AUC _{tau} (ng·h/mL)	499.2	1214.5	243.3 (149.7, 395.5)

a Historical control subjects with normal hepatic function from Cohort 3 (Part 2) of Study P2938-0212; subjects received sofosbuvir 400 mg for 7 days

GS-566500

Day 1 and Day 7 mean plasma concentration-time profiles of GS-566500 (intermediate metabolite of sofosbuvir) following dosing of sofosbuvir 400 mg QD to subjects with HCV infection and moderate or severe hepatic impairment and to historical control subjects with HCV infection and normal hepatic function (Study P2938-0212, Day 7 only) are presented in semi-logarithmic plot in [Figure 2](#).

Figure 2 GS-566500 Mean (SD) Plasma Concentration-Time Profiles



Note: Child-Pugh Class B = subjects with moderate hepatic impairment who received sofosbuvir, Child-Pugh Class C = subjects with severe hepatic impairment who received sofosbuvir, and noncirrhotic = subjects with normal hepatic function who received sofosbuvir in Study P2938-0212.

Table 4 presents Day 7 plasma and urine PK parameters of GS-566500 following administration of sofosbuvir 400 mg QD to subjects with normal hepatic function or moderate or severe hepatic impairment.

Table 4 GS-566500 Day 7 Pharmacokinetic Parameters Following Administration of Sofosbuvir 400 mg QD to Subjects with Normal Hepatic Function or Subjects with Moderate or Severe Hepatic Impairment

GS-566500 PK Parameter Mean (%CV) (N=8)	Normal Hepatic Function (Study P2938-0212) ^a	Moderate Hepatic Impairment	Severe Hepatic Impairment
Plasma PK Parameters			
AUC _{tau} (ng·hr/mL)	853.13 (45.69)	1425.18 (43.68)	1606.70 (42.18)
AUC _{last} (ng·hr/mL)	NA	1364.70 (43.55)	1545.01 (43.03)
C _{max} (ng/mL)	235.16 (38.43)	349.43 (36.21)	379.00 (42.21)
C _{last} (ng/mL)	18.41 (34.81)	18.20 (50.91)	19.08 (32.02)
T _{max} (hr) ^b	1.50 (1.00, 1.75)	1.50 (1.25, 1.75)	1.75 (1.25, 2.00)
t _{1/2} (hr) ^b	2.14 (1.99, 2.24)	2.13 (2.01, 2.39)	2.68 (2.26, 2.98)
T _{last} (hr) ^b	9.00 (9.00, 10.50)	12.00 (9.00, 12.00)	12.00 (11.93, 12.00)
Urine PK Parameters			
CL _r (L/min)	0.199 (54.2)	0.14 (23.9)	0.13 (68.0)
%Dose _e excreted	3.43 (97.8)	3.67 (38.5)	3.13 (48.2)

NA = not available

a Historical control subjects with normal hepatic function from Cohort 3 (Part 2) of Study P2938-0212; subjects received sofosbuvir 400 mg for 7 days

b Median (Q1, Q3)

Statistical comparisons of GS-566500 primary exposure parameters AUC_{tau} and C_{max}, following 7 days of sofosbuvir 400 mg QD to subjects with moderate or severe hepatic impairment or to subjects with normal hepatic function are presented in [Table 5](#). On Day 7, GS-566500 C_{max} and AUC_{tau} were 49% to 60% and 66% to 87% higher, respectively, in subjects with moderate or severe hepatic impairment than in control subjects with normal hepatic function.

5 Statistical Comparisons of GS-566500 PK Parameters for Test versus Reference Treatments

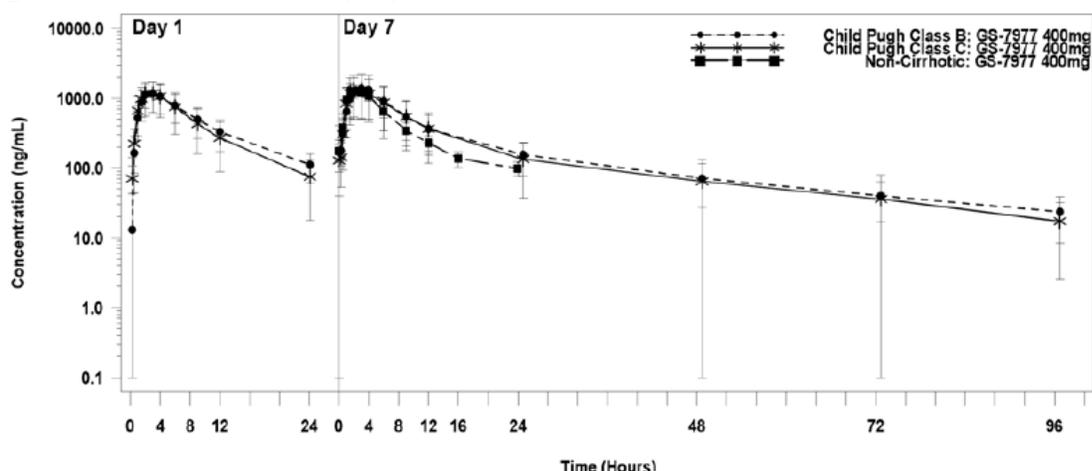
GS-566500 PK Parameters (N = 8)	Geometric Least Squares Means		Geometric Least Squares Mean Ratio (90% CI)
	Reference Treatment: Normal Hepatic Function (Study P2938-0212) ^a	Test Treatment: Moderate Hepatic Impairment	Moderate Hepatic Impairment/Normal Hepatic Function
C _{max} (ng/mL)	219.8	328.2	149.3 (105.7, 211.1)
AUC _{tau} (ng·h/mL)	778.7	1289.1	165.5 (108.3, 253.1)
	Reference Treatment: Normal Hepatic Function (Study P2938-0212) ^a	Test Treatment: Severe Hepatic Impairment	Severe Hepatic Impairment/Normal Hepatic Function
C _{max} (ng/mL)	219.8	351.2	159.8 (113.0, 225.8)
AUC _{tau} (ng·h/mL)	778.7	1456.7	187.1 (122.4, 286.01)

^a Historical control subjects with normal hepatic function from Cohort 3 (Part 2) of Study P2938-0212; subjects received sofosbuvir 400 mg for 7 days

GS-331007

[Figure 3](#) presents Day 1 and Day 7 plasma concentration-time profiles for GS-331007 following dosing of sofosbuvir 400 mg QD to subjects with HCV infection and moderate or severe hepatic impairment and to historical control subjects with HCV infection and normal hepatic function (Study P2938-0212, Day 7 only).

Figure 3 GS-331007 Mean (SD) Plasma Concentration-Time Profiles



Note: Child-Pugh Class B = subjects with moderate hepatic impairment who received sofosbuvir, Child-Pugh Class C = subjects with severe hepatic impairment who received sofosbuvir, and noncirrhotic = subjects with normal hepatic function who received sofosbuvir in Study P2938-0212.

Day 7 plasma and urine PK parameters of GS-331007 following administration of sofosbuvir 400 mg QD to subjects with normal hepatic function or with moderate or severe hepatic impairment are presented in [Table 6](#).

Table 6 GS-331007 Day 7 Pharmacokinetic Parameters Following Administration of Sofosbuvir 400 mg QD to Subjects with Normal Hepatic Function or Subjects with Moderate or Severe Hepatic Impairment

GS-331007 PK Parameter Mean (%CV) (N=8)	Normal Hepatic Function (Study P2938-0212) ^a	Moderate Hepatic Impairment	Severe Hepatic Impairment
Plasma PK Parameters			
AUC _{tau} (ng·h/mL)	9638.94 (18.72)	12560.72 (56.97)	12206.49 (63.07)
AUC _{last} (ng·h/mL)	NA	17139.60 (55.02)	16218.49 (68.48)
C _{max} (ng/mL)	1378.33 (19.16)	1441.27 (58.90)	1439.08 (59.45)
C _{tau} (ng/mL)	98.15 (23.44)	155.60 (49.74)	132.97 (71.98)
C _{last} (ng/mL)	NA	25.39 (49.61)	20.67 (54.16)
T _{max} (hr) ^b	2.00 (1.75, 3.00)	3.00 (3.00, 4.00)	2.50 (1.75, 3.00)
t _{1/2} (hr) ^b	9.42 (8.84, 12.24)	27.92 (21.76, 39.35)	28.10 (21.03, 37.45)
T _{last} (hr) ^b	23.58 (22.73, 23.97)	96.00 (96.00, 96.00)	96.00 (84.00, 96.46)
Urine PK Parameters			
CL _r (L/min)	0.25 (22.4)	0.16 (36.2)	0.15 (78.5)
%Dose _e excreted	72.6 (17.4)	54.92 (46.0)	44.14 (55.0)

NA = not available

a Historical control subjects with normal hepatic function from Cohort 3 (Part 2) of Study P2938-0212; subjects received sofosbuvir 400 mg for 7 days

b Median (Q1, Q3)

Table 7 presents statistical comparisons of GS-331007 primary exposure parameters AUC_{tau}, C_{max}, and C_{tau} following 7 days of sofosbuvir 400 mg QD to subjects with moderate or severe hepatic impairment or to subjects with normal hepatic function. On Day 7, GS-331007 C_{max} and AUC_{tau} estimates were similar in subjects with hepatic impairment and control subjects with normal hepatic function; GS-331007 C_{tau} was approximately 46% higher in subjects with moderate hepatic impairment than in subjects with normal hepatic function, and similar in subjects with severe hepatic impairment and normal hepatic function.

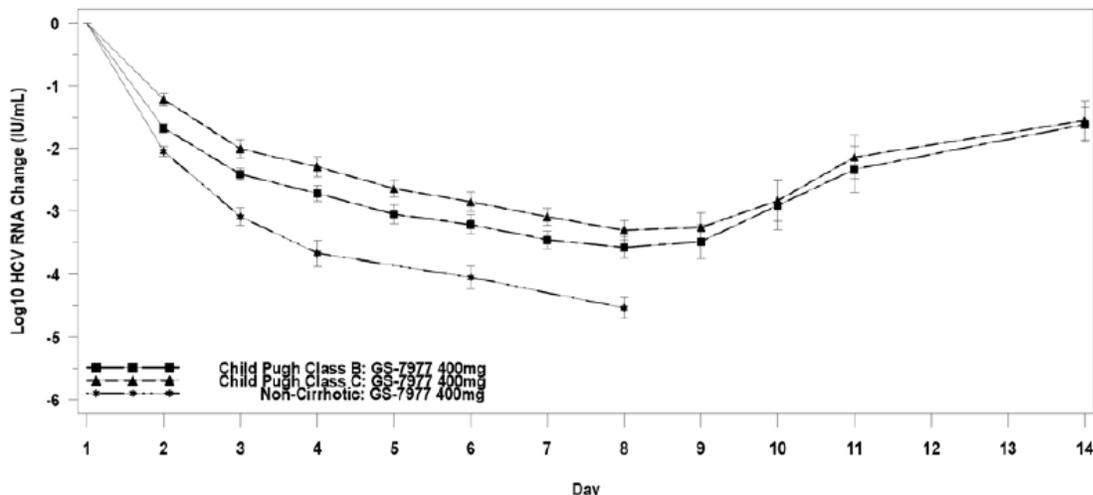
Table 7 Statistical Comparisons of GS-331007 PK Parameters for Test versus Reference Treatments

GS-331007 PK Parameters (N = 8)	Geometric Least Squares Means		Geometric Least Squares Mean Ratio (90% CI)
	Reference Treatment: Normal Hepatic Function (Study P2938-0212) ^a	Test Treatment: Moderate Hepatic Impairment	Moderate Hepatic Impairment/Normal Hepatic Function
C _{max} (ng/mL)	1358.7	1272.6	93.7 (62.6, 140.1)
AUC _{tau} (ng·h/mL)	9497.5	11162.4	117.5 (78.4, 176.3)
C _{tau} (ng/mL)	95.9	140.1	146.0 (90.8, 234.9)
	Reference Treatment: Normal Hepatic Function (Study P2938-0212) ^a	Test Treatment: Severe Hepatic Impairment	Severe Hepatic Impairment/Normal Hepatic Function
C _{max} (ng/mL)	1358.7	1230.9	90.6 (60.6, 135.5)
AUC _{tau} (ng·h/mL)	9497.5	10320.6	108.7 (72.5, 163.0)
C _{tau} (ng/mL)	95.9	104.0	108.4 (67.4, 174.4)

a Historical control subjects with normal hepatic function from Cohort 3 (Part 2) of Study P2938-0212; subjects received sofosbuvir 400 mg for 7 days

Pharmacodynamic Results: Figure 4 provides mean HCV RNA changes from baseline following multiple doses of sofosbuvir. Historical control subjects with normal hepatic function had a greater mean decrease and faster decline from baseline in HCV RNA compared with subjects with hepatic impairment in Study P2938-0515.

Figure 4 Mean (SE) HCV RNA Change From Baseline by Treatment Compared with Historical Control Subjects with Normal Hepatic Function From Study P2938-0212 Following Multiple Doses of Sofosbuvir (PD Population)



Note: Child-Pugh Class B = subjects with moderate hepatic impairment who received sofosbuvir, Child-Pugh Class C = subjects with severe hepatic impairment who received sofosbuvir, and noncirrhotic = subjects with normal hepatic function who received sofosbuvir in Study P2938-0212.

Safety: Sofosbuvir was generally well tolerated when administered to subjects with moderate or severe hepatic impairment, with no clinically significant adverse events or laboratory abnormalities.

Discussion: The higher sofosbuvir and GS-566500 exposure in hepatic impairment may be due to the decreased first-pass extraction and/or presence of portal shunts/bypasses. Despite the higher plasma exposure of sofosbuvir, modestly slower viral declines and overall smaller mean reductions in HCV RNA from baseline were observed in subjects with hepatic impairment compared with those experienced by subjects with normal hepatic function. Based on the pharmacokinetic, pharmacodynamics and safety results, no dose adjustment of sofosbuvir is recommended in subjects with mild, moderate, or severe hepatic impairment.

Conclusion: No dose adjustment of sofosbuvir is recommended in subjects with mild, moderate, or severe hepatic impairment.

4.2.3.2 P7977-0915: An Open-Label Study of Pharmacokinetics of Single Oral Doses of PSI-7977 in Subjects with Varying Degrees of Renal Function

Objectives:

- To characterize the pharmacokinetics (PK) of sofosbuvir (GS-7977; formerly PSI-7977) and metabolites after administration of single doses of sofosbuvir to subjects with varying degrees of renal impairment compared to matched healthy subjects
- To assess the safety and tolerability of single doses of sofosbuvir in subjects with varying degrees of renal impairment
- To determine the extent of removal of sofosbuvir and metabolites via hemodialysis and PK in hemodialysis subjects

Study Design: Thirty Male and female subjects with normal renal function and varying degrees of renal impairment (6 per group) were assigned to 1 of the following 5 groups based upon their

degree of renal impairment calculated using the modification of diet in renal disease (MDRD) formula to estimate the glomerular filtration rate (eGFR):

- Normal renal function group: Subjects with an eGFR > 80 mL/min/1.73 m² (Group A)
- Mild renal impairment group: Subjects with an eGFR > 50 and ≤ 80 mL/min/1.73 m² (Group B)
- Moderate renal impairment group: Subjects with an eGFR > 30 and < 50 mL/min/1.73 m² (Group C)
- Severe renal impairment group: Subjects with severe chronic renal impairment (eGFR < 30 mL/min/1.73 m²) and not on dialysis (Group D)
- End-stage renal impairment group: Subjects with end-stage renal disease (ESRD) requiring dialysis (Group E)

Subjects in the normal renal function group and in the mild, moderate, and severe renal impairment groups received 1 single oral dose of sofosbuvir 400 mg. Subjects in the end-stage renal impairment group received 2 single doses of sofosbuvir 400 mg separated by at least 2 weeks; the first treatment period (Period 1) was prior to the last dialysis session of the week and the second treatment period (Period 2) was immediately following the last dialysis session of the week.

Formulation: Sofosbuvir was administered orally as 400 mg (two 200 mg tablets, Formulation I). The lot number was OG069-P2.

PK Sampling: Serial blood samples for PK analysis (for subjects in the normal renal function and the mild, moderate, and severe renal impairment groups) were collected at: baseline (within 15 minutes prior to dosing), 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 16, 24, 48, 72, 96, and 120 hours following study drug administration.

Serial blood samples for subjects in the end-stage renal impairment group Period 1 were collected at: baseline (within 15 minutes prior to dosing), 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 16, 24, and 48 hours following study drug administration and immediately prior to next dialysis session. Samples from both the venous and arterial sides of the dialyzer were collected at the 1.5, 2, 3, 4, and 5 hour postdose timepoints. Additional samples were collected at 0.5 and 6 hours postdose for protein binding. For subjects in the end-stage renal impairment group Period 2, serial blood samples were collected at: baseline (within 15 minutes prior to dose), 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 16, 24, and 48 hours following study drug administration and immediately prior to the next dialysis session.

Timed urine collections for PK analysis occurred at 24-hour intervals postdose during the subjects stay at the clinical study unit. Baseline aliquots were also collected if available.

The entire dialysate was collected, volume was recorded, and an aliquot was taken for concentration analysis for subjects in the end-stage renal impairment group Period 1 only.

Analytical methods: Concentrations of sofosbuvir, GS-566500, and GS-331007 in plasma, urine, and dialysate samples were determined using fully validated high-performance liquid chromatography-tandem mass spectroscopy (LC/MS/MS) bioanalytical methods. All samples were analyzed in the timeframe supported by frozen stability storage data for plasma and urine. Long-term stability data for dialysates are not provided. However, because the sponsor would not recommend use of sofosbuvir in patients with ESRD at this time, the information is not

required now. The assays for sofosbuvir, GS-566500, and GS-331007 were all performed and validated by (b) (4)

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

Table 1 Summary of Quality Control (QC) Results

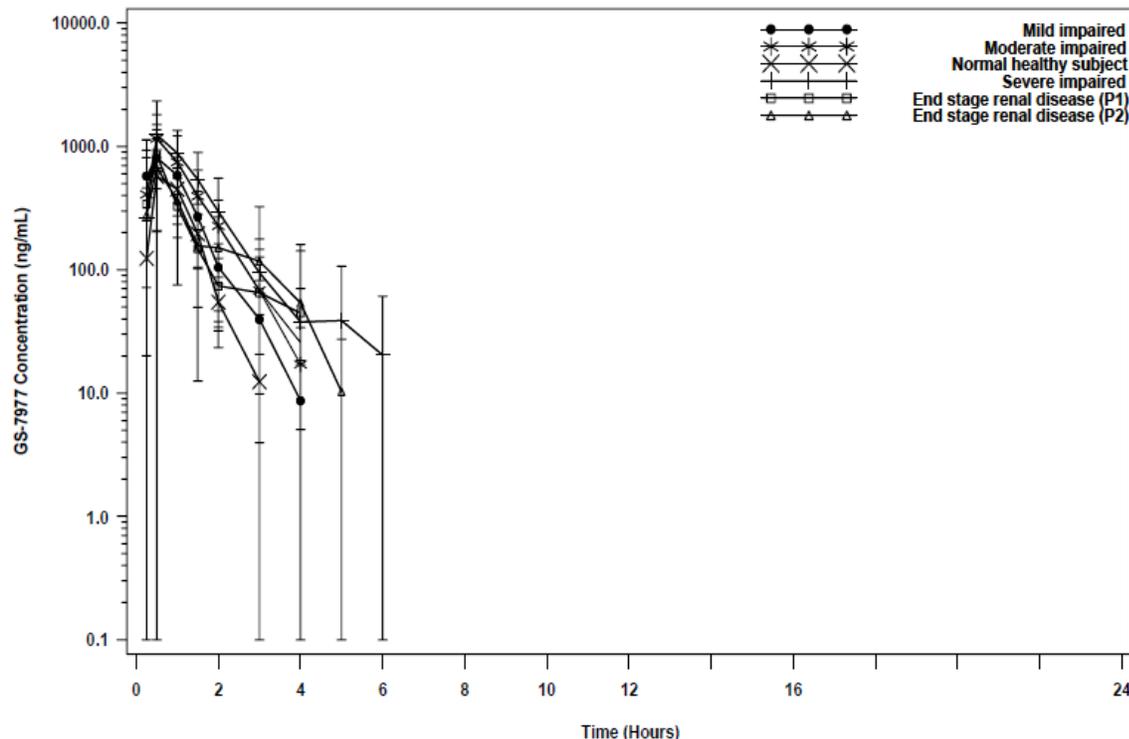
Analyte	Linear range (ng/mL)	Between Run Precision (%CV)	Between Run Bias (% Deviation)	QC samples (ng/mL)	Validation sample for stability and conditions
SOF (plasma)	5 – 5000 $R^2 > 0.993$	≤ 7.5	0.8 to 3.2	5, 15, 30, 500, and 4000	Stable for 99 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
GS-566500 (plasma)	10 – 5000 $R^2 > 0.992$	≤ 5.6	-1.8 to 3.4	10, 30, 500 and 4000	Stable for 125 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
GS-331007 (plasma)	10 – 5000 $R^2 > 0.993$	≤ 4.7	1.0 to 4.4	10, 30, 500 and 4000	Stable for 184 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
SOF (urine)	10 – 10000 $R^2 > 0.992$	≤ 8.9	0.9 to 1.8	30, 800 and 8000	Stable for 178 days at -70°C and ≥ 6 freeze/thaw cycles in plasma
GS-566500 (urine)	10 – 10000 $R^2 > 0.994$	≤ 5.3	-1.1 to 3.2	30, 800 and 8000	Stable for 133 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
GS-331007 (urine)	10 – 10000 $R^2 > 0.996$	≤ 7.2	1.8 to 6.7	30, 800 and 8000	Stable for 133 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
SOF (dialysate)	5 – 5000 $R^2 = 0.998$	n.d.	-9.3 to -5.5	5, 15, 30, 500 and 4000	Stable for ? days at -70°C and ≥ 4 freeze/thaw cycles in plasma
GS-566500 (dialysate)	10 – 5000 $R^2 = 0.999$	n.d.	-4.5 to 0	10, 30, 500 and 5000	Stable for ≥ 4 freeze/thaw cycles in plasma
GS-331007 (dialysate)	10 – 5000 $R^2 = 0.998$	n.d.	0.3 to 6.8	10, 30, 500 and 5000	Stable for ≥ 4 freeze/thaw cycles in plasma

n.d.: not determined

Pharmacokinetic Results: Sofosbuvir, GS-566500, and GS-331007 PK parameters were assessed in subjects with normal renal function; mild, moderate, and severe renal impairment; and end-stage renal disease (ESRD; during and after dialysis) after a single 400-mg dose of sofosbuvir.

Sofosbuvir: The mean sofosbuvir plasma concentration-time profiles for subjects with normal renal function; mild, moderate, and severe renal impairment; and ESRD (during and after dialysis) are shown in a semilogarithmic plot in [Figure 1](#).

Figure 1 Mean (SD) Sofosbuvir Plasma Concentration-Time Profiles for Subjects with Normal Renal Function; Mild, Moderate, and Severe Renal Impairment; and ESRD



P1-Treatment Period 1: In Period 1, subjects with ESRD received a single oral dose of study drug approximately 1 hour prior to the initiation of the last dialysis session of the week.

P2-Treatment Period 2: In Period 2, subjects with ESRD received a single oral dose of study drug immediately (within 1 h) following completion of the last dialysis session of the week.

Table 2 presents the single-dose PK parameters of sofosbuvir following administration of sofosbuvir to subjects with varying degrees of renal function. The mean sofosbuvir PK parameters V_z/F and CL/F decreased with worsening renal impairment compared with subjects with normal renal function (38% decrease for V_z/F and 64% decrease for CL/F for severe renal impairment group compared to those with normal renal function). The median $t_{1/2}$ for sofosbuvir was similar between subjects with normal renal function and mild or moderate renal impairment, and was slightly increased in subjects with severe renal impairment compared with subjects with normal renal function (median $t_{1/2}$ of 0.68 vs. 0.40 h). These data are indicative of decreased systemic clearance (evidenced by the slightly prolonged $t_{1/2}$ in severe renal impairment), and/or increased bioavailability (decreased first-pass extraction) evidenced by increased C_{max} with increased renal impairment.

Renal clearance of sofosbuvir is a minor pathway for its elimination (Study P7977-0312). Consistent with data from Study P7977-0312; in subjects with normal renal function, renal clearance accounted for approximately 1.4% of apparent oral clearance. The renal clearance of sofosbuvir (CL_r) decreased with worsening renal impairment (approximately 160, 88, 72, and 13 mL/min in subjects with normal renal function, mild, moderate, and severe renal impairment, respectively).

Table 2 Sofosbuvir Plasma and Urine Pharmacokinetic Parameters Following Administration of Sofosbuvir in Subjects with Normal Renal Function and Mild, Moderate, and Severe Renal Impairment

SOF PK Parameter	Mean (%CV)			
	Normal Renal Function (N = 6)	Mild Renal Impairment (N = 6)	Moderate Renal Impairment (N = 6)	Severe Renal Impairment (N = 6)
Plasma PK Parameters				
AUC ₀₋₂₄ (h·ng/mL)	588.80 (29.86)	961.45 (36.67)	1303.56 (50.50)	1580.91 (27.86)
AUC _{0-last} (h·ng/mL)	583.68 (29.86)	956.70 (36.90)	1297.81 (50.67)	1571.04 (28.41)
AUC _{0-inf} (h·ng/mL)	590.32 (29.93)	963.80 (36.55)	1305.10 (50.41)	1581.32 (28.14)
C _{max} (ng/mL)	715.41 (37.45)	896.21 (35.77)	1329.33 (84.03)	1305.03 (41.60)
T _{max} (h) ^a	0.50 (0.50, 1.00)	0.50 (0.25, 1.00)	0.50 (0.50, 1.00)	0.58 (0.50, 1.00)
t _{1/2} (h) ^a	0.40 (0.39, 0.47)	0.51 (0.48, 0.56)	0.46 (0.43, 0.60)	0.68 (0.49, 0.88)
V _z /F (L)	461.84 (35.98)	330.29 (36.39)	302.81 (38.92)	286.04 (43.16)
CL/F (mL/min)	12,443.59 (37.23)	7792.63 (38.05)	6391.96 (50.77)	4516.90 (29.85)
Urine PK Parameters				
CL _{renal} (mL/min)	159.85 (19.03)	87.55 (54.28)	72.13 (21.44)	13.13 (101.40)
CL _{renal} /CL/F	0.014 (23.83)	0.012 (48.76)	0.014 (43.57)	0.003 (90.98)
% Urine ^b	1.35 (24.14)	1.15 (49.16)	1.36 (43.79)	0.29 (91.24)
Overall % Urine ^b	55.86 (5.70)	61.54 (8.98)	57.34 (13.85)	43.64 (15.69)

a Median (Q1, Q3)

b Overall % Urine is the sum percents recovered for all three analytes (sofosbuvir, GS-566500, and GS-331007) corrected for molecular weight of metabolites.

Table 3 presents the single-dose PK parameters of sofosbuvir in subjects with ESRD following administration of sofosbuvir prior to and after hemodialysis. Hemodialysis modestly altered sofosbuvir mean exposure parameters, AUC, C_{max}, and CL/F (4-30% difference in the range of PK values in Period 1 relative to Period 2). Sofosbuvir V_z/F was increased by hemodialysis (approximately 47%, Period 1 relative to Period 2); however, this change should be interpreted with caution as there was a single subject exhibiting a high V_z/F in Period 2 (V_z/F for Subject 308 was 3962.69 L). Subjects with ESRD exhibited exposure (AUC_{0-inf} and C_{max}) that was more comparable to subjects with normal renal function and substantially lower than those seen in subjects with severe renal impairment. Overall, the data suggest that hemodialysis has a minimal impact on the plasma PK of sofosbuvir in subjects with ESRD.

The urinary PK parameters for sofosbuvir in subjects with ESRD were highly variable (%CV ranged from 58-114%) and unaffected by dialysis. Renal clearance was less than 0.1% of the apparent oral clearance and was substantially reduced in subjects with ESRD (CL_r was 1.8 mL/min and 0.7 mL/min in Period 1 and Period 2, respectively, compared with approximately 160 mL/min in subjects with normal renal function).

The CL_{HD} for sofosbuvir was 22.96 mL/min and the extraction ratio was 0.13 (13%). The percent of the sofosbuvir dose recovered in the dialysate was 0.13%, which indicates that hemodialysis was not a significant source of elimination of sofosbuvir from the blood. These data were consistent with the minimal changes observed in sofosbuvir plasma PK parameters.

Table 3 Sofosbuvir Plasma, Urine, and Hemodialysis Pharmacokinetic Parameters Following Administration of Sofosbuvir Prior to and After Hemodialysis in Subjects with End-Stage Renal Disease

SOF PK Parameter	Mean (%CV)	
	ESRD, Period 1 (Prior to Hemodialysis) (N = 6)	ESRD, Period 2 (After Hemodialysis) (N = 6)
Plasma PK Parameters (N = 6)		
AUC ₀₋₂₄ (h·ng/mL)	807.65 (37.86)	839.65 (45.16)
AUC _{0-last} (h·ng/mL)	798.15 (38.17)	833.07 (45.64)
AUC _{0-inf} (h·ng/mL)	785.07 (42.66) ^a	947.54 (32.89) ^a
C _{max} (ng/mL)	957.78 (60.58)	832.69 (74.33)
T _{max} (h) ^b	0.50 (0.50, 1.00)	0.79 (0.50, 2.00)
t _{1/2} (h) ^b	0.60 (0.51, 0.60) ^a	0.63 (0.41, 0.88) ^a
CL/F (mL/min)	10,120.90 (48.05) ^a	7,778.81 (36.90) ^a
V _z /F (L)	568.50 (44.99) ^a	1062.37 (153.21) ^a
Urine PK Parameters (N = 4)		
CL _{renal} (mL/min)	1.81 (70.43)	0.71 (113.79)
CL _{renal} /CL/F	< 0.001 (76.34)	< 0.001 (110.59)
% Urine	0.03 (76.00)	0.01 (111.14)
Overall % Urine ^c	10.65 (69.09)	12.21 (58.40)
Hemodialysis PK Parameters (N = 6)		
AUC _{HD} (h·ng/mL)	330.51 (84.17)	—
CL _{HD} (mL/min)	22.96 (51.27)	—
ER	0.13 (44.95)	—
% Dialysate ^c	0.13 (101.25)	—
Overall % Dialysate ^c	19.44 (30.78)	—

a n = 5

b median (Q1, Q3)

c Overall % Urine or % Dialysate are the sum percents recovered for all 3 analytes (sofosbuvir, GS-566500, and GS-331007) in urine or dialysate, corrected for the molecular weight of metabolites.

The overall mean percentage of unbound sofosbuvir in plasma was comparable in subjects with normal renal function ($17.6 \pm 2.5\%$) and subjects with ESRD in Period 1 ($15.3 \pm 2.1\%$).

The correlations between creatinine clearance (CL_{cr}) and sofosbuvir AUC_{0-inf} and C_{max} are presented in [Figure 2](#). Regression analysis of sofosbuvir AUC_{0-inf} versus CL_{cr} indicated a significant negative correlation between these parameters (Pearson correlation coefficient of -0.412 , $p = 0.026$). The slope of the regression line (-6.65) implies a predicted increase of 6.65 h·ng/mL in sofosbuvir AUC_{0-inf} for each 1 mL/min decrease in CL_{cr}. The negative correlation between sofosbuvir C_{max} and CL_{cr} was not significant ($p = 0.247$).

Figure 2 Plasma AUC_{0-inf} and C_{max} for Sofosbuvir versus Renal Function (CL_{cr}) Following Administration of Sofosbuvir in Subjects With Normal Renal Function; Mild, Moderate, and Severe Renal Impairment; and End-Stage Renal Disease

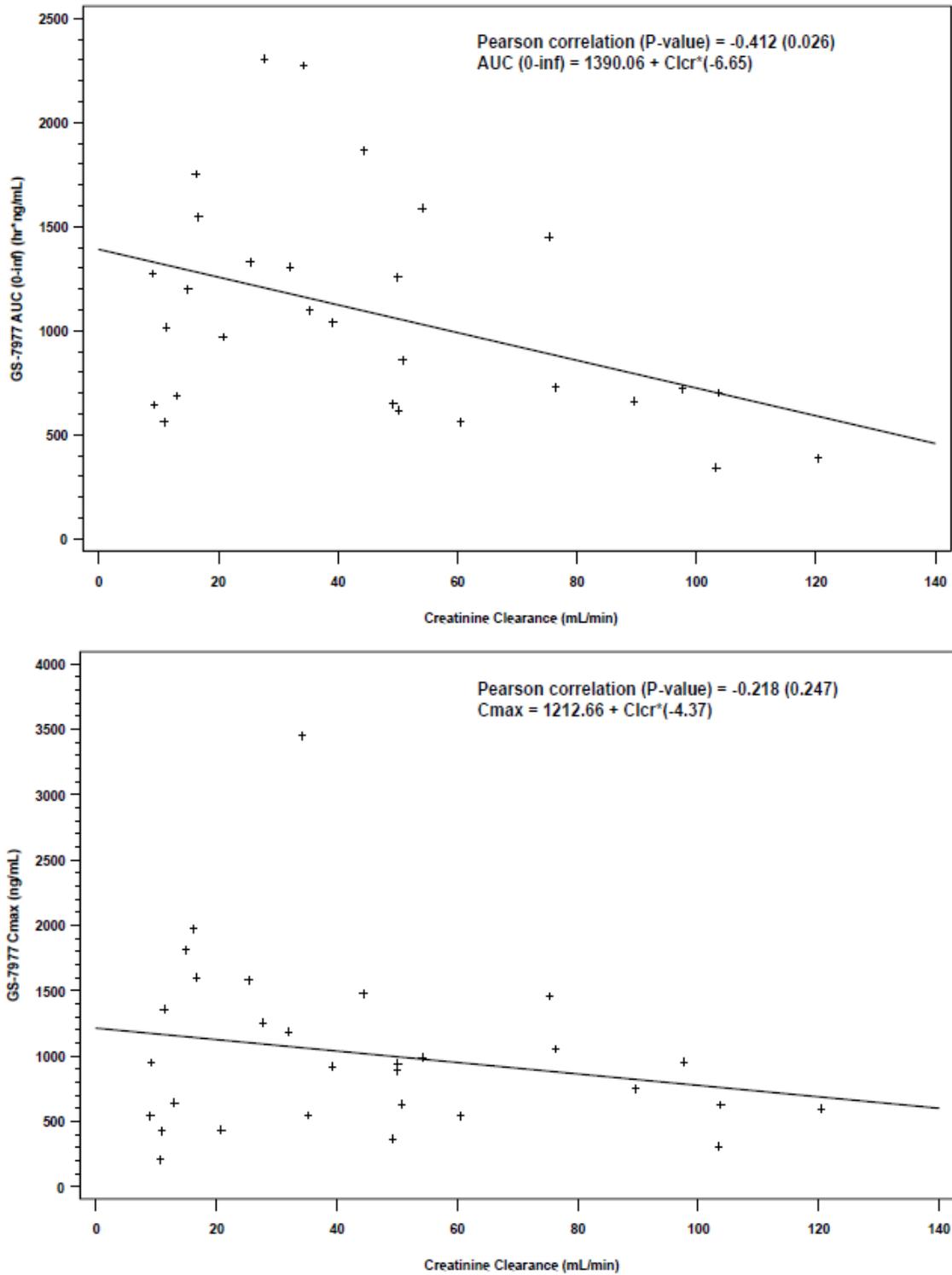


Table 4 presents the GLSM ratios and corresponding 90% CIs of the sofosbuvir PK parameters AUC_{0-inf} and C_{max} following administration of sofosbuvir in subjects with mild, moderate, and severe renal impairment and ESRD (prior to and after dialysis) compared with subjects with normal renal function. The GLSM ratios and associated 90% CIs show increased exposure of sofosbuvir in subjects with varying degrees of renal impairment compared with subjects with normal renal function.

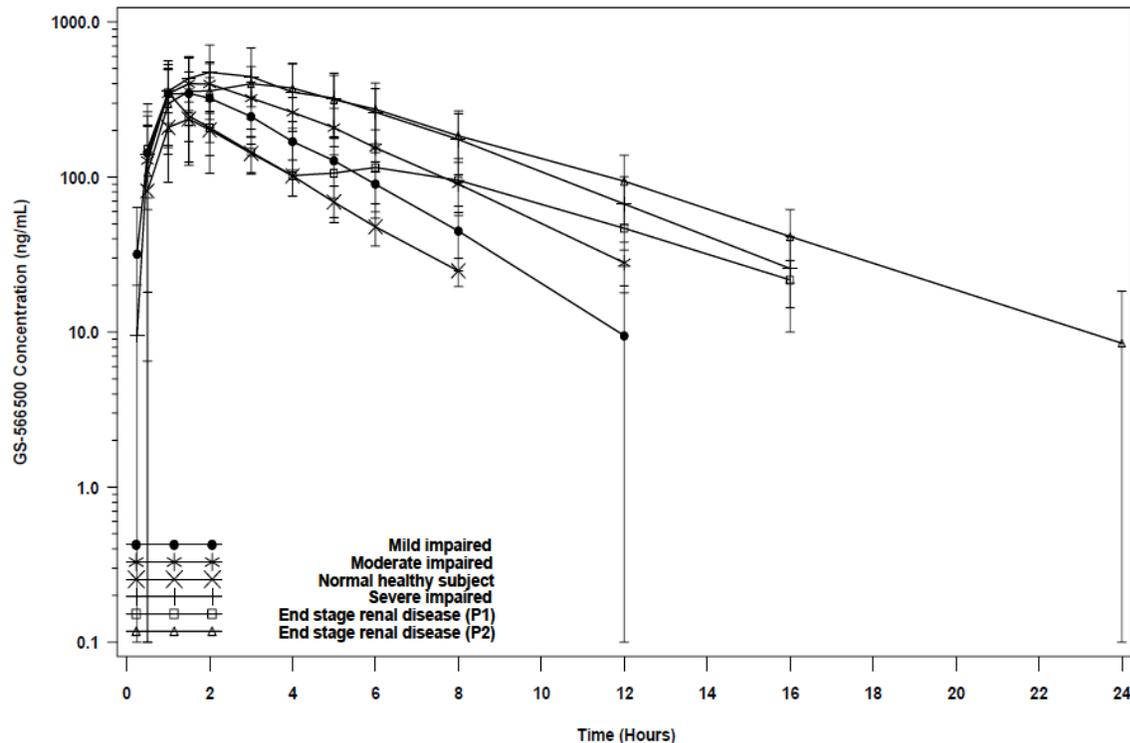
Table 4 Statistical Evaluations of Sofosbuvir Plasma Pharmacokinetic Parameters Following Administration of Sofosbuvir in Subjects with Normal Renal Function; Mild, Moderate, and Severe Renal Impairment; and End-Stage Renal Disease Prior to and After Hemodialysis

SOF PK Parameter	GLSM	%GLSM Ratio (Impaired/Normal)	90% Confidence Interval
Normal Renal Function (N = 6)			
C _{max}	666.4	—	—
AUC _{0-inf} (h· ng/mL)	564.4	—	—
Mild Renal Impairment (N = 6)			
C _{max}	852.0	127.9	(69.8, 234.2)
AUC _{0-inf} (h· ng/mL)	908.8	161.0	(108.5, 238.9)
Moderate Renal Impairment (N = 6)			
C _{max}	1022.9	153.5	(83.8, 281.2)
AUC _{0-inf} (h· ng/mL)	1167.1	206.8	(139.4, 306.8)
Severe Renal Impairment (N = 6)			
C _{max}	1178.8	176.9	(96.6, 324.1)
AUC _{0-inf} (h· ng/mL)	1528.8	270.9	(182.6, 401.9)
ESRD Period 1 (Prior to Hemodialysis) (N = 6)			
C _{max}	805.5	120.9	(66.0, 221.4)
AUC _{0-inf} (h· ng/mL)	721.7	127.9	(84.5, 193.4)
ESRD Period 2 (After Hemodialysis) (N = 6)			
C _{max}	652.3	97.9	(53.4, 179.3)
AUC _{0-inf} (h· ng/mL)	902.9	160.0	(105.8, 242.0)

GS-566500

The mean GS-566500 plasma concentration-time profiles for subjects with normal renal function; mild, moderate, and severe renal impairment; and ESRD (during and after dialysis) are shown in a semilogarithmic plot in [Figure 3](#).

Figure 3 Mean (SD) GS-566500 Plasma Concentration-Time Profiles for Subjects with Normal Renal Function; Mild, Moderate, and Severe Renal Impairment; and ESRD



P1-Treatment Period 1: In Period 1, subjects with ESRD received a single oral dose of study drug approximately 1 hour prior to the initiation of the last dialysis session of the week.

P2-Treatment Period 2: In Period 2, subjects with ESRD received a single oral dose of study drug immediately (within 1 h) following completion of the last dialysis session of the week.

Table 5 presents the single-dose PK parameters of GS-566500 following administration of sofosbuvir to subjects with varying degrees of renal function.

Table 5 GS-566500 Plasma and Urine Pharmacokinetic Parameters Following Administration of Sofosbuvir in Subjects with Normal Renal Function and Mild, Moderate, and Severe Renal Impairment

GS-566500 PK Parameter	Mean (%CV)			
	Normal Renal Function (N = 6)	Mild Renal Impairment (N = 6)	Moderate Renal Impairment (N = 6)	Severe Renal Impairment (N = 6)
Plasma Pharmacokinetic Parameters				
AUC ₀₋₂₄ (h·ng/mL)	869.33 (23.46)	1477.91 (32.16)	2115.24 (32.94)	3199.91 (41.32)
AUC _{0-last} (h·ng/mL)	832.83 (25.30)	1430.49 (32.67)	2067.99 (33.42)	3091.17 (41.68)
AUC _{0-inf} (h·ng/mL)	887.93 (22.55)	1498.82 (32.07)	2135.79 (32.23)	3218.10 (40.91)
C _{max} (ng/mL)	248.44 (25.37)	370.46 (37.02)	426.11 (36.55)	507.59 (40.49)
T _{max} (h) ^a	1.25 (1.00, 1.50)	1.00 (1.00, 2.00)	1.50 (1.50, 2.00)	2.00 (1.50, 2.00)
t _{1/2} (h) ^a	2.18 (1.90, 2.30)	2.08 (1.82, 2.30)	2.39 (2.25, 2.60)	3.02 (2.53, 3.43)
Urine Pharmacokinetic Parameters				
CL _{renal} (mL/min)	133.71 (38.71)	93.54 (26.29)	61.28 (30.07)	26.20 (71.31)
% Urine ^b	2.00 (15.43)	2.41 (23.71)	2.30 (22.10)	1.30 (50.33)

a median (Q1, Q3)

b % Urine is the percent of dose recovered as GS-566500 in the urine corrected for molecular weight of GS-566500.

Table 6 presents the single-dose PK parameters of GS-566500 in subjects with ESRD following administration of sofosbuvir prior to and after hemodialysis. Hemodialysis decreased the mean AUC_{0-inf} for GS-566500 by approximately 50% and the C_{max} for GS-566500 by approximately 22%. These data suggest that hemodialysis has a substantial impact on the removal of GS-566500 from plasma in subjects with ESRD. The CL_{HD} for GS-566500 was 144.79 mL/min, the extraction ratio was 0.68 (68%) and the percent of the sofosbuvir dose recovered in the dialysate as GS-566500 (1.77%), suggesting that hemodialysis was a source of elimination of GS-566500 from the blood. These data are consistent with the changes observed in GS-566500 plasma PK parameters.

Table 6 GS-566500 Plasma, Urine, and Hemodialysis Pharmacokinetic Parameters Following Administration of Sofosbuvir Prior to and After Hemodialysis in Subjects with End-Stage Renal Disease

GS-566500 PK Parameter	Mean (%CV)	
	ESRD Period 1 (Prior to Hemodialysis) (N = 6)	ESRD Period 2 (After Hemodialysis) (N = 6)
Plasma PK Parameters (N = 6)		
AUC ₀₋₂₄ (h·ng/mL)	1600.59 (18.50)	3237.87 (34.71)
AUC _{0-last} (h·ng/mL)	1527.09 (18.92)	3189.57 (36.16)
AUC _{0-inf} (h·ng/mL)	1647.47 (18.37)	3301.08 (35.24)
C _{max} (ng/mL)	379.67 (43.00)	484.14 (37.96)
T _{max} (h) ^a	1.00 (1.00, 1.00)	2.00 (1.50, 3.00)
t _{1/2} (h) ^a	3.73 (3.44, 3.79)	3.81 (3.38, 4.20)
Urine PK Parameters (N = 4)		
CL _{renal} (mL/min)	4.27 (101.87)	2.83 (129.06)
% Urine	0.14 (92.73)	0.16 (119.04)
Hemodialysis PK Parameters (N = 6)		
AUC _{HD} (h·ng/mL)	635.38 (20.80)	—
CL _{HD} (mL/min)	144.79 (15.24)	—
ER	0.68 (10.24)	—
% Dialysate ^b	1.77 (23.03)	—

a median (Q1, Q3)

b % Urine or Dialysate are the percent of dose recovered in urine or dialysate, corrected for molecular weight of GS-566500.

The correlations between CL_{cr} and GS-566500 AUC_{0-inf} and C_{max} are presented in [Figure 4](#). Regression analysis of GS-566500 AUC_{0-inf} versus CL_{cr} identified a significant negative correlation (Pearson correlation coefficient of -0.671; p < 0.001).

Figure 4 Plasma AUC_{0-inf} and C_{max} for GS-566500 Versus Renal Function (CL_{cr}) Following Administration of Sofosbuvir in Subjects With Normal Renal Function; Mild, Moderate, and Severe Renal Impairment; and End-Stage Renal Disease

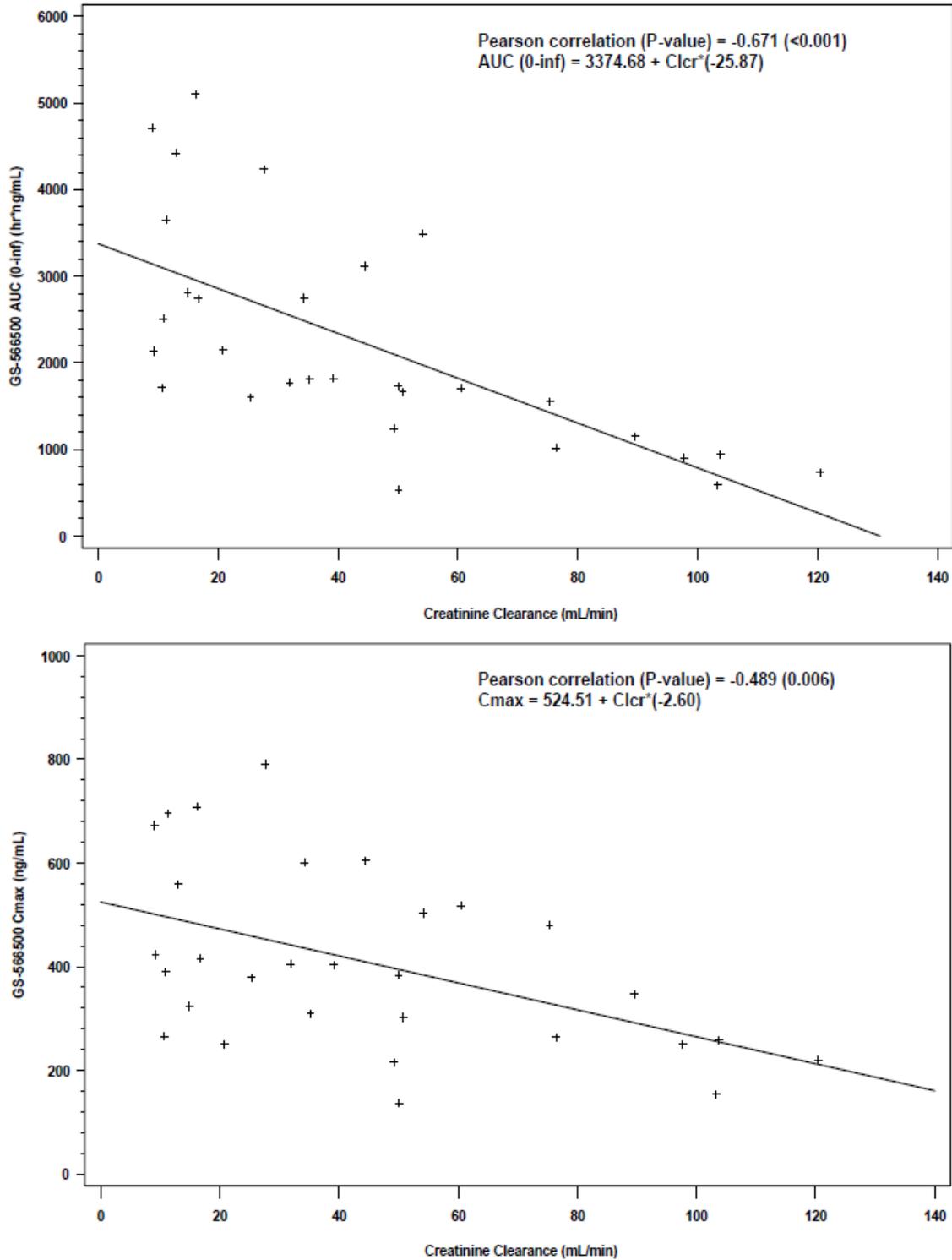


Table 7 presents the GLSM ratios and corresponding 90% CIs of GS-566500 PK parameters AUC_{0-inf} and C_{max} following administration of sofosbuvir in subjects with mild, moderate, and

severe renal impairment and ESRD (prior to and after dialysis) compared with subjects with normal renal function. The GLSM ratios and associated 90% CIs indicate increased exposure of GS-566500 in subjects with varying degrees of renal impairment compared with subjects with normal renal function.

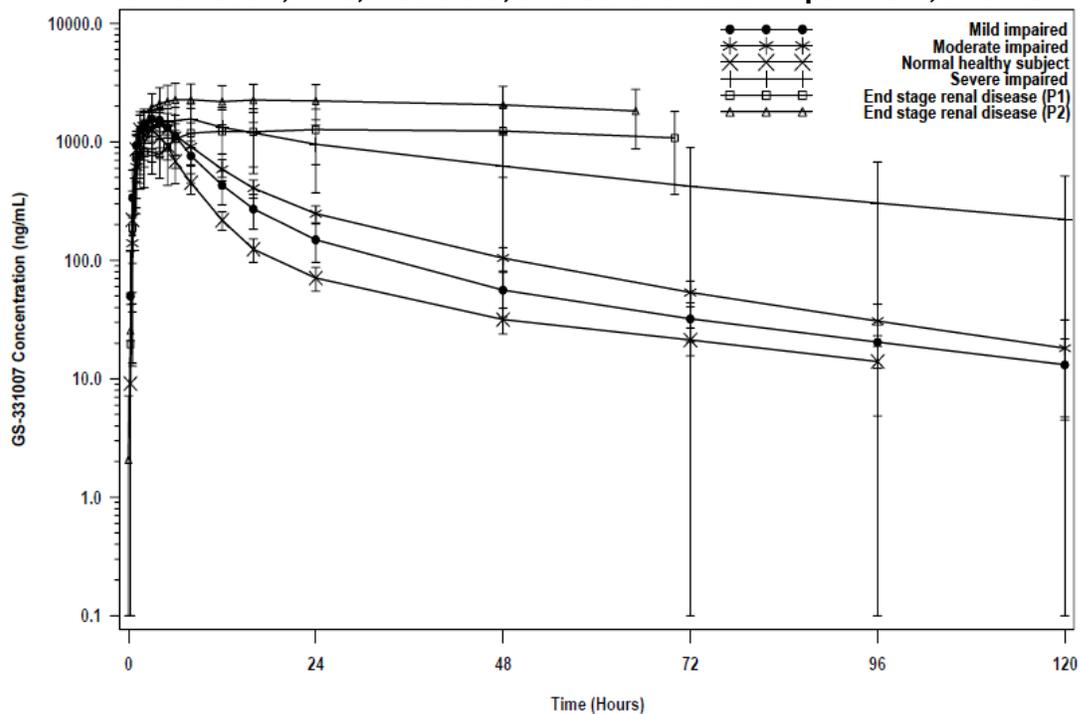
Table 7 Statistical Evaluations of GS-566500 Plasma Pharmacokinetic Parameters Following Administration of Sofosbuvir in Subjects with Normal Renal Function; Mild, Moderate, and Severe Renal Impairment; and End-Stage Renal Disease Prior to and After Hemodialysis

Normal Renal Function (N = 6)			
C_{max}	241.4	—	—
$AUC_{0-inf}(h \cdot ng/mL)$	867.7	—	—
Mild Renal Impairment (N = 6)			
C_{max}	341.5	141.5	(95.2, 210.2)
$AUC_{0-inf}(h \cdot ng/mL)$	1394.4	160.7	(113.1, 228.3)
Moderate Renal Impairment (N = 6)			
C_{max}	400.5	165.9	(111.7, 246.4)
$AUC_{0-inf}(h \cdot ng/mL)$	2042.3	235.4	(165.7, 334.4)
Severe Renal Impairment (N = 6)			
C_{max}	472.2	195.6	(131.7, 290.6)
$AUC_{0-inf}(h \cdot ng/mL)$	2985.3	344.1	(242.1, 488.9)
ESRD Period 1 (Prior to Hemodialysis) (N = 6)			
C_{max}	353.2	146.3	(98.5, 217.4)
$AUC_{0-inf}(h \cdot ng/mL)$	1620.2	186.7	131.4, 265.3
ESRD Period 2 (After Hemodialysis) (N = 6)			
C_{max}	453.7	187.9	(126.5, 279.2)
$AUC_{0-inf}(h \cdot ng/mL)$	3115.9	359.1	252.7, 510.3

GS-331007

The mean GS-331007 plasma concentration-time profiles for subjects with normal renal function; mild, moderate, and severe renal impairment; and ESRD (during and after dialysis) are shown in a semilogarithmic plot in [Figure 5](#).

Figure 5. Mean (SD) GS-331007 Plasma Concentration-Time Profiles for Subjects with Normal Renal Function; Mild, Moderate, and Severe Renal Impairment; and ESRD



P1-Treatment Period 1: In Period 1, subjects with ESRD received a single oral dose of study drug approximately 1 hour prior to the initiation of the last dialysis session of the week.

P2-Treatment Period 2: In Period 2, subjects with ESRD received a single oral dose of study drug immediately (within 1 h) following completion of the last dialysis session of the week.

Table 8 presents the single-dose PK parameters of GS-331007 following administration of sofosbuvir to subjects with varying degrees of renal impairment.

Table 8 GS-331007 Plasma and Urine Pharmacokinetic Parameters Following Administration of Sofosbuvir in Subjects with Normal Renal Function and Mild, Moderate, and Severe Renal Impairment

GS-331007 PK Parameter	Mean (%CV)			
	Normal Renal Function (N = 6)	Mild Renal Impairment (N = 6)	Moderate Renal Impairment (N = 6)	Severe Renal Impairment (N = 6)
Plasma Pharmacokinetic Parameters				
AUC ₀₋₂₄ (h· ng/mL)	9539.48 (24.21)	14,456.59 (10.53)	15,759.61 (26.24)	28,717.29 (35.45)
AUC _{0-last} (h· ng/mL)	11,865.85 (21.34)	18,573.25 (12.37)	23,063.94 (21.11)	74,414.98 (70.06)
AUC _{0-inf} (h· ng/mL)	12,743.92 (19.05)	19,644.66 (14.25)	24,100.55 (23.33)	92,563.96 (85.89)
C _{max} (ng/mL)	1357.51 (42.29)	1643.74 (16.28)	1463.61 (33.20)	1736.74 (23.03)
T _{max} (h) ^a	2.00 (1.50, 3.00)	3.00 (3.00, 4.00)	4.00 (3.03, 4.00)	6.50 (4.00, 8.00)
t _{1/2} (h) ^a	33.42 (26.63, 45.12)	35.62 (33.94, 44.33)	29.36 (25.22, 33.49)	38.01 (26.70, 48.38)
Urine Pharmacokinetic Parameters				
CL _{renal} (mL/min)	150.79 (21.99)	103.01 (18.36)	78.48 (21.38)	28.92 (68.03)
% Urine ^b	52.51 (5.44)	57.98 (10.65)	53.68 (14.69)	42.05 (14.85)

a median (Q1, Q3)

b % Urine is the percent of dose recovered as GS-331007 in the urine corrected for molecular weight of GS-331007.

Table 9 presents the single-dose PK parameters for GS-331007 in subjects with ESRD following administration of sofosbuvir prior to and after hemodialysis. Hemodialysis decreased the mean AUC₀₋₂₄ for GS-331007 by 47% and the C_{max} by 39% (AUC₀₋₂₄ rather than AUC_{0-inf} was used in this analysis due to the flat terminal elimination phase for GS-331007). These data suggest that hemodialysis has a substantial impact on the removal of GS-331007 from plasma in subjects with ESRD.

Table 9 Plasma, Urine and Hemodialysis Pharmacokinetic Parameters of GS-331007 Following Administration of Sofosbuvir Prior to and After Hemodialysis in Subjects with End-Stage Renal Disease

GS-331007 PK Parameter	Mean (%CV)	
	ESRD Period 1 (Prior to Hemodialysis) (N = 6)	ESRD Period 2 (After Hemodialysis) (N = 6)
Plasma PK Parameters (N = 6)		
AUC ₍₀₋₂₄₎ (h·ng/mL)	26,349.78 (47.43)	49,572.77 (33.99)
AUC _(0-last) (h·ng/mL)	84,894.36 (53.44)	141,055.99 (41.18)
AUC _(0-inf) (h·ng/mL)	225,947.12 (78.60) ^a	358,352.14 (70.70) ^a
C _{max} (ng/mL)	1471.85 (39.53)	2419.18 (34.97)
T _{max} (h) ^b	30.00 (3.00, 48.00)	15.00 (6.00, 24.00)
t _{1/2} (h) ^b	84.20 (45.90, 338.58) ^a	88.18 (41.08, 199.08) ^a
Urine PK Parameters (N = 4)		
CL _{renal} (mL/min)	4.60 (108.37)	5.53 (121.42)
% Urine ^c	10.48 (68.82)	12.05 (58.12)
Hemodialysis PK Parameters (N = 6)		
AUC _{HD} (h·ng/mL)	3181.59 (24.06)	–
CL _{HD} (mL/min)	178.68 (16.92)	–
ER	0.53 (5.18)	–
% Dialysate ^c	17.53 (33.70)	–

a n = 3

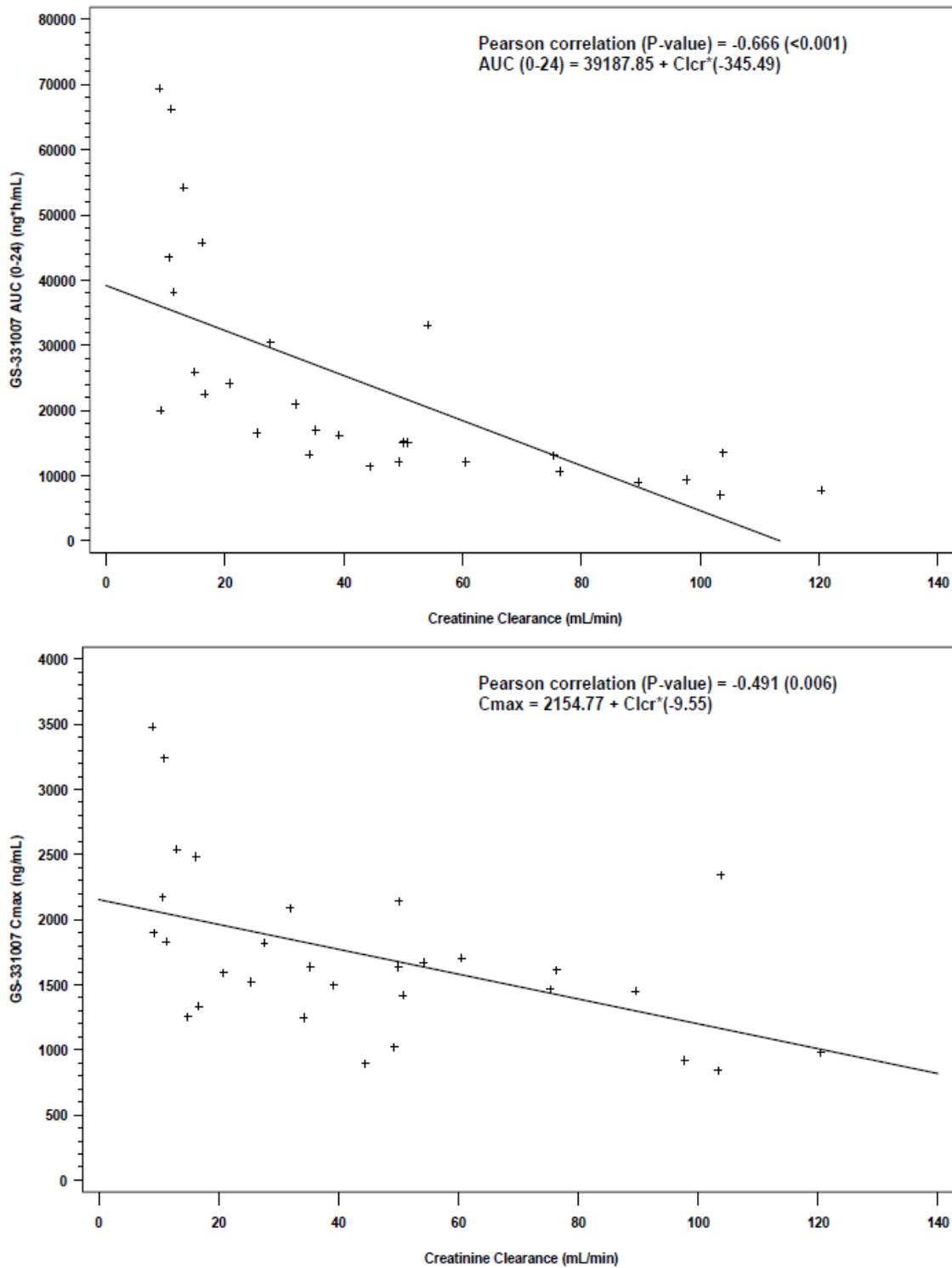
b median (Q1, Q3)

c % Urine or % Dialysate are the percent of dose recovered in urine or dialysate, corrected for molecular weight of GS-331007.

GS-331007 showed minimal binding to plasma proteins and there was no difference between subjects with normal renal function (unbound fraction: 93.3 ± 6.2%) and subjects with ESRD in Period 1 (unbound fraction: 95.5 ± 9.1%)

The correlations between CL_{cr} and GS-331007 AUC₀₋₂₄ and C_{max} are presented in [Figure 6](#). Regression analysis of GS-331007 AUC₀₋₂₄ versus CL_{cr} identified a negative correlation (Pearson correlation coefficient of -0.666; p < 0.001).

Figure 6 Plasma AUC₀₋₂₄ and C_{max} for GS-331007 Versus Renal Function (CL_{cr}) Following Administration of Sofosbuvir in Subjects With Normal Renal Function; Mild, Moderate, and Severe Renal Impairment; and End-Stage Renal Disease



[Table 10](#) presents the GLSM ratios and corresponding 90% CIs of the GS-331007 PK parameters AUC₀₋₂₄ and C_{max} following administration of sofosbuvir in subjects with mild, moderate, and severe renal impairment, and ESRD (prior to and after dialysis) compared with subjects with normal renal function. The GLSM ratios and associated 90% CIs indicate increased exposure of GS-331007 in subjects with varying degrees of renal impairment compared with subjects with normal renal function. The sponsor used AUC₀₋₂₄ rather than AUC_{0-inf} in this analysis due to the flat terminal elimination phase for GS-331007. However, the ratio estimated based on AUC₀₋₂₄ would underestimate the real magnitude of the renal effect. [Table 11](#) shows the GLSM ratios and corresponding 90% CIs of the GS-331007 AUC_{0-inf}.

Table 10 Statistical Evaluations of GS-331007 Plasma Pharmacokinetic Parameters Following Administration of Sofosbuvir in Subjects With Normal Renal Function; Mild, Moderate, and Severe Renal Impairment; and End-Stage Renal Disease Prior to and After Hemodialysis

GS-331007 PK Parameter	GLSM	%GLSM Ratio (Impaired/Normal)	90% Confidence Interval
Normal Renal Function (N = 6)			
C _{max}	1267.3	—	—
AUC ₀₋₂₄ (h· ng/mL)	9322.9	—	—
Mild Renal Impairment (N = 6)			
C _{max}	1627.3	128.4	(94.3, 174.9)
AUC ₀₋₂₄ (h· ng/mL)	14,387.0	154.3	(114.4, 208.2)
Moderate Renal Impairment (N = 6)			
C _{max}	1394.6	110.0	(80.8, 149.9)
AUC ₀₋₂₄ (h· ng/mL)	15,312.0	164.2	(121.7, 221.6)
Severe Renal Impairment (N = 6)			
C _{max}	1702.8	134.4	(98.6, 183.0)
AUC ₀₋₂₄ (h· ng/mL)	27,278.2	292.6	(216.8, 394.8)
ESRD Period 1 (Prior to Hemodialysis) (N = 6)			
C _{max}	1399.0	110.4	(81.0, 150.4)
AUC ₀₋₂₄ (h· ng/mL)	24,419.2	261.9	(194.1, 353.4)
ESRD Period 2 (After Hemodialysis) (N = 6)			
C _{max}	2286.0	180.4	(132.4, 245.7)
AUC ₀₋₂₄ (h· ng/mL)	46,957.8	503.7	(373.3, 679.6)

Table 11: Statistical Evaluations of GS-331007 AUC0-inf Following Administration of Sofosbuvir in Subjects with Normal Renal Function; Mild, Moderate, and Severe Renal Impairment; and End-Stage Renal Disease Prior to and After Hemodialysis

GS-331007 PK Parameter	GLSM	%GLSM Ratio (Impaired/Normal)	90% Confidence Interval
Normal Renal Function (N = 6)			
AUC0-inf	12541	-	-
Mild Renal Impairment (N = 6)			
AUC0-inf	19482.3	155.3	(88.3, 273.2)
Moderate Renal Impairment (N = 6)			
AUC0-inf	23601.1	188.2	(107.0, 330.9)
Severe Renal Impairment (N = 6)			
AUC0-inf	69043.1	550.5	(313.1, 968.0)
ESRD Period 1 (Prior to Hemodialysis) (N = 6)			
AUC0-inf	173461	1383.2	(692.9, 2761.0)
ESRD Period 2 (After Hemodialysis) (N = 6)			
AUC0-inf	272336.2	2171.6	(1087.9, 4334.8)

GS-331007 Cmax was 28.4%, 10.0%, and 34.4% higher in subjects with mild, moderate, and severe renal impairment, respectively. In subjects with ESRD, the Cmax for GS-331007 was 10.4 % and 80.4% higher than in subjects with normal renal function when sofosbuvir was administered prior to and after hemodialysis, respectively. GS-331007 AUC0-24 was increased 54.3%, 64.2%, and 192.6% in subjects with mild, moderate, and severe renal impairment, respectively, compared with subjects with normal renal function. In subjects with ESRD, GS-331007 AUC0-24 was increased 161.9% and 403.7% when sofosbuvir was administered prior to and after hemodialysis, respectively. GS-331007 AUC0-inf was increased 55.3%, 88.2%, and 450.5% in subjects with mild, moderate, and severe renal impairment, respectively, compared with subjects with normal renal function. In subjects with ESRD, GS-331007 AUC0-inf was increased 1283% and 2071.6% when sofosbuvir was administered prior to and after hemodialysis, respectively.

Overall, the increase in plasma exposure of sofosbuvir and its 2 circulating metabolites was less than 3-fold in subjects with mild and moderate renal impairment compared with subjects with normal renal function. Similar exposures of GS-7977 and metabolites have been observed in clinical studies including drug-drug interaction, thorough QT, safety, efficacy, and special population studies with no safety signals. Additionally, safety margins from toxicology studies continue to remain adequate; therefore, dose adjustment of sofosbuvir is not warranted in mild-to-moderate renal impairment.

Conclusion: SOF can be used in patients with mild-to-moderate renal impairment without dose adjustment, but should not be used in patients with severe RI or ESRD.

4.2.4 Extrinsic Factors

4.2.4.1 GS-US-334-0131 (DDI portion): A Phase 1, Open-label, Pharmacokinetic Drug-Drug Interaction Study between GS-7977 and Antiretrovirals Efavirenz/Emtricitabine/Tenofovir Disoproxil Fumarate (EFV/FTC/TDF), a Boosted Protease Inhibitor, Darunavir/Ritonavir (DRV/r), an Integrase Inhibitor, Raltegravir (RAL), and Non-Nucleoside Reverse Transcriptase Inhibitor, Rilpivirine (RPV)

Objectives:

- To evaluate the pharmacokinetics (PK) of sofosbuvir (GS-7977) on coadministration with efavirenz/emtricitabine/tenofovir disoproxil fumarate (EFV/FTC/TDF; Atripla® [ATR]), ritonavir (RTV; /r)-boosted darunavir (DRV/r), raltegravir (RAL), and rilpivirine (RPV) relative to administration of sofosbuvir alone
- To evaluate the PK of tenofovir (TFV), FTC, EFV, DRV, RTV, RAL, and RPV on coadministration with sofosbuvir relative to the administration of these agents alone
- To evaluate the safety and tolerability of coadministration of sofosbuvir and human immunodeficiency virus (HIV) medications ATR (nucleoside, nucleotide, and nonnucleoside reverse transcriptase inhibitors), DRV/r (boosted protease inhibitor), RAL (integrase inhibitor), and RPV (nonnucleoside reverse transcriptase inhibitor)

Study Design: Subjects were enrolled in 1 of the following 5 cohorts (only Cohorts 1 to 4 are reviewed here):

Cohort 1: Subjects received a single dose (400 mg) of sofosbuvir (Day 1; Treatment A) followed by a 3-day washout period (Days 2–4), ATR (EFV 600 mg/FTC 200 mg/TDF 300 mg once daily) for 14 days (Days 5–18; Treatment B), and a single dose of sofosbuvir+ATR (Day 19; Treatment C).

Cohort 2: Subjects received a single dose (400 mg) of sofosbuvir (Day 1; Treatment D) followed by a 3-day washout period (Days 2–4), DRV/r (800 mg/100 mg once daily) for 10 days (Days 5–14; Treatment E), and a single dose of sofosbuvir+DRV/r (Day 15; Treatment F).

Cohort 3: Subjects received a single dose (400 mg) of sofosbuvir (Day 1; Treatment G) followed by a 3-day washout period (Days 2–4), RAL (400 mg twice daily) for 10 days (Days 5–14; Treatment H), and a single dose of sofosbuvir+RAL (Day 15; Treatment I).

Cohort 4: Subjects received a single dose (400 mg) of sofosbuvir (Day 1; Treatment J) followed by a 3-day washout period (Days 2–4), RPV (25 mg once daily) for 10 days (Days 5–14; Treatment K), and a single dose of sofosbuvir+RPV (Day 15; Treatment L).

Cohort 5: Subjects received a single dose (400 mg) of (b) (4) sofosbuvir (Day 1; Treatment M) followed by a 4-day washout period (Days 2–5).

Seventy-two subjects were enrolled in the first 4 cohorts. The doses of study drug(s) and administration regimens were the same for all subjects within a cohort. For subjects who received study drug(s) under fasting conditions (Cohorts 1, 3, and 5), dosing occurred after an overnight fast of at least 8 hours. For subjects who received study drug(s) with food (Cohorts 2 and 4), dosing occurred within 5 minutes of the morning meal.

Formulation: Sofosbuvir was administered orally as 400 mg (1 x 400 mg tablets, (b) (4) The lot number was 11J111-P1. ATR, DRV, RTV, RAL, and RPV were commercially available formulation.

PK Sampling: Serial blood samples were collected relative to dosing at the following time points:

Cohort 1:

- Days 1 and 19: 0 (predose) and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 4.5, 5, 6, 8, 10, 12, 24, 36, 48, 72, and 96 hours postdose
- Day 18: 0 (predose) and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 4.5, 5, 6, 8, 10, 12, and 24 hours postdose

Cohorts 2, 3, and 4:

- Days 1 and 15 (relative to morning dose): 0 (predose) and 0.5, 1, 1.5, 2, 3, 4, 4.5, 5, 6, 8, 10, 12, 24, 36, 48, 72, and 96 hours postdose
- Day 14 (relative to morning dose): 0 (predose) and 0.5, 1, 1.5, 2, 3, 4, 4.5, 5, 6, 8, 10, 12, and 24 hours postdose

Analytical Methods: Concentrations of sofosbuvir, GS-566500, GS-331007, EFV, FTC, TFV, DRV, RTV, RPV, and RAL were determined using fully validated high-performance liquid chromatography-tandem mass spectroscopy (LC/MS/MS) bioanalytical methods. All samples were analyzed within the time frames supported by frozen stability storage data. The assays for sofosbuvir, GS-566500, GS-331007, FTC, TFV, EFV, DRV, RTV, and RPV were performed and validated by (b) (4). Raltegravir was analyzed using validated methods by (b) (4).

The standard curve and QC data indicated that the plasma assay method for GS-566500, GS-331007, EFV, FTC, TFV, DRV, RTV, RPV, and RAL were precise and accurate as shown in the following table. For SOF, there was contamination in Run 29, which led to higher between-run %CV. Samples in Run 29 were repeated in Run 36 and shows reasonable precision.

Table 1 Summary of Quality Control (QC) Results –Study GS-US-334-0131

Analyte	Linear range (ng/mL)	Between Run Precision (%CV)	Between Run Bias (% Deviation)	QC samples (ng/mL)	Validation sample for stability and conditions
SOF	5 – 5000 R ² > 0.991	≤ 26.9	-3.8 to 3.8	15, 30, 500 and 4000	Stable for 377 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
GS-566500	10 – 5000 R ² > 0.988	≤ 9.9	0.0 to 2.0	30, 500 and 4000	Stable for 308 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
GS-331007	10 – 5000 R ² > 0.991	≤ 4.7	-0.4 to 1.8	30, 500 and 4000	Stable for 308 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
EFV	5 – 5000 R ² > 0.992	≤ 7.7	0.3 to 6.6	15, 200, 750, and 4000	Stable for 127 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
FTC	5 – 3000 R ² > 0.997	≤ 2.3	-3.7 to 2.0	15, 150, 600 and 2400	Stable for 190 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
TFV	5 – 3000 R ² > 0.996	≤ 4.8	-2.5 to 3.9	15, 150, 600 and 2400	Stable for 190 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
DRV	20 -10,000 R ² > 0.993	≤ 6.8	-5.1 to 0.4	60, 800, and 9000	Stable for 301 days at -

					70°C and ≥ 4 freeze/thaw cycles in plasma
RTV	5 - 2500 R ² > 0.996	≤ 2.8	-2.8 to -1.6	15, 400 and 2000	Stable for 721 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
RPV	1 - 500 R ² > 0.996	≤ 4.9	-1.3 to 1.8	3, 40, 400	Stable for 56 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
RAL	1 - 1500 R ² > 0.997	≤ 20.6	-4.3 to 2.9	3, 100, 1200, and 7500	Stable for 1303 days at -70°C and ≥ 4 freeze/thaw cycles in plasma

Pharmacokinetic Results:

Tenofovir: Figure 1 shows mean (SD) plasma concentration-time profiles for TFV when administered as a component of ATR alone and in combination with a single dose of sofosbuvir.

Figure 1 Mean (SD) Tenofovir Plasma Concentration-Time Profiles Following Administration of Atripla Alone and in Combination with Single Dose of Sofosbuvir

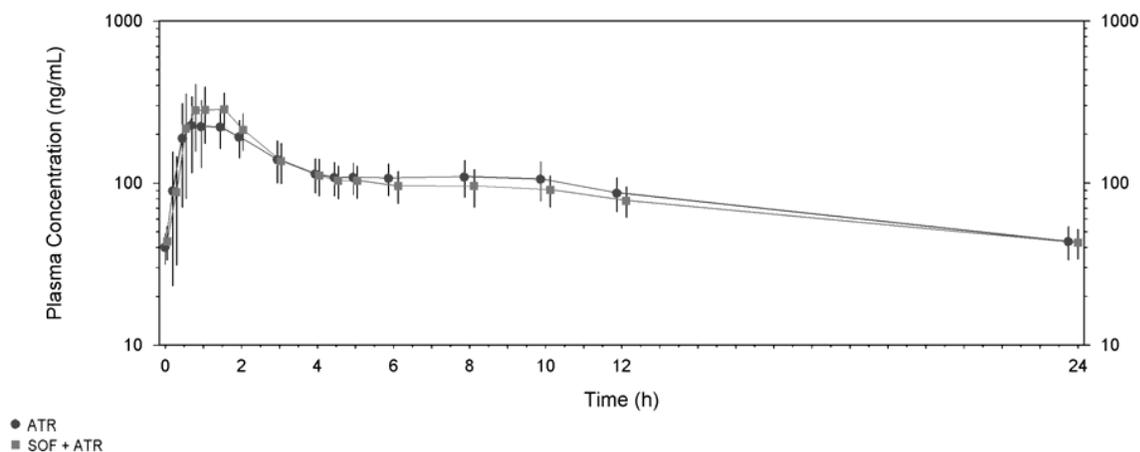


Table 2 presents TFV PK parameters following administration of ATR alone and in combination with a single dose of sofosbuvir.

Table 2 Tenofovir Plasma Pharmacokinetic Parameters Following Administration of Atripla Alone and in Combination (healthy subjects) with Single Dose of Sofosbuvir

TFV PK Parameter	Mean (%CV)	
	ATR (N = 16)	SOF+ATR (N = 16)
AUC _{tau} (h·ng/mL)	2265.7 (22.6)	2206.5 (20.3)
C _{max} (ng/mL)	274.3 (32.0)	343.0 (29.6)
C _{tau} (ng/mL)	43.7 (23.1)	42.9 (20.6)
T _{max} (h) ^a	1.25 (0.75, 1.50)	1.29 (0.75, 1.50)
t _{1/2} (h) ^a	11.64 (11.07, 11.87)	13.61 (12.70, 15.05)
T _{last} (h) ^a	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)

a Median (Q1, Q3)

Table 3 presents statistical comparisons of the primary TFV PK parameters following administration of ATR alone and when coadministered with a single dose of sofosbuvir. The 90% CIs of the GLSM ratios for TFV AUC_{tau} and C_{tau} were within 80% to 125%. However, coadministration of ATR and sofosbuvir increased TFV C_{max} by 25% compared with ATR alone (343.0 vs. 274.3 ng/mL).

Note: The sponsor indicated that no dose adjustment is necessary because TFV C_{max} increased by 30% when TDF was coadministered with elvitegravir/cobicistat and did not require TDF dose modification. The Viread label includes clinical recommendations (monitoring for tenofovir-associated adverse reactions) for those drugs that increase the AUC of TFV, or increased AUC coupled with increased C_{min} and/or C_{max}. For the two drugs (indinavir and tacrolimus) that increase only the TFV C_{max} by ~14%, no additional monitoring or precautionary language is presented in the label. Thus, a similar magnitude of change (~25%) in TFV C_{max} caused by SOF would not appear to necessitate precautionary language in the SOF label. However, it should be noted that if SOF is coadministered with Stribild™ (elvitegravir, cobicistat, emtricitabine, tenofovir disoproxil fumarate), the increase in tenofovir's exposure could be additive.

Table 3 Statistical Comparisons of Tenofovir Plasma Pharmacokinetic Parameters Following Administration of Atripla Alone and in Combination with Single Dose of Sofosbuvir

TFV PK Parameter	GLSM		%GLSM Ratio (90% CI) SOF+ATR / ATR
	ATR (N = 16)	SOF+ATR (N = 16)	
AUC _{tau} (h·ng/mL)	2213.31	2163.58	97.75 (91.09, 104.9)
C _{max} (ng/mL)	263.36	329.74	125.2 (108.4, 144.7)
C _{tau} (ng/mL)	42.59	41.99	98.61 (91.30, 106.5)

Emtricitabine: Figure 2 shows mean (SD) plasma concentration-time profiles for FTC when administered as a component of ATR alone and in combination with a single dose of sofosbuvir.

Figure 2 Mean (SD) Emtricitabine Plasma Concentration-Time Profiles Following Administration of Atripla Alone and in Combination with Single Dose of Sofosbuvir

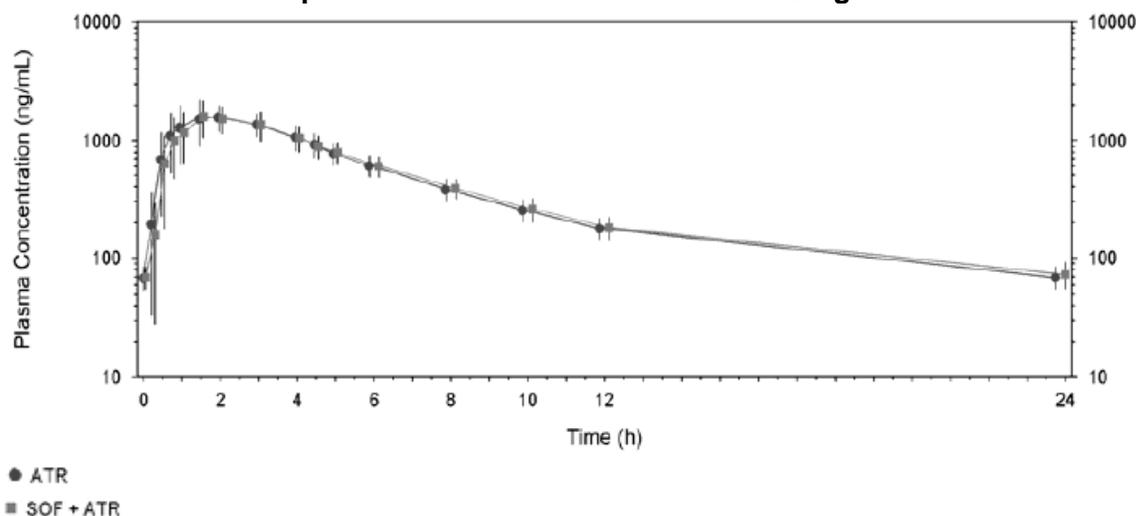


Table 4 presents FTC PK parameters following administration of ATR alone and in combination with a single dose of sofosbuvir.

Table 4 Emtricitabine Plasma Pharmacokinetic Parameters Following Administration of Atripla Alone and in Combination with Single Dose of Sofosbuvir

FTC PK Parameters	Mean (%CV)	
	ATR (N = 16)	SOF+ATR (N = 16)
AUC _{tau} (h·ng/mL)	9904.2 (17.6)	9808.8 (17.1)
C _{max} (ng/mL)	1837.1 (28.6)	1780.3 (27.2)
C _{tau} (ng/mL)	69.2 (22.0)	73.0 (25.6)
T _{max} (h) ^a	2.00 (1.50, 2.52)	1.50 (1.50, 2.50)
t _{1/2} (h) ^a	7.90 (7.13, 8.69)	8.13 (7.17, 8.91)
T _{last} (h) ^a	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)

a Median (Q1, Q3)

Table 5 presents statistical comparisons of the primary FTC PK parameters following administration of ATR alone and in combination with a single dose of SOF. The 90% CIs of the GLSM ratios for FTC AUC_{tau}, C_{max}, and C_{tau} were within the boundaries of 80% to 125%, indicating that there are not likely to be any clinically relevant effects on FTC PK when ATR is coadministered with SOF.

Table 5 Statistical Comparisons of Emtricitabine Plasma Pharmacokinetic Parameters Following Administration of Atripla Alone and in Combination with Single Dose of Sofosbuvir

FTC PK Parameter	GLSM		%GLSM Ratio (90% CI) SOF+ATR / ATR
	ATR (N = 16)	SOF+ATR (N = 16)	
AUC _{tau} (h·ng/mL)	9756.30	9672.66	99.14 (93.71, 104.9)
C _{max} (ng/mL)	1770.57	1716.31	96.94 (87.59, 107.3)
C _{tau} (ng/mL)	67.33	70.18	104.2 (97.59, 111.3)

Efavirenz: Figure 3 shows mean (SD) plasma concentration-time profiles for EFV when administered as a component of ATR alone and in combination with a single dose of sofosbuvir. The plasma concentration-time profiles for EFV were similar when ATR was administered alone and when coadministered with single dose of sofosbuvir.

Figure 3 Mean (SD) Efavirenz Plasma Concentration-Time Profiles Following Administration of Atripla Alone and in Combination with Single Dose of Sofosbuvir

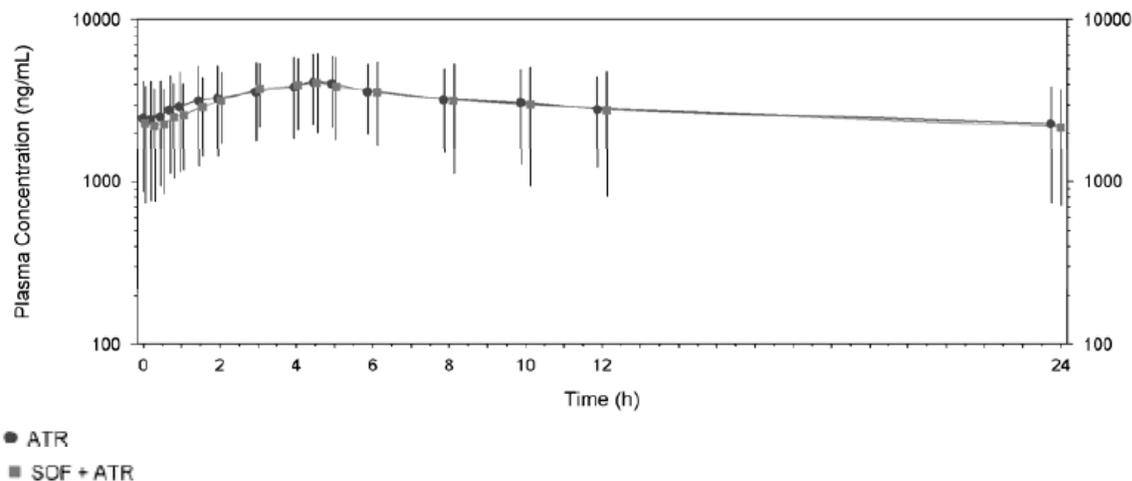


Table 6 presents EFV PK parameters following administration of ATR alone and in combination with a single dose of sofosbuvir. The measured PK parameters of EFV were similar when ATR was administered alone and in combination with sofosbuvir.

Table 6 Efavirenz Plasma Pharmacokinetic Parameters Following Administration of Atripla Alone and in Combination with Single Dose of Sofosbuvir

EFV PK Parameter	Mean (%CV)	
	ATR (N = 16)	SOF+ATR (N = 16)
AUC _{tau} (h·ng/mL)	70,905.0 (56.3)	69,114.0 (60.8)
C _{max} (ng/mL)	4407.7 (45.2)	4250.5 (50.7)
C _{tau} (ng/mL)	2312.6 (68.0)	2203.4 (67.5)
T _{max} (h) ^a	4.25 (3.00, 4.50)	4.00 (3.00, 4.50)
t _{1/2} (h) ^a	29.62 (22.84, 42.81)	28.60 (23.40, 44.10)
T _{last} (h) ^a	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)

a Median (Q1,Q3)

Table 7 presents statistical comparisons of the primary EFV PK parameters following administration of ATR alone and in combination with a single dose of sofosbuvir. The 90% CIs of the GLSM ratios for EFV AUC_{tau}, C_{max}, and C_{tau} were within the boundaries of 80% to 125%, indicating that there are not likely to be any clinically relevant effects on EFV PK when ATR is coadministered with single dose of sofosbuvir.

Table 7 Statistical Comparisons of Efavirenz Plasma Pharmacokinetic Parameters Following Administration of Atripla Alone and in Combination with Single Dose of Sofosbuvir

EFV PK Parameter	GLSM		%GLSM Ratio (90% CI) SOF+ATR / ATR
	ATR (N = 16)	SOF+ATR (N = 16)	
AUC _{tau} (h·ng/mL)	63,589.75	61,319.26	96.43 (90.66, 102.6)
C _{max} (ng/mL)	4077.08	3862.25	94.73 (84.56, 106.1)
C _{tau} (ng/mL)	1990.84	1907.40	95.81 (93.40, 98.28)

Darunavir: Figure 4 shows mean (SD) plasma concentration-time profiles for DRV when DRV/r was administered alone and in combination with a single dose of sofosbuvir. The plasma concentration-time profiles for DRV were similar when DRV/r was administered alone and when coadministered with sofosbuvir.

Figure 4 Mean (SD) Darunavir Plasma Concentration-Time Profiles Following Administration of Ritonavir-Boosted Darunavir Alone and in Combination with Single Dose of Sofosbuvir

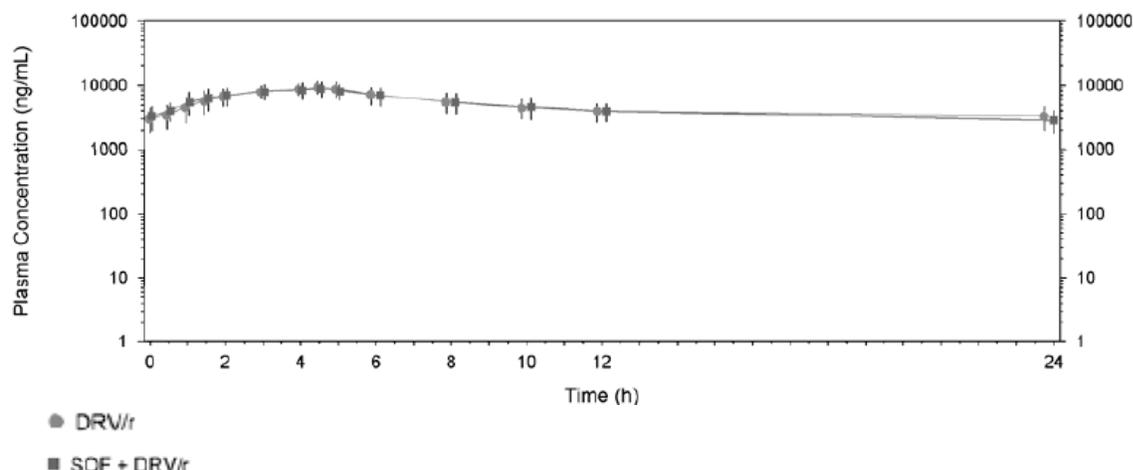


Table 8 presents DRV PK parameters following administration of DRV/r alone and in combination with a single dose of sofosbuvir. AUC_{tau}, C_{max}, C_{tau}, and T_{max} for DRV were similar when DRV/r was administered alone and when coadministered with sofosbuvir. Median t_{1/2} for DRV decreased 42% when coadministered with sofosbuvir (16.59 vs. 28.36 h); however, this difference should be interpreted with caution, as the half-life of DRV was highly variable in this study

Table 8 Darunavir Plasma Pharmacokinetic Parameters Following Administration of Ritonavir-Boosted Darunavir Alone and in Combination with Single Dose of Sofosbuvir

DRV PK Parameter	Mean (%CV)	
	DRV/r (N = 18)	SOF+DRV/r (N = 18)
AUC _{tau} (h·ng/mL)	116,335.0 (25.8)	113,161.8 (26.2)
C _{max} (ng/mL)	9740.3 (20.3)	9481.5 (19.8)
C _{tau} (ng/mL)	3328.7 (40.4)	2860.2 (38.6)
T _{max} (h) ^a	4.50 (4.00, 4.53)	4.50 (3.00, 4.50)
t _{1/2} (h) ^a	28.36 (16.25, 37.01)	16.59 (14.64, 19.56)
T _{last} (h) ^a	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)

a Median (Q1, Q3)

Table 9 presents statistical comparisons of the primary DRV PK parameters following administration of DRV/r alone and in combination with a single dose of sofosbuvir. The 90% CIs of the GLSM ratios for DRV AUC_{tau}, and C_{max} were within the predetermined equivalence boundaries of 80% to 120%, only C_{tau} has the lower 90% CI below 80%. A single dose of sofosbuvir is not expected to cause clinically relevant effects on DRV PK when the two drugs are coadministered. However, the half-life of DRV was highly variable in this study and only the effect of single dose of SOF was evaluated.

Table 9 Statistical Comparisons of Darunavir Plasma Pharmacokinetic Parameters Following Administration of Ritonavir-Boosted Darunavir Alone and in Combination with Single Dose of Sofosbuvir

DRV PK Parameter	GLSM		%GLSM Ratio (90% CI) SOF+DRV/r / DRV/r
	DRV/r (N = 18)	SOF+DRV/r (N = 18)	
AUC _{tau} (h·ng/mL)	112,759.65	109,338.18	96.97 (94.20, 99.81)
C _{max} (ng/mL)	9548.09	9290.70	97.30 (94.06, 100.7)
C _{tau} (ng/mL)	3079.40	2661.34	86.42 (77.65, 96.19)

Ritonavir: Figure 5 shows mean (SD) plasma concentration-time profiles of RTV when administered as a component of DRV/r alone and when coadministered with a single dose of sofosbuvir. The plasma concentration-time profiles for RTV were similar when DRV/r was administered alone and coadministered with sofosbuvir.

Figure 5 Mean (SD) Ritonavir Plasma Concentration-Time Profiles Following Administration of Ritonavir-Boosted Darunavir Alone and in Combination with Single Dose of Sofosbuvir

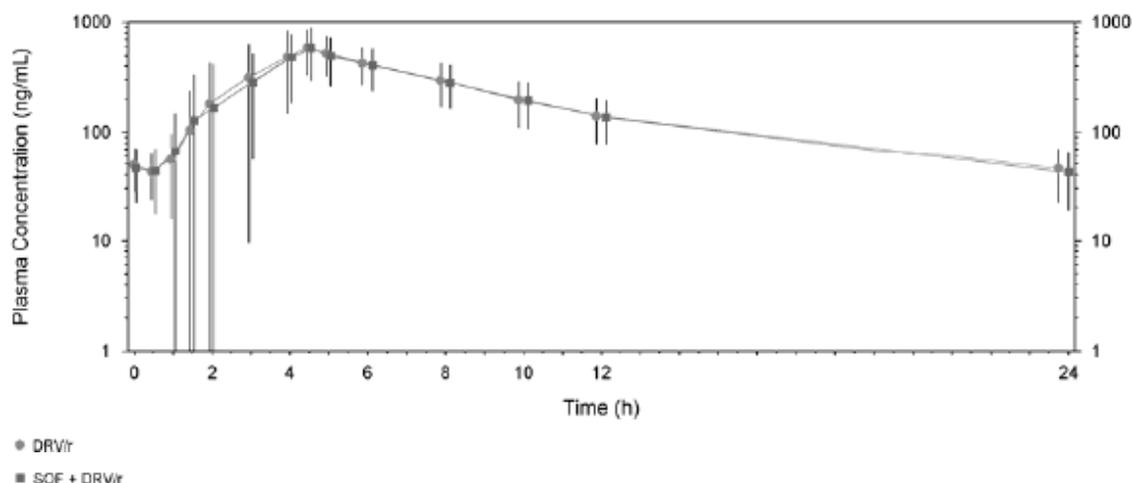


Table 10 presents RTV PK parameters following administration of DRV/r alone and in combination with a single dose of sofosbuvir.

Table 10 Ritonavir Plasma Pharmacokinetic Parameters Following Administration of Ritonavir-Boosted Darunavir Alone and in Combination with Single Dose of Sofosbuvir

RTV PK Parameter	Mean (%CV)	
	DRV/r (N = 18)	SOF+DRV/r (N = 18)
AUC _{tau} (h ng/mL)	4399.6 (45.2)	4217.1 (46.3)
C _{max} (ng/mL)	630.4 (50.2)	608.5 (46.4)
C _{tau} (ng/mL)	45.8 (51.5)	42.1 (54.9)
T _{max} (h) ^a	4.50 (4.50, 5.00)	4.50 (4.50, 4.50)
t _{1/2} (h) ^a	5.95 (5.62, 6.42)	5.83 (5.51, 6.14)
T _{last} (h) ^a	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)

a Median (Q1, Q3)

Table 11 presents statistical comparisons of the primary RTV PK parameters following administration of DRV/r alone and in combination with a single dose of sofosbuvir. The 90% CIs of the GLSM ratios for RTV AUC_{tau}, C_{max}, and C_{tau} were within the predetermined equivalence boundaries of 70% to 143%, indicating that there were no clinically relevant effects on RTV PK when DRV/r was coadministered with single dose of sofosbuvir.

Table 11 Statistical Comparisons of Ritonavir Plasma Pharmacokinetic Parameters Following Administration of Ritonavir-Boosted Darunavir Alone and in Combination with Single Dose of Sofosbuvir

RTV PK Parameter	GLSM		%GLSM Ratio (90% CI) SOF+DRV/r / DRV/r
	DRV/r (N = 18)	SOF+DRV/r (N = 18)	
AUC _{tau} (h ng/mL)	4056.03	3852.68	94.99 (88.15, 102.4)
C _{max} (ng/mL)	576.37	546.58	94.83 (84.35, 106.6)
C _{tau} (ng/mL)	40.22	36.75	91.38 (86.05, 97.03)

Raltegravir: Figure 6 shows mean (SD) plasma concentration-time profiles for RAL when administered alone and when coadministered with a single dose of sofosbuvir. The mean RAL concentration-time profiles were variable, with an apparent elongation of the distribution phase of the profile when RAL was coadministered with sofosbuvir. The profiles should be interpreted with caution, as the variability of RAL concentration was as high as 219% CV.

Figure 6 Mean (SD) Raltegravir Plasma Concentration-Time Profiles Following Administration of Raltegravir Alone and in Combination with Single Dose of Sofosbuvir

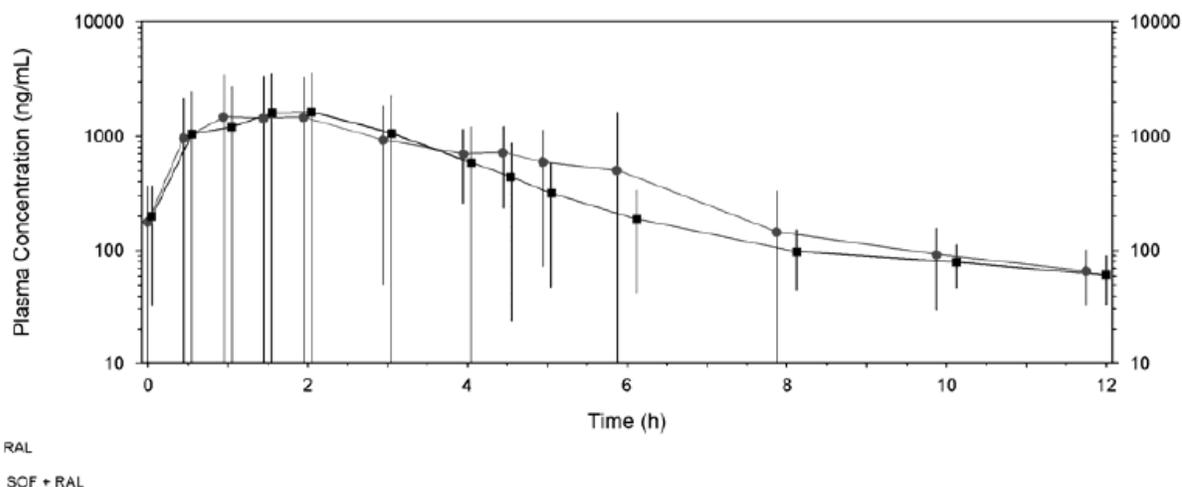


Table 12 presents RAL PK parameters following administration of RAL alone and in combination with a single dose of sofosbuvir.

Table 12 Raltegravir Plasma Pharmacokinetic Parameters Following Administration of Raltegravir Alone and in Combination with Single Dose of Sofosbuvir

RAL PK Parameter	Mean (%CV)	
	RAL (N = 19)	SOF+RAL (N = 19)
AUC _{tau} (h ng/mL)	6480.2 (74.6)	5785.9 (100.1)
C _{max} (ng/mL)	2346.2 (83.9)	1839.2 (113.2)
C _{tau} (ng/mL)	66.6 (51.0)	61.7 (45.6)
T _{max} (h) ^a	2.00 (1.00, 4.50)	1.50 (1.00, 2.00)
t _{1/2} (h) ^a	3.39 (2.89, 5.51)	5.70 (3.29, 9.58)
T _{last} (h) ^a	12.00 (12.00, 12.00)	12.00 (12.00, 12.00)

a Median (Q1, Q3)

Table 13 presents statistical comparisons of the primary RAL PK parameters following administration of RAL alone and in combination with a single dose of sofosbuvir. 90% CIs of the GLSM ratios for RAL C_{tau} were within the prespecified boundary of 80% to 125%. The 90% CIs for AUC_{tau} and C_{max} were not within the boundary. The GLSM ratios for RAL AUC_{tau} and C_{max} decreased 27% and 43%, respectively, when RAL was coadministered with sofosbuvir. The decrease in RAL exposure was comparable to that observed when RAL was coadministered with tipranavir/r (18%, 24%, and 54% decreases in C_{max}, AUC₀₋₁₂, and C₁₂, respectively) or EFV (36%, 36%, and 21% decrease in C_{max}, AUC_{inf}, and C₁₂, respectively), which did not necessitate RAL dose adjustment, and suggests that the decreases observed in AUC_{tau} and C_{max} when RAL is coadministered with sofosbuvir do not necessitate RAL dose adjustment. In addition, the RAL dose may not need adjustment when coadministered with SOF because raltegravir has highly variable PK with a wide therapeutic window. Furthermore, preliminary results from the ongoing Phase 3 study in HIV/HCV co-infected subjects (GS-US-334-0123) show that 45 of the 46 subjects on a SOF plus raltegravir-containing ARV regimen

have maintained HIV virologic suppression. A single subject (Subject 4262-8725; HCV genotype 3) receiving ARV treatment with raltegravir and emtricitabine/tenofovir DF had HIV-1 virologic rebound during the study. For this subject, HIV-1 RNA was not detected from baseline through Week 8, but was detected at Week 12 (no Week 10 assessment). Per the investigator (data on file), this subject had poor adherence to HIV medications at the time of HIV virologic rebound. In addition, this subject had HCV virologic relapse and may not have adhered to study drug, as evidenced by a lack of decline in hemoglobin concentration and a lack of increase in reticulocyte count during the treatment period.

Table 13 Statistical Comparisons of Raltegravir Plasma Pharmacokinetic Parameters Following Administration of Raltegravir Alone and in Combination with Sofosbuvir

RAL PK Parameter	GLSM		%GLSM Ratio (90% CI) SOF+RAL / RAL
	RAL (N = 19)	SOF+RAL (N = 19)	
AUC _{tau} (h·ng/mL)	4739.90	3464.51	73.09 (58.65, 91.09)
C _{max} (ng/mL)	1536.39	881.89	57.40 (43.69, 75.41)
C _{tau} (ng/mL)	59.19	56.25	95.04 (80.98, 111.6)

Rilpivirine: Figure 7 shows mean (SD) plasma concentration-time profiles for RPV when administered alone and when coadministered with a single dose of sofosbuvir. The plasma concentration-time profiles for RPV were similar when RPV was administered alone and in combination with single dose of sofosbuvir.

Figure 7 Mean (SD) Rilpivirine Plasma Concentration-Time Profiles Following Administration of Rilpivirine Alone and in Combination with Single Dose of Sofosbuvir

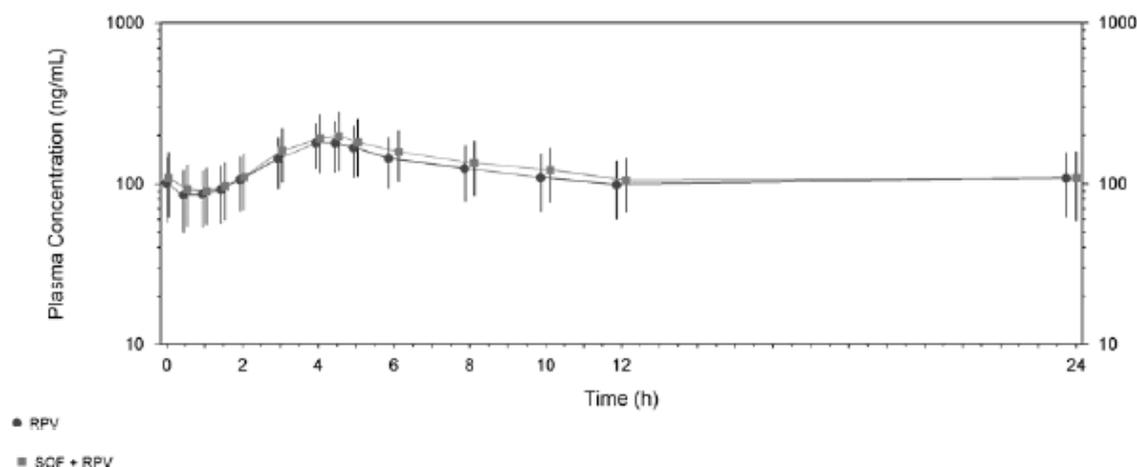


Table 14 presents RPV PK parameters following administration of RPV alone and in combination with a single dose of sofosbuvir. The measured PK parameters of RPV were similar when RPV was administered alone and when coadministered with single dose of sofosbuvir.

Table 14 Rilpivirine Plasma Pharmacokinetic Parameters Following Administration of Rilpivirine Alone and in Combination with Single Dose of Sofosbuvir

RPV PK Parameter	Mean (%CV)	
	RPV (N = 17)	SOF+RPV (N = 17)
AUC _{tau} (h·ng/mL)	2771.1 (36.7)	2927.8 (37.1)
C _{max} (ng/mL)	195.1 (28.8)	210.3 (35.5)
C _{tau} (ng/mL)	109.4 (43.1)	108.7 (45.8)
T _{max} (h) ^a	4.00 (4.00, 4.50)	4.50 (4.00, 4.50)
t _{1/2} (h) ^a	42.21 (28.41, 51.88) ^b	31.39 (26.04, 39.64)
T _{last} (h) ^a	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)

a Median (Q1, Q3)

b n = 16

Table 15 presents statistical comparisons of the primary RPV PK parameters following administration of RPV alone and in combination with a single dose of sofosbuvir. The 90% CIs of the GLSM ratios for RPV AUC_{tau}, C_{max}, and C_{tau} were within the boundaries of 80% to 125%, indicating that there are not likely to be any clinically relevant effects on RPV PK when RPV is coadministered with sofosbuvir.

Table 15. GS-US-334-0131: Statistical Evaluations of Rilpivirine Plasma Pharmacokinetic Parameters Following Administration of Rilpivirine Alone and in Combination with Sofosbuvir

RPV PK Parameter	GLSM		%GLSM Ratio (90% CI) SOF+RPV / RPV
	RPV (N = 17)	SOF+RPV (N = 17)	
AUC _{tau} (h·ng/mL)	2592.37	2733.78	105.5 (102.3, 108.7)
C _{max} (ng/mL)	188.09	198.04	105.3 (96.66, 114.7)
C _{tau} (ng/mL)	99.30	98.16	98.86 (94.44, 103.5)

Sofosbuvir: Figure 8 shows mean (SD) plasma concentration-time profiles for a single dose of sofosbuvir administered alone and in combination with ATR dosed to steady state (both under fasting conditions). The plasma concentration-time profiles for single dose of sofosbuvir were similar when sofosbuvir was administered alone or in combination with ATR.

Figure 8 Mean (SD) Sofosbuvir Plasma Concentration-Time Profiles Following Administration of Single Dose of Sofosbuvir Alone and in Combination with Atripla

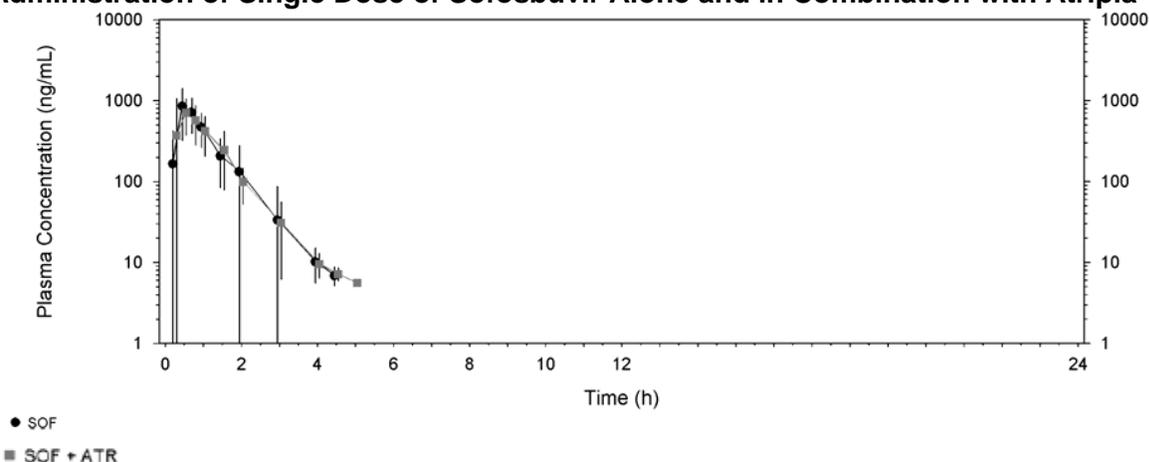


Figure 9 shows mean (SD) plasma concentration-time profiles for a single dose of sofosbuvir administered alone and in combination with DRV/r dosed to steady state (both under fed conditions). Sofosbuvir plasma concentration reached C_{max} at median T_{max} of 2.0 h (consistent with administration of sofosbuvir with food) and then rapidly decreased in a monoexponential manner. The shape of the composite curve in Figure 9 should be interpreted with caution, as the 10- and 12-hour time points are representative of 3 and 1 subjects, respectively (for all other subjects, plasma concentration was BLQ).

Figure 9 Mean (SD) Sofosbuvir Plasma Concentration-Time Profiles Following Administration of Sofosbuvir Single Dose Alone and in Combination with Ritonavir-Boosted Darunavir

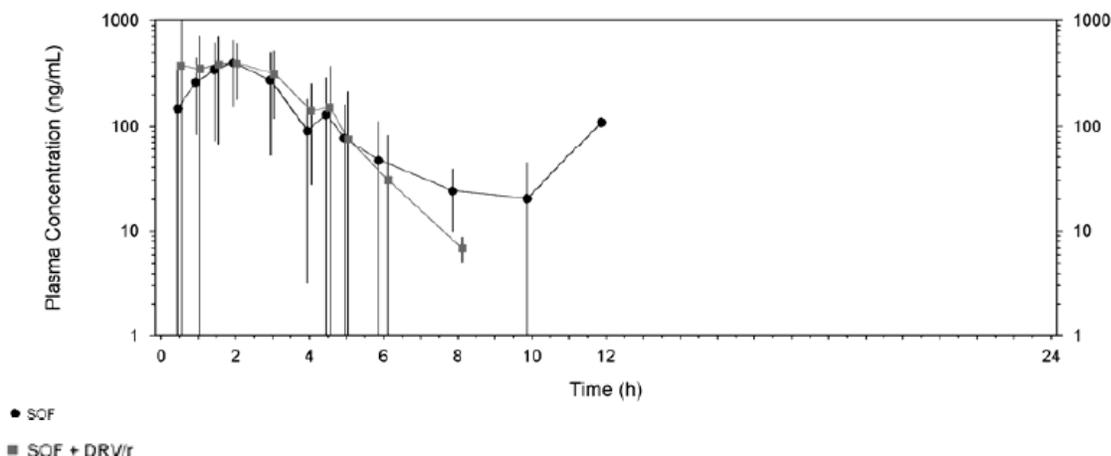


Figure 10 shows mean (SD) plasma concentration-time profiles for a single dose of sofosbuvir administered alone and in combination with RAL dosed to steady state (both under fasting conditions). The plasma concentration-time profiles for SOF were similar when SOF was administered alone and when coadministered with RAL.

Figure 10 Mean (SD) Sofosbuvir Plasma Concentration-Time Profiles Following Administration of Single Dose of Sofosbuvir Alone and in Combination with Raltegravir

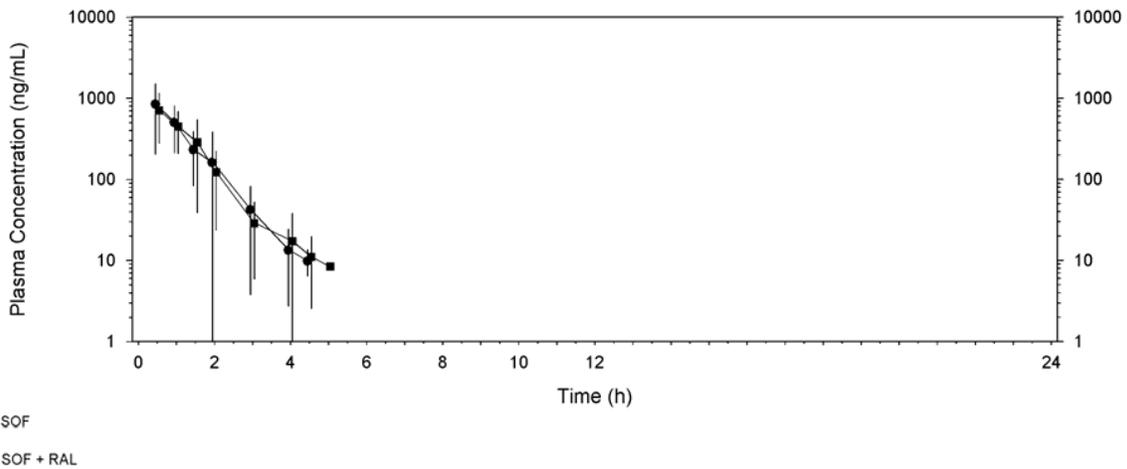
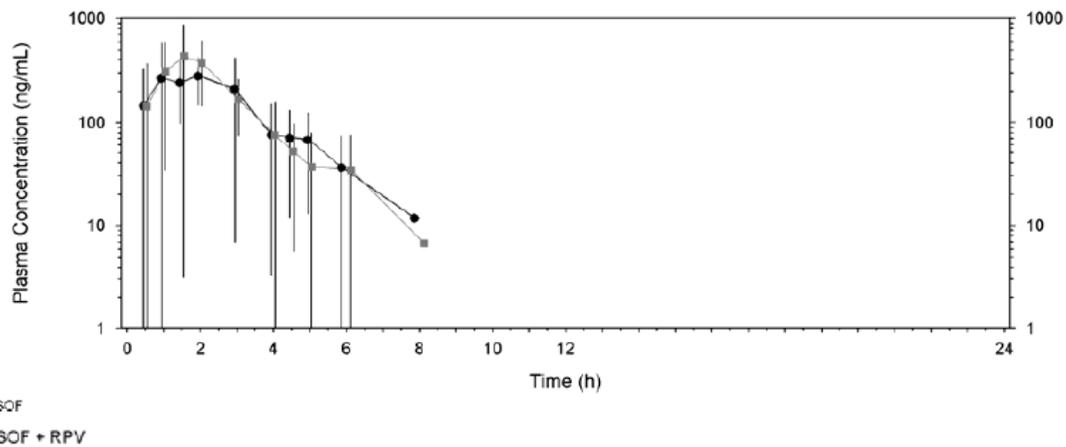


Figure 11 shows mean (SD) plasma concentration-time profiles for a single dose of sofosbuvir administered alone and in combination with RPV dosed to steady state (both under fed conditions).

Figure 11 Mean (SD) Sofosbuvir Plasma Concentration-Time Profiles Following Administration of Single Dose of Sofosbuvir Alone and in Combination with Rilpivirine



Concentration-time profiles show that there is large inter-subject variability of SOF concentrations for most time points.

Table 16 presents sofosbuvir plasma PK parameters following administration of a single dose of sofosbuvir alone and in combination with ATR, DRV/r, RAL, or RPV.

Table 16 Sofosbuvir Plasma Pharmacokinetic Parameters Following Administration of Single Dose of Sofosbuvir Alone and in Combination with Antiretroviral Agents

SOF PK Parameter	Cohort 1 Mean (%CV)		Cohort 2 Mean (%CV)	
	SOF (N = 17)	SOF+ATR (N = 16)	SOF (N = 19)	SOF+DRV/r (N = 18)
AUC _{last} (h·ng/mL)	822.3 (42.3)	772.8 (41.7)	1093.7 (48.5)	1318.6 (30.9)
AUC _{inf} (h·ng/mL)	828.3 (41.9)	779.8 (41.1)	1140.4 (51.8)	1327.9 (30.6)
C _{max} (ng/mL)	1023.2 (47.7)	877.1 (64.0)	544.4 (48.3)	773.4 (55.8)
T _{max} (h) ^a	0.50 (0.50, 0.75)	0.50 (0.50, 0.75)	2.00 (1.02, 2.00)	2.00 (1.50, 3.00)
t _{1/2} (h) ^a	0.43 (0.40, 0.47)	0.48 (0.40, 0.53)	0.58 (0.48, 0.88)	0.52 (0.46, 0.63)
SOF PK Parameter	Cohort 3 Mean (%CV)		Cohort 4 Mean (%CV)	
	SOF (N = 19)	SOF+RAL (N = 19)	SOF (N = 17)	SOF+RPV (N = 17)
AUC _{last} (h·ng/mL)	868.7 (51.4)	800.4 (39.7)	833.2 (31.2)	935.9 (38.5)
AUC _{inf} (h·ng/mL)	876.1 (50.9)	806.8 (39.3)	846.7 (30.2)	943.5 (37.9)
C _{max} (ng/mL)	942.9 (58.5)	806.8 (45.1)	462.9 (56.4)	587.5 (66.6)
T _{max} (h) ^a	0.50 (0.50, 1.00)	0.50, (0.50, 1.00)	2.00 (1.00, 3.00)	1.50 (1.50, 2.00)
t _{1/2} (h) ^a	0.43 (0.36, 0.48)	0.41 (0.33, 0.48)	0.63 (0.44, 0.86)	0.46 (0.36, 0.63)

a Median (Q1, Q3)

Table 17 presents statistical comparisons of the primary sofosbuvir PK parameters following administration of a single dose of sofosbuvir alone and in combination with ATR, DRV/r, RAL, or RPV. Coadministration with ATR decreased sofosbuvir C_{max} by 19%, which was not considered a clinically relevant change. Coadministration of DRV/r increased sofosbuvir AUC_{last}, AUC_{inf}, and C_{max} by 37%, 34%, and 45%, respectively. The effect of DRV/r on the PK of sofosbuvir was likely a result of Pgp inhibition by DRV/r. The increases are not considered clinically relevant. Coadministration of sofosbuvir and RPV was associated with a 20% increase in sofosbuvir C_{max} that was not considered clinically relevant.

Table 17 Statistical Comparisons of Sofosbuvir Plasma Pharmacokinetic Parameters Following Administration of Single Dose of Sofosbuvir Alone and in Combination with Antiretroviral Agents

SOF PK Parameters	GLSM		%GLSM Ratio (90% CI)
	SOF+ATR (N = 16)	SOF (N = 16)	
Cohort 1			SOF+ATR / SOF
AUC _{last} (h·ng/mL)	719.28	766.90	93.79 (75.39, 116.7)
AUC _{inf} (h·ng/mL)	727.43	773.37	94.06 (76.02, 116.4)
C _{max} (ng/mL)	760.01	939.77	80.87 (59.62, 109.7)
Cohort 2	SOF+DRV/r (N = 18)	SOF (N = 18)	SOF+DRV/r / SOF
AUC _{last} (h·ng/mL)	1258.69	918.43	137.05 (115.7, 162.4)
AUC _{inf} (h·ng/mL)	1268.56	949.09	133.66 (112.1, 159.4)
C _{max} (ng/mL)	683.95	471.29	145.12 (109.78, 191.9)
Cohort 3	SOF+RAL (N = 19)	SOF (N = 19)	SOF+RAL / SOF
AUC _{last} (h·ng/mL)	742.51	784.33	94.67 (81.80, 109.6)
AUC _{inf} (h·ng/mL)	749.79	792.87	94.57 (81.87, 109.2)
C _{max} (ng/mL)	729.07	835.59	87.25 (70.75, 107.6)
Cohort 4	SOF+RPV (N = 17)	SOF (N = 17)	SOF+RPV / SOF
AUC _{last} (h·ng/mL)	873.81	793.73	110.1 (93.94, 129.0)
AUC _{inf} (h·ng/mL)	883.07	809.15	109.1 (93.57, 127.3)
C _{max} (ng/mL)	480.88	398.88	120.6 (89.64, 162.1)

GS-566500: Figure 12 shows mean (SD) plasma concentration-time profiles for GS-566500 following administration of a single dose of sofosbuvir alone and in combination with ATR dosed to steady state (both under fasting conditions). GS-566500 plasma concentration rapidly reached C_{max} at median T_{max} of 1.0 h to 1.5 h (consistent with administration of sofosbuvir under fasting conditions) and then rapidly decreased in a monoexponential manner.

Figure 12 Mean (SD) GS-566500 Plasma Concentration-Time Profiles Following Administration of Single Dose of Sofosbuvir Alone and in Combination with Atripla

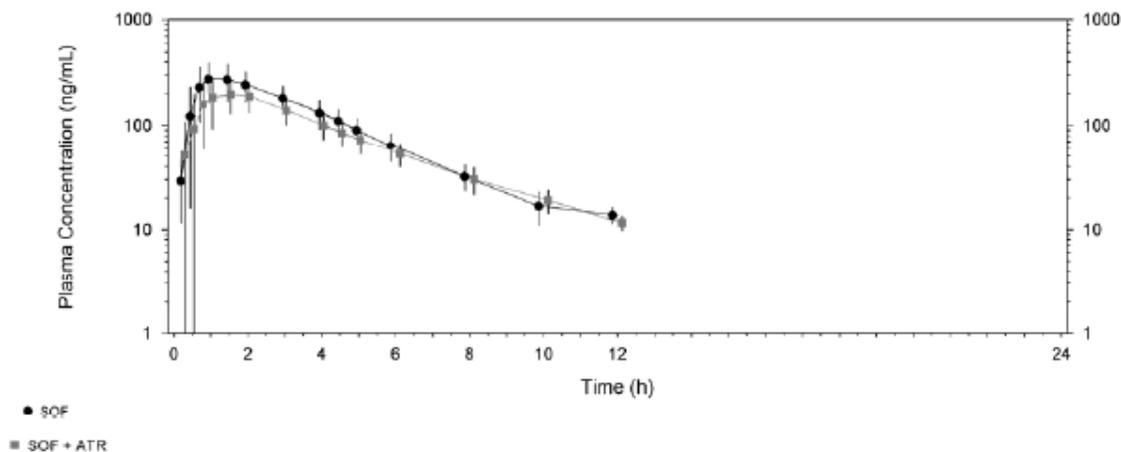


Figure 13 shows mean (SD) plasma concentration-time profiles for GS-566500 following administration of a single dose of sofosbuvir alone and in combination with DRV/r dosed to steady state (both under fed conditions). GS-566500 plasma concentration reached C_{max} at median T_{max} of 3.0 h (consistent with administration of sofosbuvir with food) and then rapidly decreased in a monoexponential manner.

Figure 13 Mean (SD) GS-566500 Plasma Concentration-Time Profiles Following Administration of Sofosbuvir Alone and in Combination with Ritonavir-Boosted Darunavir

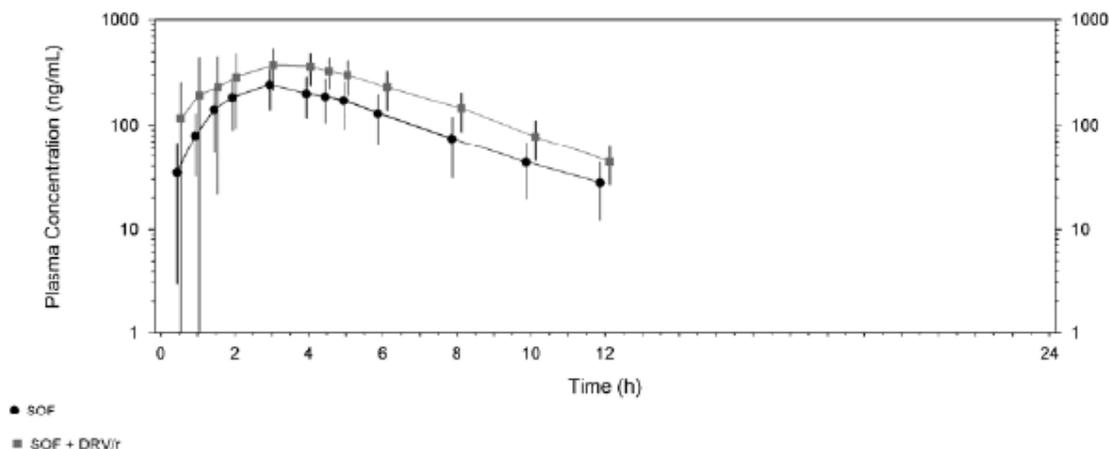


Figure 14 shows mean (SD) plasma concentration-time profiles of GS-566500 following administration of a single dose of sofosbuvir alone and in combination with RAL dosed to steady state (both under fasting conditions). GS-566500 plasma concentration rapidly reached C_{max} at median T_{max} of 1.0 h to 1.5 h (consistent with administration of sofosbuvir under fasting conditions) and then rapidly decreased in a monoexponential manner.

Figure 14. GS-US-334-0131: Mean (SD) GS-56650 Plasma Concentration-Time Profiles Following Administration of Single Dose of Sofosbuvir Alone and in Combination with Raltegravir

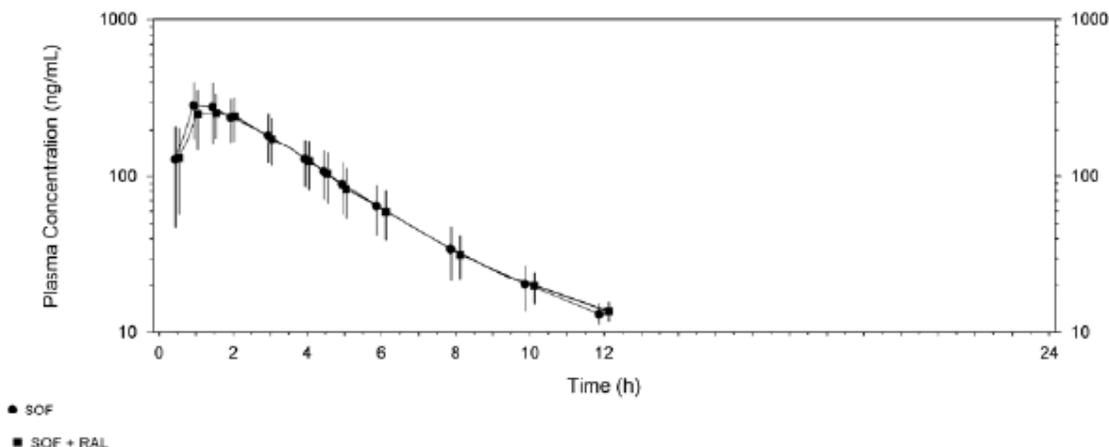


Figure 15 shows mean (SD) plasma concentration-time profiles of GS-56650 following administration of a single dose of sofosbuvir alone and in combination with RPV dosed to steady state (both under fed conditions). GS-56650 plasma concentration reached C_{max} at median T_{max} of 3.0 h (consistent with administration of sofosbuvir with food) and then rapidly decreased in a monoexponential manner.

Figure 15. GS-US-334-0131: Mean (SD) GS-56650 Plasma Concentration-Time Profiles Following Administration of Single Dose of Sofosbuvir Alone and in Combination with Rilpivirine

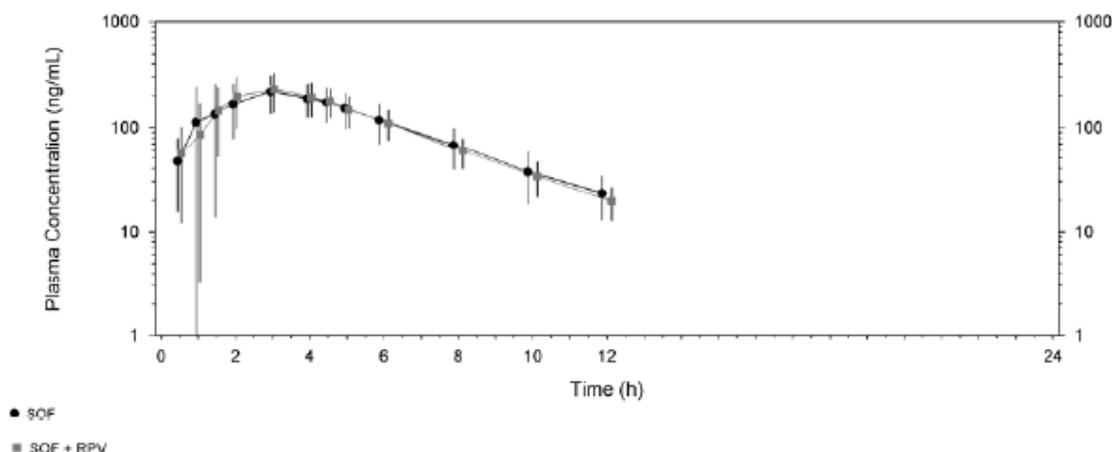


Table 18 presents GS-56650 plasma PK parameters following administration of single doses of sofosbuvir alone and in combination with ATR, DRV/r, RAL, or RPV dosed to steady state. In general the trends and magnitudes of changes in sofosbuvir exposure when coadministered with the ARVs were reflected in the PK parameters of GS-56650.

Table 18 GS-566500 Plasma Pharmacokinetic Parameters Following Administration of Single Dose of Sofosbuvir Alone and in Combination with Antiretroviral Agents

GS-566500 PK Parameter	Cohort 1 Mean (%CV)		Cohort 2 Mean (%CV)	
	SOF (N = 17)	SOF+ATR (N = 16)	SOF (N = 19)	SOF+DRV/r (N = 18)
AUC _{inst} (h·ng/mL)	1096.1 (28.8)	848.3 (30.2)	1302.8 (37.2)	2266.8 (29.6)
AUC _{inf} (h·ng/mL)	1139.1 (27.8)	895.5 (28.4)	1403.9 (36.9)	2427.7 (29.4)
C _{max} (ng/mL)	308.2 (37.5)	218.9 (37.7)	266.2 (38.4)	468.0 (34.9)
T _{max} (h) ^a	1.00 (1.00, 1.50)	1.50 (1.00, 1.75)	3.00 (3.00, 3.00)	3.00 (3.00, 4.00)
t _{1/2} (h) ^a	2.11 (1.91, 2.26)	2.46 (2.19, 2.52)	2.35 (2.15, 2.62)	2.53 (2.37, 2.65)
GS-566500 PK Parameter	Cohort 3 Mean (%CV)		Cohort 4 Mean (%CV)	
	SOF (N = 19)	SOF+RAL (N = 19)	SOF (N = 17)	SOF+RPV (N = 17)
AUC _{inst} (h·ng/mL)	1115.0 (28.5)	1058.7 (31.4)	1207.6 (26.0)	1190.4 (27.3)
AUC _{inf} (h·ng/mL)	1158.6 (27.5)	1108.5 (30.0)	1286.1 (25.9)	1257.1 (26.4)
C _{max} (ng/mL)	315.6 (29.2)	284.7 (30.2)	255.9 (38.1)	262.7 (35.0)
T _{max} (h) ^a	1.50 (1.00, 1.50)	1.00 (1.00, 1.50)	3.00 (2.00, 4.00)	3.00 (3.00, 4.00)
t _{1/2} (h) ^a	2.25 (2.03, 2.45)	2.39 (2.20, 2.62)	2.35 (2.24, 2.45)	2.30 (2.21, 2.52)

a Median (Q1, Q3)

Table 19 presents statistical comparisons of the primary GS-566500 PK parameters following administration of a single dose of sofosbuvir alone and in combination with ATR, DRV/r, RAL, or RPV. In general the trends and magnitudes of change in sofosbuvir exposure when coadministered with the ARVs were reflected in the PK parameters of GS-566500. These changes are not considered clinically significant.

Table 19. GS-US-334-0131: Statistical Comparisons of GS-566500 Plasma Pharmacokinetic Parameters Following Administration of Single Dose of Sofosbuvir Alone and in Combination with Antiretroviral Agents

GS-566500 PK Parameter	GLSM		%GLSM Ratio (90% CI)
	SOF+ATR (N = 16)	SOF (N = 16)	
Cohort 1			SOF+ATR / SOF
AUC _{last} (h·ng/mL)	808.20	1039.02	77.78 (67.50, 89.63)
AUC _{inf} (h·ng/mL)	858.72	1084.24	79.20 (69.37, 90.42)
C _{max} (ng/mL)	204.88	283.77	72.20 (62.24, 83.75)
Cohort 2	SOF+DRV/r (N = 18)	SOF (N = 18)	SOF+DRV/r / SOF
AUC _{last} (h·ng/mL)	2161.43	1190.51	181.6 (163.6, 201.5)
AUC _{inf} (h·ng/mL)	2316.73	1286.27	180.1 (163.6, 198.3)
C _{max} (ng/mL)	440.45	244.70	180.0 (155.9, 207.8)
Cohort 3	SOF+RAL (N = 19)	SOF (N = 19)	SOF+RAL / SOF
AUC _{last} (h·ng/mL)	994.30	1071.41	92.80 (83.05, 103.7)
AUC _{inf} (h·ng/mL)	1050.08	1116.29	94.07 (85.14, 103.9)
C _{max} (ng/mL)	268.64	302.29	88.87 (78.73, 100.3)
Cohort 4	SOF+RPV (N = 17)	SOF Alone (N = 17)	SOF+RPV / SOF
AUC _{last} (h·ng/mL)	1147.01	1166.65	98.32 (88.50, 109.2)
AUC _{inf} (h·ng/mL)	1213.75	1242.68	97.67 (88.17, 108.2)
C _{max} (ng/mL)	246.84	239.74	103.0 (87.75, 120.8)

GS-331007: Figure 16 shows mean (SD) plasma concentration-time profiles for GS-331007 following administration of a single dose of sofosbuvir alone and in combination with ATR dosed to steady state (both under fasting conditions). GS-331007 plasma concentration reached C_{max} at median T_{max} of 2.0 h (consistent with administration of sofosbuvir under fasting conditions) and then decreased in a biexponential manner.

Figure 16 Mean (SD) GS-331007 Plasma Concentration-Time Profiles Following Administration of Single Dose of Sofosbuvir Alone and in Combination with Atripla

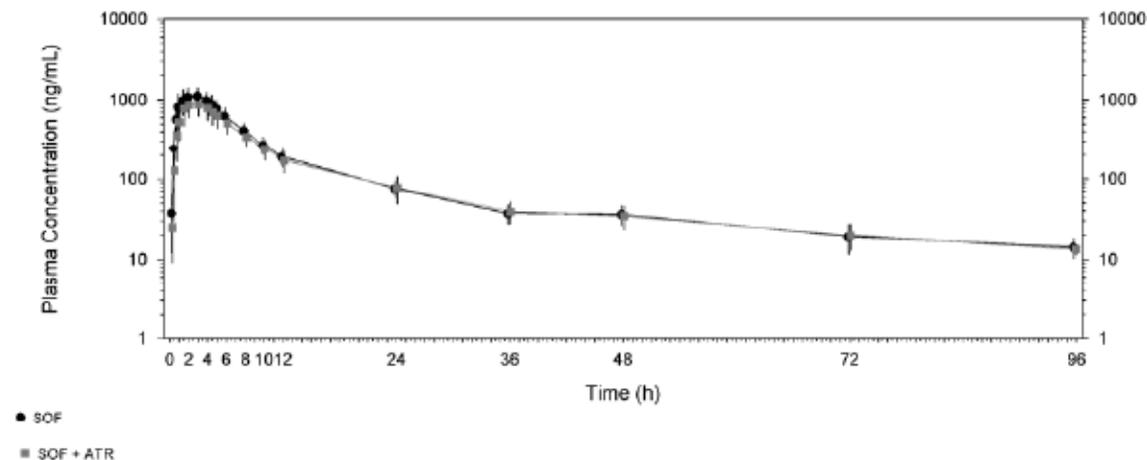


Figure 17 shows mean (SD) plasma concentration-time profiles for GS-331007 following administration of sofosbuvir alone and in combination DRV/r dosed to steady state (both under fed conditions). GS-331007 plasma concentration reached C_{max} at median T_{max} of 4.0 h to 4.5 h (consistent with administration of sofosbuvir with food) and then decreased in a biexponential manner.

Figure 17 Mean (SD) GS-331007 Plasma Concentration-Time Profiles Following Administration of Single Dose of Sofosbuvir Alone and in Combination with Ritonavir-Boosted Darunavir

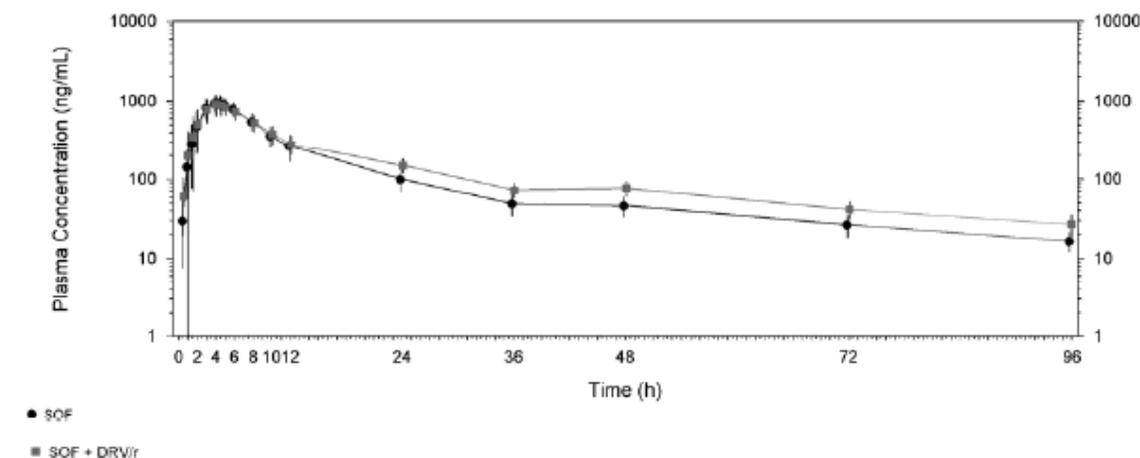


Figure 8 shows mean (SD) plasma concentration-time profiles of GS-331007 following administration of a single dose of sofosbuvir alone and in combination with RAL dosed to steady state (both under fasting conditions). GS-331007 plasma concentration reached C_{max} at median T_{max} of 3.0 h (consistent with administration of sofosbuvir under fasting conditions) and then decreased in a biexponential manner.

Figure 8 Mean (SD) GS-331007 Plasma Concentration-Time Profiles Following Administration of Single Dose of Sofosbuvir Alone and in Combination with Raltegravir

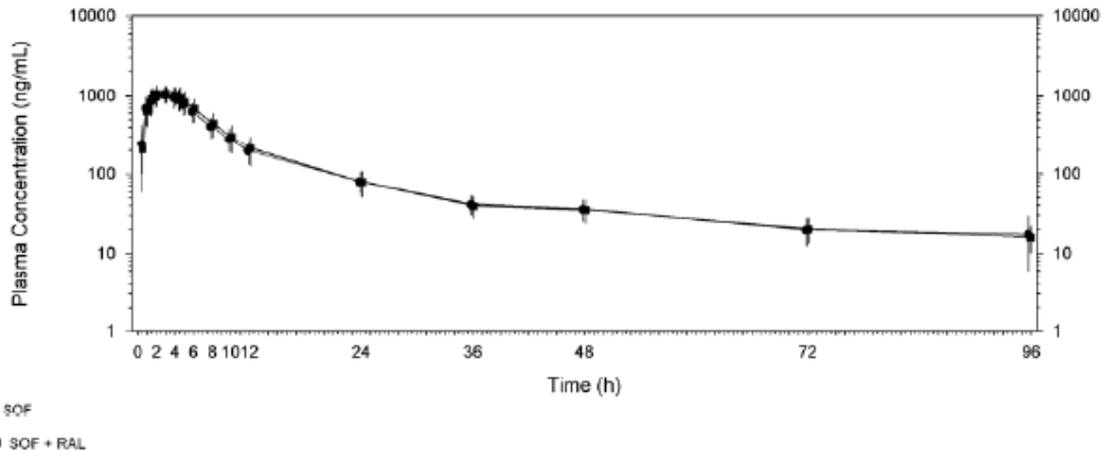


Figure 19 shows mean (SD) plasma concentration-time profiles for GS-33107 following administration of a single dose of sofosbuvir alone and in combination with RPV dosed to steady state (both under fed conditions). GS-33107 plasma concentration reached C_{max} at median T_{max} of 4.0 h to 4.5 h (consistent with sofosbuvir administration with food) and then decreased in a biexponential manner.

Figure 19 Mean (SD) GS-33107 Plasma Concentration-Time Profiles Following Administration of Single Dose of Sofosbuvir Alone and in Combination with Rilpivirine

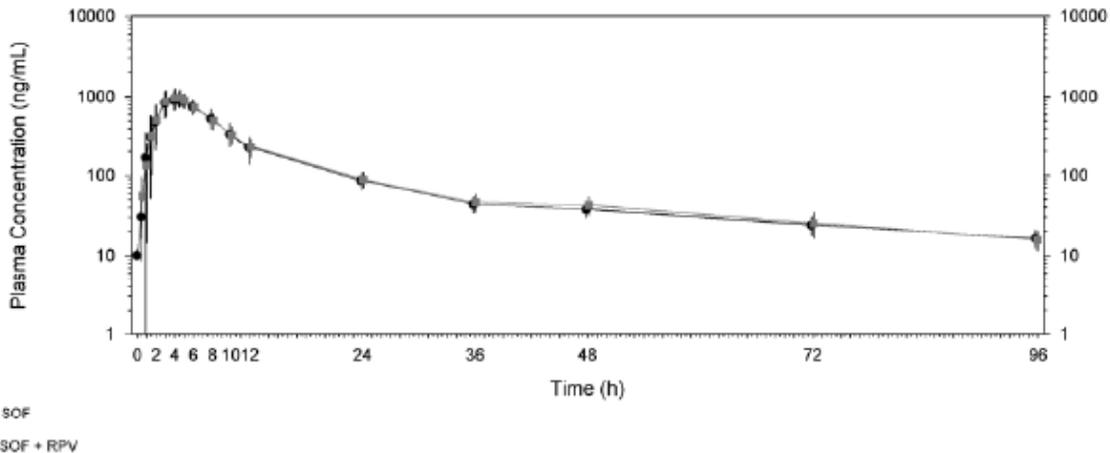


Table 20 presents GS-33107 plasma PK parameters following administration of single doses of sofosbuvir alone and in combination with ATR, DRV/r, RAL, or RPV.

Table 20 GS-331007 Plasma Pharmacokinetic Parameters Following Administration of Single Dose of Sofosbuvir Alone and in Combination with Antiretroviral Agents

GS-331007 PK Parameter	Cohort 1 Mean (%CV)		Cohort 2 Mean (%CV)	
	SOF (N = 17)	SOF+ATR (N = 16)	SOF (N = 19)	SOF+DRV/r (N = 18)
AUC _{last} (h·ng/mL)	10,669.3 (18.8)	9014.4 (27.0)	11,002.2 (16.4)	13,203.5 (16.1)
AUC _{inf} (h·ng/mL)	11,253.0 (18.4)	9620.6 (25.5)	11,833.5 (16.4)	14,678.8 (16.8)
C _{max} (ng/mL)	1254.1 (24.2)	965.0 (26.8)	1015.4 (20.7)	988.1 (19.8)
T _{max} (h) ^a	2.00 (1.50, 3.00)	2.00 (1.50, 3.00)	4.50 (4.00, 4.50)	4.00 (4.00, 4.50)
t _{1/2} (h) ^a	26.15 (23.08, 31.17)	24.34 (21.44, 32.07)	29.80 (24.80, 36.51)	35.51 (30.74, 39.85)
GS-331007 PK Parameter	Cohort 3 Mean (%CV)		Cohort 4 Mean (%CV)	
	SOF (N = 19)	SOF+RAL (N = 19)	SOF (N = 17)	SOF+RPV (N = 17)
AUC _{last} (h·ng/mL)	10,515.5 (21.3)	10,762.6 (22.9)	10,441.6 (18.4)	10,680.1 (18.5)
AUC _{inf} (h·ng/mL)	11,274.0 (21.4)	11,541.7 (20.9)	11,377.9 (17.8)	11,465.0 (19.2)
C _{max} (ng/mL)	1097.3 (22.6)	1193.1 (22.3)	1002.3 (22.1)	1061.1 (22.2)
T _{max} (h) ^a	3.00 (1.50, 3.00)	3.00 (2.00, 4.00)	4.50 (4.00, 5.00)	4.00 (3.00, 4.50)
t _{1/2} (h) ^a	27.77 (24.93, 34.26)	29.80 (24.79, 33.35)	33.46 (31.92, 40.99)	35.79 (31.28, 37.64)

a Median (Q1, Q3)

Table 21 presents statistical comparisons of the primary GS-331007 PK parameters following administration of a single dose of sofosbuvir alone and in combination with ATR, DRV/r, RAL, or RPV. Based on 80%-125% boundaries of 90% CIs of the GLSM ratio for GS-331007, RAL and RPV do not affect the PK of GS-331007. However, ATR reduced GS-331007 AUC_{inf} and C_{max} by 16% and 23% respectively; DRV/r increased GS-331007 AUC_{inf} by 24%. These magnitudes of effect are not considered clinically significant.

Table 21 Statistical Comparisons of GS-331007 Plasma Pharmacokinetic Parameters Following Administration of Single Dose of Sofosbuvir Alone and in Combination with Antiretroviral Agents

GS-331007 PK Parameter	GLSM		%GLSM Ratio (90% CI)
	SOF+ATR (N = 16)	SOF (N = 16)	
Cohort 1			SOF+ATR / SOF
AUC _{last} (h·ng/mL)	8718.10	10,582.30	82.81 (75.40, 90.94)
AUC _{inf} (h·ng/mL)	9332.34	11,119.92	83.92 (76.29, 92.32)
C _{max} (ng/mL)	930.84	1213.46	76.71 (69.80, 84.31)
Cohort 2	SOF+DRV/r (N = 18)	SOF (N = 18)	SOF+DRV/r / SOF
AUC _{last} (h·ng/mL)	13,036.01	10,954.37	119.0 (113.2, 125.1)
AUC _{inf} (h·ng/mL)	14,472.46	11,713.36	123.6 (117.6, 129.8)
C _{max} (ng/mL)	968.53	995.14	97.33 (90.06, 105.2)
Cohort 3	SOF+RAL (N = 19)	SOF (N = 19)	SOF+RAL / SOF
AUC _{last} (h·ng/mL)	10,481.61	10,282.08	101.9 (95.68, 108.6)
AUC _{inf} (h·ng/mL)	11,295.35	11,022.07	102.5 (96.89, 108.4)
C _{max} (ng/mL)	1163.81	1070.60	108.7 (98.92, 119.5)
Cohort 4	SOF+RPV (N = 17)	SOF (N = 17)	SOF+RPV / SOF
AUC _{last} (h·ng/mL)	10,513.32	10,270.35	102.4 (98.17, 106.7)
AUC _{inf} (h·ng/mL)	11,273.19	11,201.86	100.6 (97.07, 104.3)
C _{max} (ng/mL)	1038.02	980.09	105.9 (98.64, 113.7)

Conclusion: Patients can take regimens containing tenofovir, raltegravir, rilpivirine, or darunavir/r in combination with SOF without any dose adjustments.

4.2.4.2 P7977-1910 (Part A): Drug Interaction Study between GS-7977 and Antiretroviral Therapy (ARV) Combinations of Efavirenz, Tenofovir and Emtricitabine; Efavirenz, Zidovudine and Lamivudine; Atazanavir/ritonavir, Tenofovir and Emtricitabine; Darunavir/ritonavir, Tenofovir and Emtricitabine; Raltegravir, Tenofovir and Emtricitabine in Human Immunodeficiency Virus and Hepatitis C Virus (HIV/HCV) Co-infected Patients.

Objectives:

- Evaluate whether SOF significantly influenced the PK parameters of atazanavir/ritonavir (ATV/RTV [r]), efavirenz (EFV), tenofovir disoproxil fumarate (TDF), emtricitabine (FTC), zidovudine (ZDV), lamivudine (3TC), darunavir/r (DRV/r), or raltegravir (RAL) in HIV/HCV coinfecting subjects
- Evaluate whether ATV/r, EFV, TDF, FTC, ZDV, 3TC, DRV/r, or RAL significantly affected the PK parameters of SOF and its metabolites, GS-566500 and GS-331007, in HIV/HCV coinfecting subjects as compared to historical data (Study P2938-0212)

Study Design: This was an open-label, single-sequence study consisting of 5 cohorts of HIV/HCV co-infected subjects using 1 of the following 5 ARV combinations for a minimum of 4 weeks (with no interruptions or dose changes) prior to 7 days of dosing of SOF in combination with their HIV drug regimens:

Cohort 1: EFV/FTC/TDF (Atripla®, ATR)

Cohort 2: EFV (600 mg once daily)+ZDV/3TC (300 mg/ 150 mg twice daily)_

Cohort 3: ATV/r (400 mg/100 mg once daily)+FTC/TDF (Truvada®, TVD)

Cohort 4: DRV/r (800 mg/100 mg once daily)+TVD

Cohort 5: RAL(400 mg twice daily) +TVD

Subjects continued their HIV drug regimen for 7 more days after the 7 days of 400 mg once daily of SOF combination treatment period. Subjects were enrolled only in the cohort corresponding to the ARV regimen that the subject had been receiving to manage their HIV infection. Medications were administered under fasting conditions for Cohorts 1 and 2; and were administered with food for Cohorts 3, 4, and 5.

Subjects: A total of 34 HIV/HCV co-infected subjects (males and females who were ≥ 21 years old) were enrolled, but only 30 subjects were included in the PK analysis set.

Formulation: SOF 200-mg (Lot 11G086-P1) or 400-mg (Lots DC1203B1 and DC1204B1) tablets were used. Commercially available formulation were used for ART medications were used with the following lot numbers: ATR (000291, 020100443), EFV (1K68788A, 2B70526A), TVD (000357, GKWX, GWYM, 000679), ZDV/3TC (30216178A), RTV (103032E, 130252E), ATV (1J5033A, 1K5013A), DRV (2E6851, 2E6854), and RAL (H004691, H011486).

PK Sampling: Blood samples were obtained to determine plasma concentrations and calculate PK parameters of ARV agents and SOF and its metabolites, GS-566500 (formerly PSI-352707) and GS-331007 (formerly PSI-6206), at the following time points:

Analyte	Day(s)	Sample Time (relative to dosing)
ZDV, 3TC, RAL	0 and 7	0 (predose) and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours postdose ^{ab}
TDF, FTC, ATV, RTV, EFV, DRV	0 and 7	0 (predose) and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 hours postdose ^{ab}
SOF, GS-566500, GS-331007	7 ^b	0 (predose) and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 48, 72, and 168 (Day 14) hours post SOF+ARV dose

a Sampling on Day 0 occurred post ARV dose but before SOF dosing.

b Sampling on Day 7 occurred post SOF+ARV dose.

Analytical Methods: Concentrations of SOF, GS-566500, GS-331007, ZDV, 3TC, RAL, ATV, RTV, FTC, TFV (the measurable analyte of TDF), EFV, and DRV in plasma samples were determined using validated high-performance liquid chromatography-tandem mass spectroscopy (LC/MS/MS) bioanalytical methods. All samples were analyzed in the timeframe supported by frozen stability storage data. The assays for SOF, GS-566500, GS-331007, ZDV, 3TC, ATV, RTV, FTC, TFV, EFV, and DRV were all performed and validated by (b) (4). The assay for RAL was performed and validated by (b) (4).

The standard curve and QC data indicated that the plasma assay method for SOF, GS-566500, GS-331007, ZDV, 3TC, RAL, ATV, RTV, FTC, TFV, EFV, and DRV were precise and accurate as shown in the following table.

Table 1 Summary of Quality Control (QC) Results –Study P7977-1910

Analyte	Linear range (ng/mL)	Between Run Precision (%CV)	Between Run Bias (% Deviation)	QC samples (ng/mL)	Validation sample for stability and conditions
SOF	5 – 5000 R ² > 0.994	≤ 7.6	-9.5 to 5.7	5, 15, 30, 500 and 4000	Stable for 377 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
GS-566500	10 – 5000 R ² > 0.995	≤ 10.6	0.5 to 2.3	10, 30, 500 and 4000	Stable for 308 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
GS-331007	10 – 5000 R ² > 0.995	≤ 5.6	-1.2 to 2.4	30, 500 and 4000	Stable for 308 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
EFV	5 – 5000 R ² > 0.992	≤ 7.7	0.3 to 6.6	15, 200, 750, and 4000	Stable for 127 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
ZDV	2 - 2000 R ² > 0.995	≤ 6.4	-3.2 to 1.6	2, 5, 15, 500 and 18000	Stable for 183 days at -70°C and ≥ 3 freeze/thaw cycles in plasma
3TC	2 - 2000 R ² > 0.998	≤ 7.3	-3.6 to -2.0	2, 5, 15, 500 and 18000	Stable for 310 days at -70°C and ≥ 3 freeze/thaw cycles in plasma
RAL	1 – 1500 R ² > 0.997	≤ 3.8	-3.3 to 4.0	3, 100, 1200, and 7500 (dilution=10)	Stable for 1303 days at -70°C and ≥ 4 freeze/thaw cycles in plasma
ATV	10 – 5000	≤ 6.1	0.5 to 7.0	30, 800, 400,	Stable for 721 days at -

	$R^2 > 0.996$			and 4000 (diluted)	70°C and ≥ 5 freeze/thaw cycles in plasma
RTV	5 - 2500 $R^2 > 0.990$	≤ 4.2	1.8 to 6.0	15, 400 and 2000	Stable for 721 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
FTC	5 - 3000 $R^2 > 0.997$	≤ 15.0	-2.0 to 4.4	15, 150, 600 and 2400	Stable for 190 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
TFV	5 - 3000 $R^2 > 0.994$	≤ 18.3	-7.2 to 3.5	15, 150, 600 and 2400	Stable for 190 days at -70°C and ≥ 5 freeze/thaw cycles in plasma
DRV	20 - 10,000 $R^2 > 0.997$	≤ 6.3	-3.2 to 0.7	60, 800, and 9000	Stable for 301 days at -70°C and ≥ 4 freeze/thaw cycles in plasma

Pharmacokinetic Results:

Efavirenz:

Figure 1 and Figure 2 show mean (SD) plasma concentration-time profiles for EFV following administration of ATR and EFV+ZDV/3TC alone and in combination with SOF. Great variability was noted due to the small sample sizes.

Figure 1 Mean (SD) Efavirenz Plasma Concentration-Time Profiles Following Administration of Atripla Alone and in Combination with Sofosbuvir (Multiple Doses)

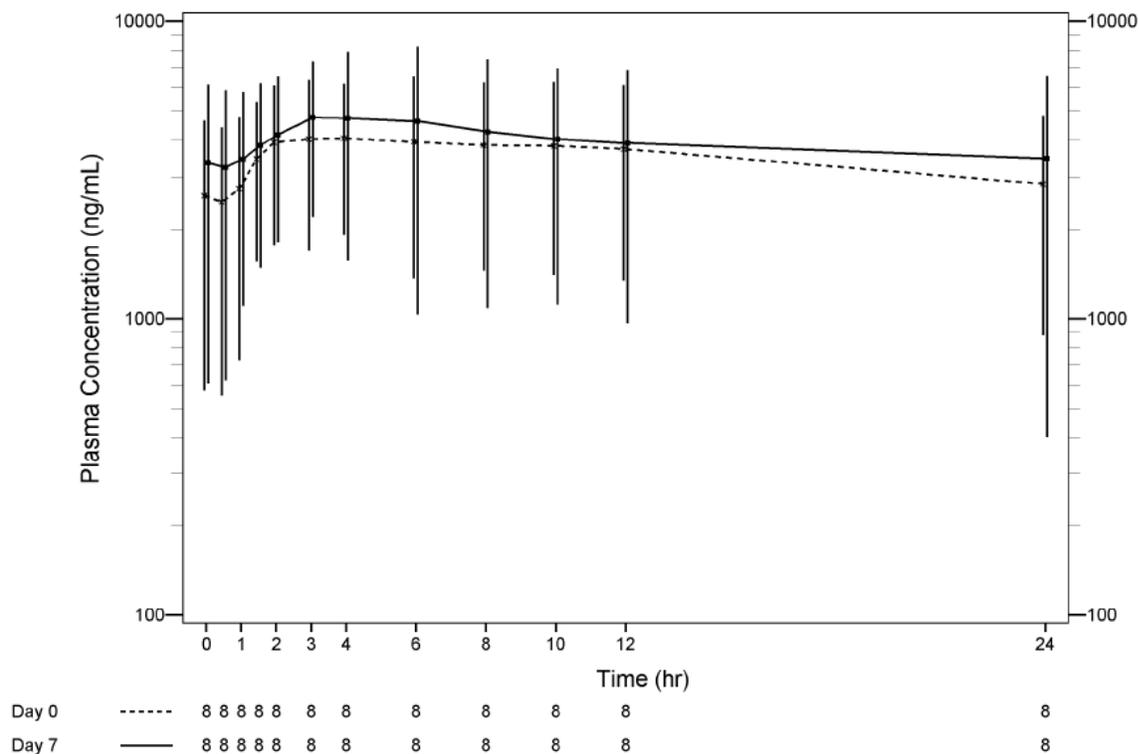


Figure 2 Mean (SD) Efavirenz Plasma Concentration-Time Profiles Following Administration of Efavirenz+Zidovudine/Lamivudine Alone and in Combination with Sofosbuvir (Multiple Doses)

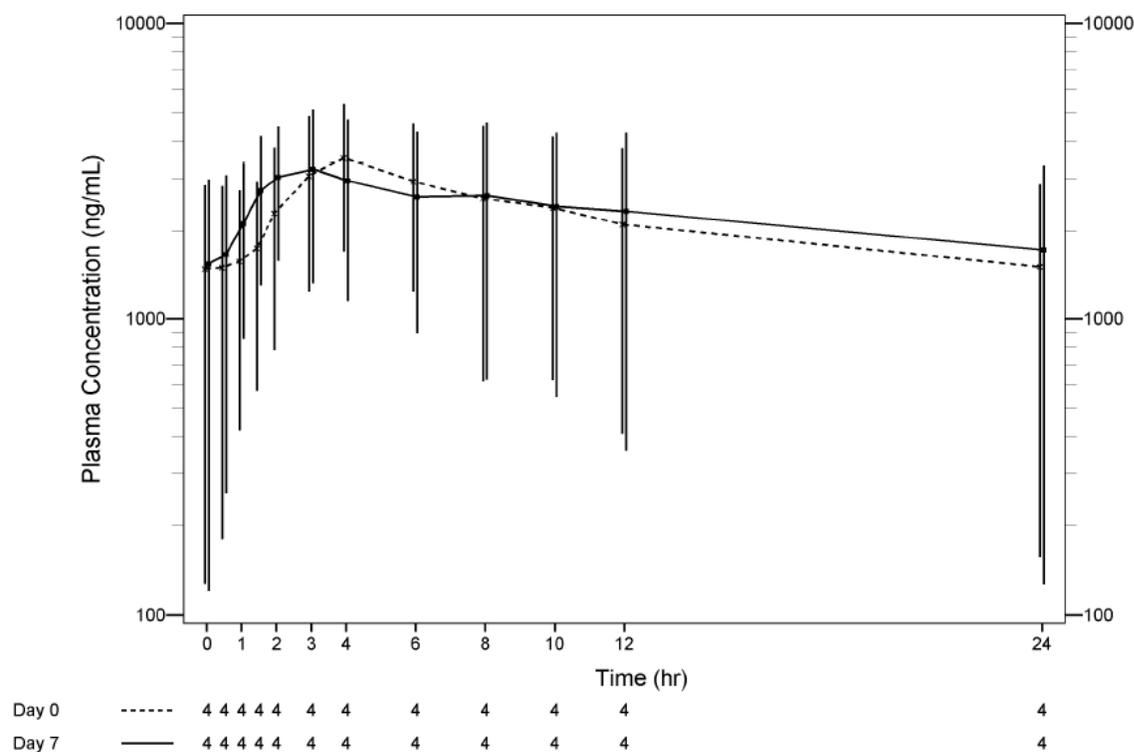


Table 2 presents EFV PK parameters following administration of ATR or EFV+ZDV/3TC alone and in combination with SOF.

Table 2 Efavirenz Plasma Pharmacokinetic Parameters Following Administration of Antiretroviral Agents Alone and in Combination with Sofosbuvir (Multiple Doses)

EFV PK Parameter	Mean (%CV)			
	Cohort 1 (N = 8)		Cohort 2 (N = 4)	
	Day 0 ATR	Day 7 SOF+ATR	Day 0 EFV+ZDV/3TC	Day 7 SOF+EFV+ZDV/3TC
AUC _{tau} (h·ng/mL)	84,260.2 (61.8)	95,094.4 (74.9)	52,053.1 (70.7)	55,152.3 (75.2)
C _{max} (ng/mL)	4930.3 (46.1)	5423.8 (60.5)	3527.0 (51.7)	3469.5 (49.2)
C _{tau} (ng/mL)	2852.2 (69.1)	3476.2 (88.4)	1510.5 (89.6)	1722.2 (92.6)
T _{max} (h) ^a	5.00 (2.50, 10.00)	3.08 (3.08, 5.08)	4.00 (4.00, 4.00)	2.58 (1.83, 3.58)
t _{1/2} (h) ^a	28.44 (18.20, 48.13)	44.28 (26.12, 63.5)	18.38 (9.50, 27.09)	19.75 (14.09, 36.89)
T _{last} (h) ^a	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)

a Median (Q1, Q3)

Table 3 presents statistical comparisons of the primary EFV PK parameters following administration of ATR or EFV+ ZDV/3TC alone and in combination with SOF. The data indicate

that the effect of SOF on EFV exposures is not clinically significant. The result is consistent with the effect of single dose of SOF on the PK of EFV observed in Study GS-US-334-0131.

Table 3 Statistical Comparisons of Efavirenz Plasma Pharmacokinetic Parameters Following Administration of Antiretroviral Agents Alone and in Combination with Sofosbuvir (Multiple Doses)

EFV PK Parameter	GLSM		%GLSM Ratio (90% CI)
	Day 0	Day 7	
Cohort 1	ATR (N = 8)	SOF+ATR (N = 8)	SOF+ATR / ATR
AUC _{tau} (h·ng/mL)	71,761.37	76,690.78	106.9 (94.77, 120.5)
C _{max} (ng/mL)	4501.02	4714.27	104.7 (90.72, 120.9)
C _{tau} (ng/mL)	2312.99	2523.06	109.1 (90.18, 131.9)
Cohort 2	EFV+ZDV/3TC (N = 4)	SOF+EFV+ZDV/3TC (N = 4)	SOF+EFV+ZDV/3TC / EFV+ZDV/3TC
AUC _{tau} (h·ng/mL)	34,124.21	36,750.47	107.7 (93.60, 123.9)
C _{max} (ng/mL)	2944.87	3051.92	103.6 (78.52, 136.8)
C _{tau} (ng/mL)	720.70	878.41	121.9 (83.71, 177.5)

Tenofovir:

Figure 3 through Figure 6 show mean (SD) plasma concentration-time profiles for TFV following administration of ATR, ATV/r+TVD, DRV/r+TVD, or RAL+TVD alone and in combination with SOF. Tenofovir AUC was comparable for all treatments; C_{max} was slightly increased following administration of SOF+ATR and SOF+ATV/r+TVD.

Figure 3 Mean (SD) Tenofovir Plasma Concentration-Time Profiles Following Administration of Atripla Alone and in Combination with Sofosbuvir (Multiple Doses)

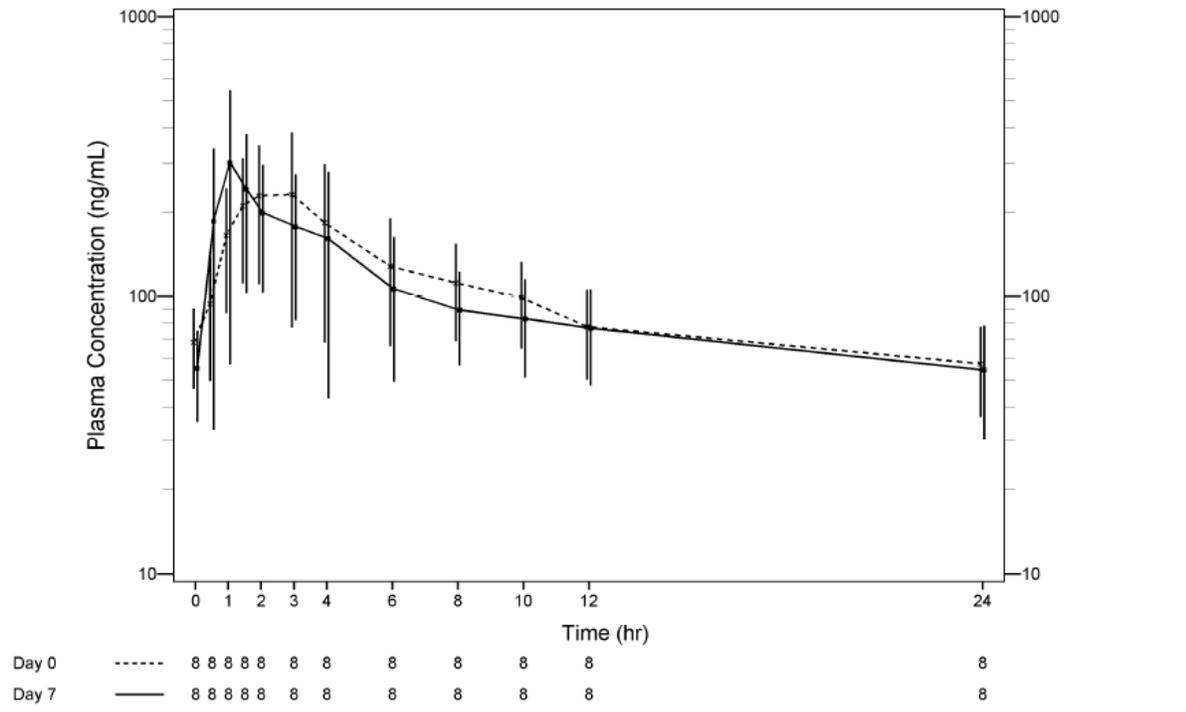


Figure 4 Mean (SD) Tenofovir Plasma Concentration-Time Profiles Following Administration of Ritonavir-Boosted Atazanavir+Truvada Alone and in Combination with Sofosbuvir (Multiple Doses)

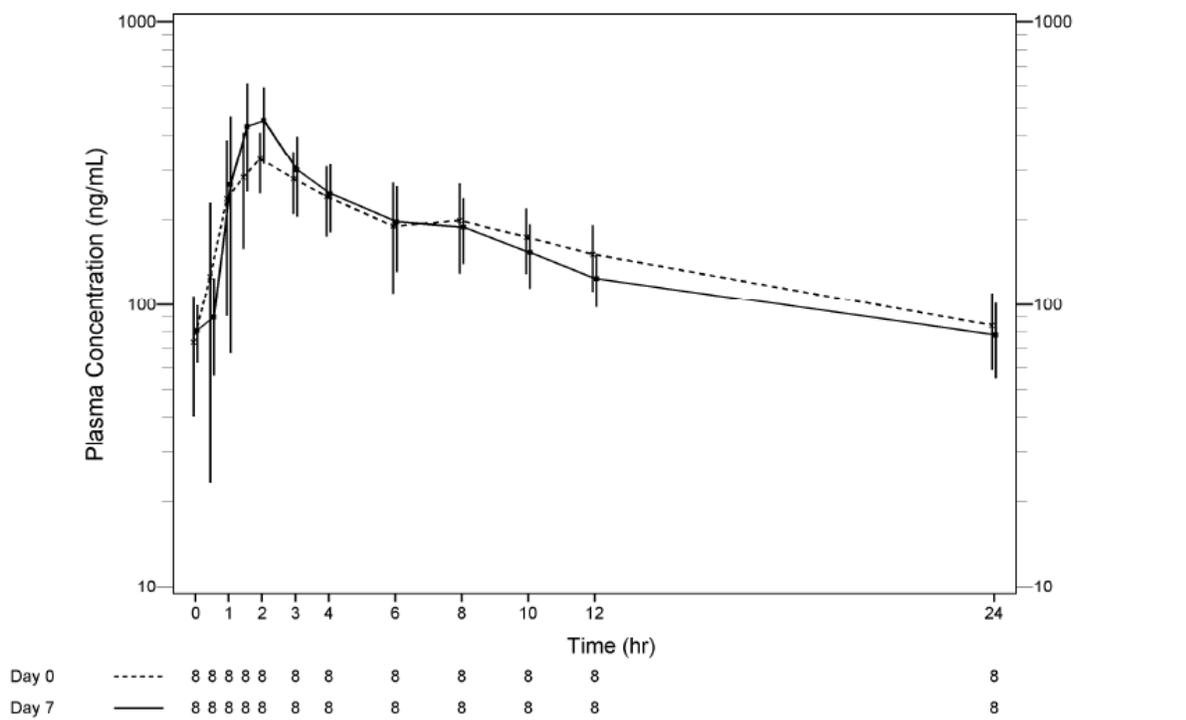


Figure 5 Mean (SD) Tenofovir Plasma Concentration-Time Profiles Following Administration of Ritonavir-Boosted Darunavir+Truvada Alone and in Combination with Sofosbuvir (Multiple Doses)

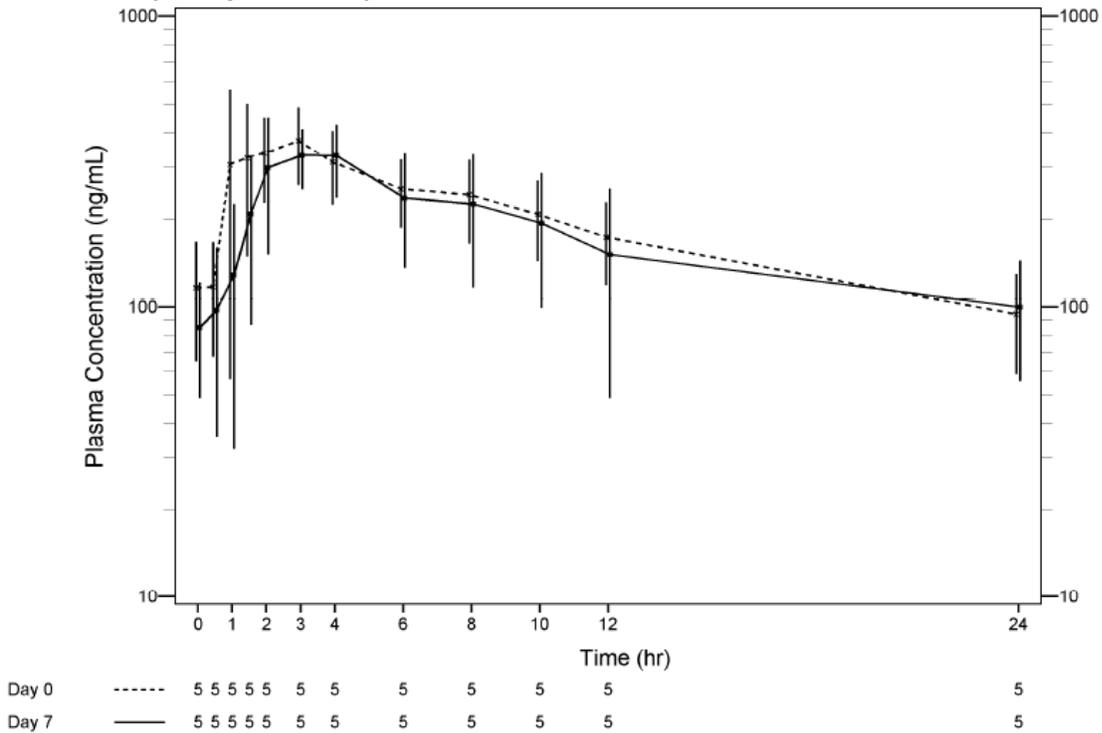


Figure 6 Mean (SD) Tenofovir Plasma Concentration-Time Profiles Following Administration of Raltegravir+Truvada Alone and in Combination with Sofosbuvir (Multiple Doses)

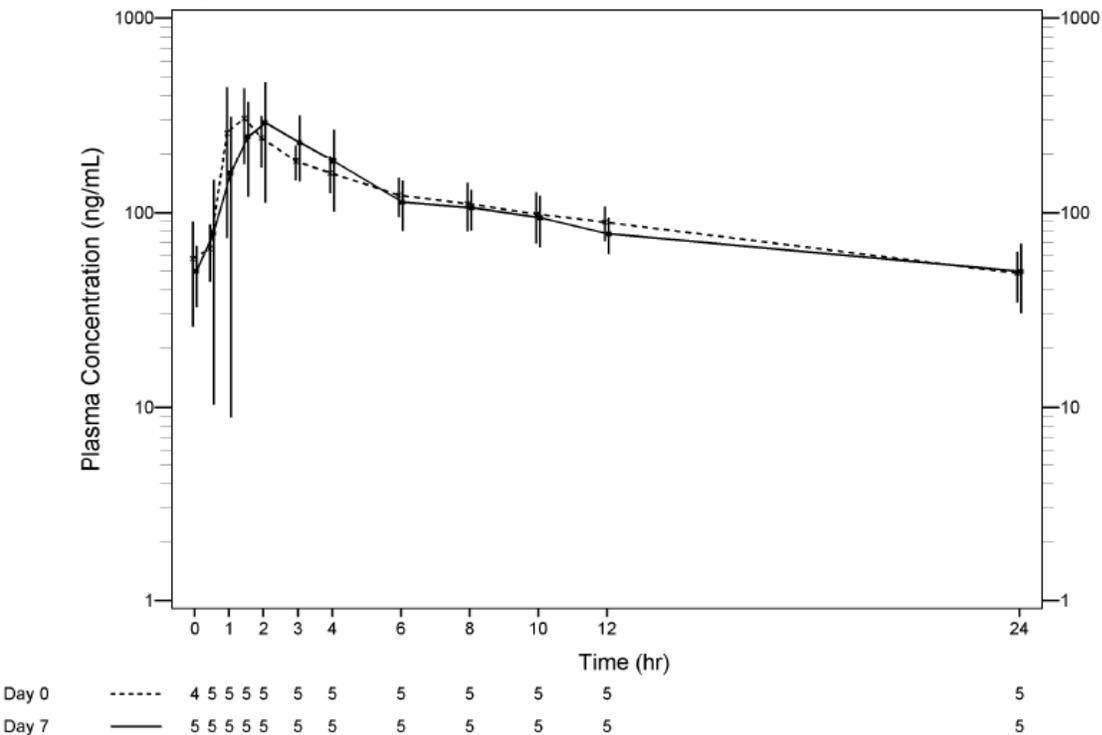


Table 4 presents TFV PK parameters following administration of TDF as a component of ARV regimens alone and in combination with SOF. The primary TFV PK parameters (mean AUC_{tau}, C_{max}, and C_{tau} and median T_{max} and t_{1/2}) were comparable following administration of ATR, ATV/r+TVD, DRV/r+TVD, or RAL+TVD alone and when coadministered SOF, with the exception of slight increases in TFV C_{max} following administration of SOF+ATR and SOF+ATV/r+TVD.

Table 4 Tenofovir Plasma Pharmacokinetic Parameters Following Administration of Antiretroviral Agents Alone and in Combination with Sofosbuvir

TFV PK Parameter	Mean (%CV)			
	Cohort 1 (N = 8)		Cohort 3 (N = 8)	
	Day 0 ATR	Day 7 SOF+ATR	Day 0 ATV/r+TVD	Day 7 SOF+ATV/r+TVD
AUC _{tau} (h·ng/mL)	2475.1 (40.8)	2351.2 (40.9)	3862.3 (25.9)	3793.0 (22.0)
C _{max} (ng/mL)	278.1 (47.5)	372.5 (56.0)	367.3 (19.6)	519.8 (23.1)
C _{tau} (ng/mL)	57.3 (35.4)	54.4 (43.8)	84.2 (30.2)	78.1 (29.7)
T _{max} (h) ^a	2.50 (1.50, 3.00)	1.33 (1.08, 2.33)	2.00 (1.25, 2.50)	1.58 (1.58, 2.08)
t _{1/2} (h) ^a	19.72 (16.64, 22.19)	20.80 (19.23, 24.03)	11.88 (10.81, 17.16)	15.06 (12.82, 16.61)
T _{last} (h) ^a	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
	Cohort 4 (N = 5)		Cohort 5 (N = 5)	
	Day 0 DRV/r+TVD	Day 7 SOF+DRV/r+TVD	Day 0 RAL+TVD	Day 7 SOF+RAL+TVD
	AUC _{tau} (h·ng/mL)	4626.1 (29.4)	4202.9 (45.8)	2482.7 (15.1)
C _{max} (ng/mL)	450.8 (36.1)	368.0 (30.2)	354.1 (33.7)	365.3 (30.5)
C _{tau} (ng/mL)	94.2 (37.5)	99.8 (44.2)	48.9 (29.1)	49.9 (38.7)
T _{max} (h) ^a	3.00 (1.50, 3.00)	3.08 (3.08, 3.08)	1.50 (1.50, 2.00)	2.08 (1.08, 4.08)
t _{1/2} (h) ^a	12.58 (10.19, 12.67)	12.92 (12.06, 13.83)	13.75 (11.53, 18.68)	15.16 (11.76, 15.91)
T _{last} (h) ^a	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)

^a Median (Q1, Q3)

Table 5 presents statistical comparisons of the primary TFV PK parameters following administration of TDF as a component of ARV regimens alone and in combination with SOF. Administration of ATR, ATV/r+TVD, DRV/r+TVD, or RAL+TVD alone or coadministered with SOF resulted in comparable TFV AUC_{tau} and C_{tau}. Tenofovir C_{max} was comparable following coadministration of SOF with DRV/r+TVD or RAL+TVD and increased 35% to 40% following coadministration of SOF with ATR or ATV/r+TVD. A similar increase in TFV C_{max} was observed in Study GS-US-334-0131 following coadministration of single dose of SOF with ATR.

Table 5 Statistical Comparisons of TFV Plasma Pharmacokinetic Parameters Following Administration of Antiretroviral Agents Alone and in Combination with Sofosbuvir

TFV PK Parameter	GLSM		%GLSM Ratio (90% CI)
	Day 0	Day 7	
Cohort 1	ATR (N =8)	SOF+ATR (N =8)	SOF+ATR / ATR
AUC _{tau} (h·ng/mL)	2308.60	2185.41	94.66 (87.42, 102.5)
C _{max} (ng/mL)	247.21	332.67	134.6 (109.6, 165.3)
C _{tau} (ng/mL)	54.45	50.24	92.27 (81.38, 104.6)
Cohort 3	ATV/r+TVD (N = 8)	SOF+ATV/r+TVD (N = 8)	SOF+ATV/r+TVD / ATV/r+TVD
AUC _{tau} (h·ng/mL)	3735.10	3706.98	99.25 (83.79, 117.6)
C _{max} (ng/mL)	361.44	507.45	140.4 (114.53, 172.1)
C _{tau} (ng/mL)	80.74	75.19	93.13 (75.64, 114.7)
Cohort 4	DRV/r+TVD (N = 5)	SOF+DRV/r+TVD (N = 5)	SOF+ DRV/r+TVD / DRV/r+TVD
AUC _{tau} (h·ng/mL)	4468.74	3896.73	87.20 (73.04, 104.11)
C _{max} (ng/mL)	425.30	354.57	83.37 (63.67, 109.17)
C _{tau} (ng/mL)	89.34	92.00	103.0 (75.43, 140.6)
Cohort 5	RAL+TVD (N = 5)	SOF+RAL+TVD (N = 5)	SOF+ RAL+TVD / RAL+TVD
AUC _{tau} (h·ng/mL)	2460.72	2355.72	95.73 (72.22, 126.9)
C _{max} (ng/mL)	337.87	353.03	104.5 (68.04, 160.5)
C _{tau} (ng/mL)	47.50	47.05	99.05 (68.74, 142.7)

Emtricitabine

Figure 7 through Figure 10 show mean (SD) plasma concentration-time profiles for FTC following administration of ATR, ATV/r+TVD, DRV/r+TVD, or RAL+TVD alone and in combination with SOF. The plasma concentration-time profiles for FTC were comparable following administration of ATR, ATV/r+TVD, DRV/r+TVD, or RAL+TVD alone (Day 0) and coadministered with SOF (Day 7); FTC T_{max} was slightly later following administration of SOF+DRV/r+TVD.

Figure 7 Mean (SD) Emtricitabine Plasma Concentration-Time Profiles Following Administration of Atripla Alone and in Combination with Sofosbuvir

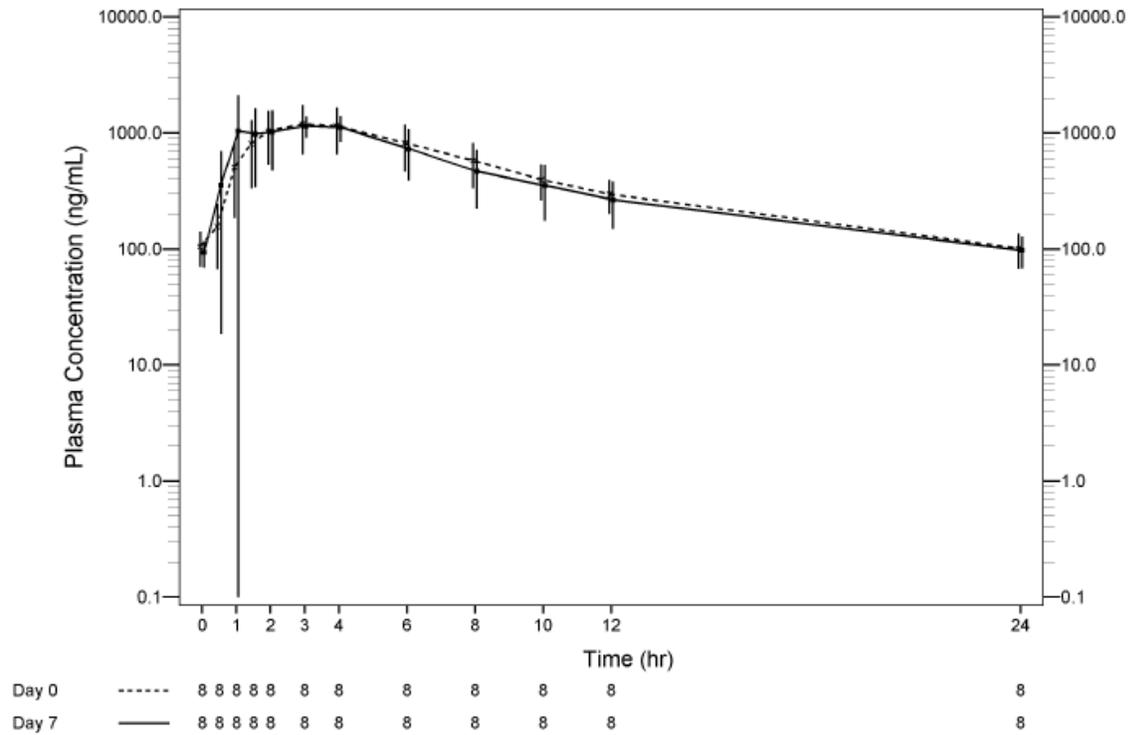


Figure 8 Mean (SD) Emtricitabine Plasma Concentration-Time Profiles Following Administration of Ritonavir-Boosted Atazanavir+Truvada Alone and in Combination with Sofosbuvir

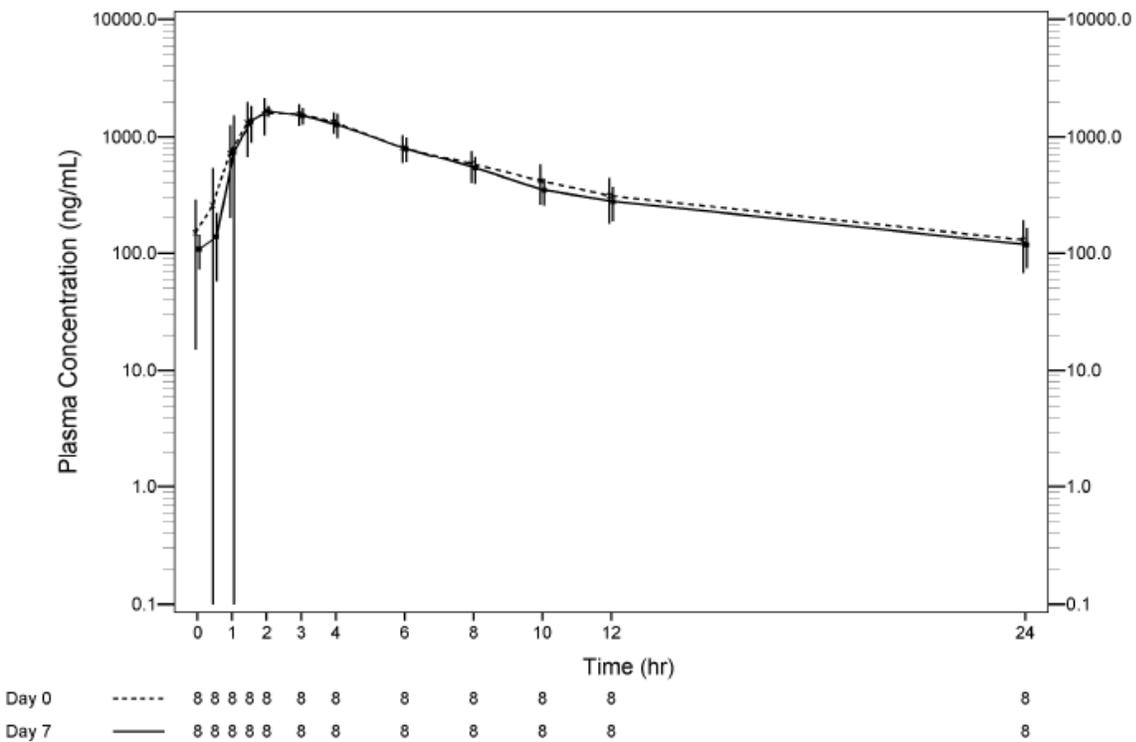


Figure 9 Mean (SD) Emtricitabine Plasma Concentration-Time Profiles Following Administration of Ritonavir-Boosted Darunavir+Truvada Alone and in Combination with Sofosbuvir

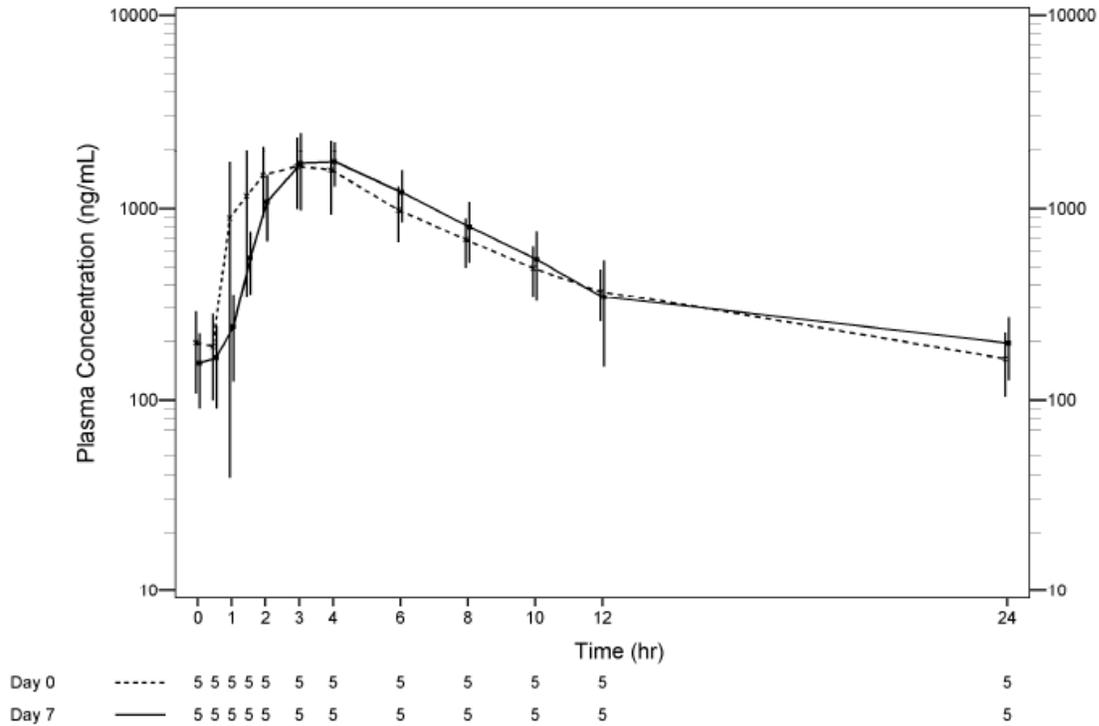


Figure 10 Mean (SD) Emtricitabine Plasma Concentration-Time Profiles Following Administration of Raltegravir+Truvada Alone and in Combination with Sofosbuvir

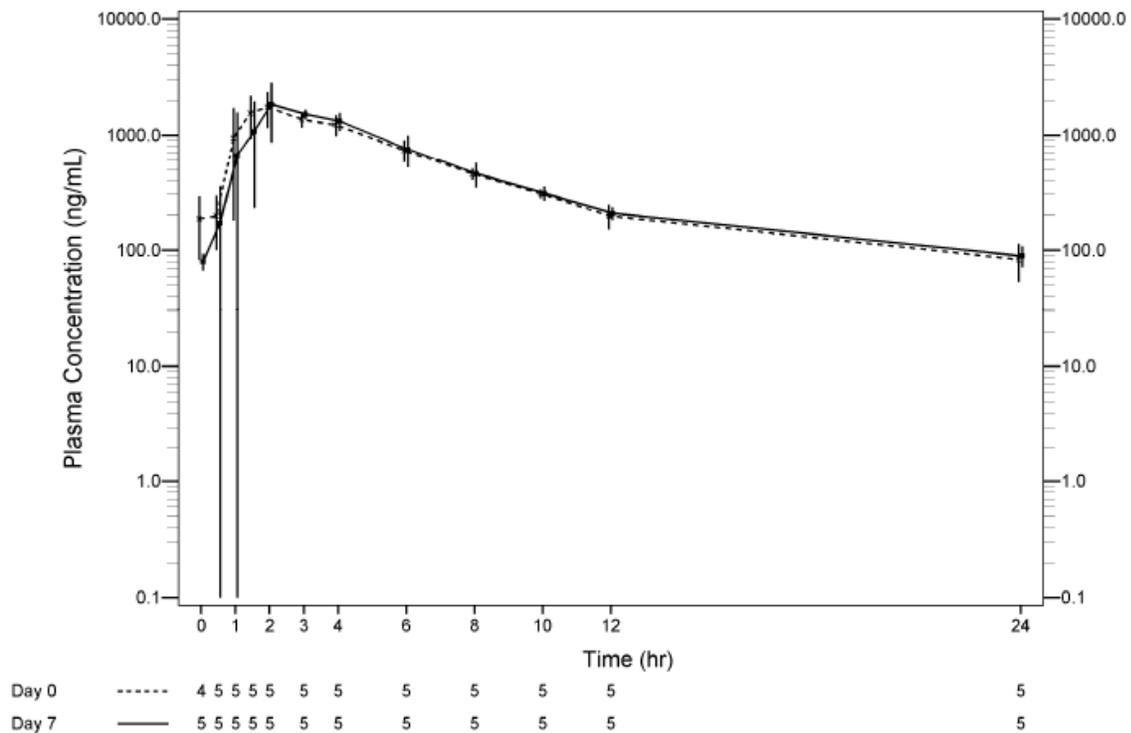


Table 6 presents FTC PK parameters following administration of FTC as a component of ARV regimens alone and in combination with SOF. The primary FTC PK parameters (mean AUC_{tau}, C_{max}, and C_{tau} and median T_{max} and t_{1/2}) were comparable following administration of ATR, ATV/r+TVD, DRV/r+TVD, or RAL+TVD alone and when coadministered with SOF, with the exception of slight increases in FTC T_{max} following administration of SOF+DRV/r+TVD.

Table 6 Emtricitabine Plasma Pharmacokinetic Parameters Following Administration of Antiretroviral Agents Alone and in Combination with Sofosbuvir

FTC PK Parameter	Mean (%CV)			
	Cohort 1 (N = 8)		Cohort 3 (N = 8)	
	Day 0 ATR	Day 7 SOF+ATR	Day 0 ATV/r+TVD	Day 7 SOF+ATV/r+TVD
AUC _{tau} (h·ng/mL)	10,497.5 (33.0)	10,144.8 (27.9)	12,263.1 (27.3)	11,564.9 (15.0)
C _{max} (ng/mL)	1291.4 (36.2)	1533.1 (54.1)	1791.4 (16.0)	1797.9 (12.9)
C _{tau} (ng/mL)	101.6 (33.3)	98.0 (30.0)	129.2 (47.4)	117.8 (37.2)
T _{max} (h) ^a	3.00 (3.00, 4.00)	2.08 (1.33, 3.58)	2.00 (2.00, 2.50)	2.08 (1.58, 3.08)
t _{1/2} (h) ^a	6.71 (6.18, 7.36)	7.77 (7.43, 8.59)	7.86 (7.38, 8.25)	7.70 (6.77, 8.97)
T _{last} (h) ^a	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)
FTC PK Parameter	Cohort 4 (N = 5)		Cohort 5 (N = 5)	
	Day 0 DRV/r+TVD	Day 7 SOF+DRV/r+TVD	Day 0 RAL+TVD	Day 7 SOF+RAL+TVD
	AUC _{tau} (h·ng/mL)	13,952.5 (23.3)	14,109.7 (31.9)	10,604.9 (8.2)
C _{max} (ng/mL)	2007.7 (28.9)	1842.2 (35.0)	1940.2 (17.0)	2213.5 (17.8)
C _{tau} (ng/mL)	163.5 (36.4)	197.4 (36.0)	83.2 (35.8)	89.4 (20.3)
T _{max} (h) ^a	3.00 (2.00, 4.00)	4.08 (4.08, 4.08)	2.00 (2.00, 2.00)	2.08 (2.08, 2.08)
t _{1/2} (h) ^a	7.46 (7.35, 7.46)	8.12 (7.61, 8.74)	7.20 (5.64, 8.27)	7.80 (6.85, 8.78)
T _{last} (h) ^a	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)

a Median (Q1, Q3)

Table 7 presents statistical comparisons of the primary FTC PK parameters following administration of FTC as a component of ARV regimens alone and in combination with SOF. FTC AUC, C_{max}, C_{tau} was comparable following coadministration of SOF with all FTC containing regimens except 19% and 14% increase on C_{max} following administration of SOF+ATR and SOF+RAL+TVD, respectively; and 20% increase on C_{tau} following administration of SOF+DRV/r+TVD. These differences are not expected to be clinically significant.

Table 7 Statistical Comparisons of Emtricitabine Plasma Pharmacokinetic Parameters Following Administration of Antiretroviral Regimens Alone and in Combination with Sofosbuvir

FTC PK Parameter	GLSM		%GLSM Ratio (90% CI)
	Day 0	Day 7	
Cohort 1	ATR (N = 8)	SOF+ATR (N = 8)	SOF+ATR / ATR
AUC _{tau} (h·ng/mL)	9884.60	9823.05	99.38 (85.20, 115.9)
C _{max} (ng/mL)	1177.53	1406.09	119.4 (85.68, 166.4)
C _{tau} (ng/mL)	96.51	93.99	97.39 (90.03, 105.4)
Cohort 3	ATV/r+TVD (N = 8)	SOF+ATV/r+TVD (N = 8)	SOF+ATV/r+TVD / ATV/r+TVD
AUC _{tau} (h·ng/mL)	11,888.99	11,452.65	96.33 (80.21, 115.7)
C _{max} (ng/mL)	1772.23	1784.05	100.7 (87.71, 115.5)
C _{tau} (ng/mL)	117.93	110.74	93.90 (79.06, 111.5)
Cohort 4	DRV/r+TVD (N = 5)	SOF+DRV/r+TVD (N = 5)	SOF+ DRV/r+TVD / DRV/r+TVD
AUC _{tau} (h·ng/mL)	13,655.18	13,577.28	99.43 (85.62, 115.5)
C _{max} (ng/mL)	1941.10	1767.47	91.06 (80.67, 102.8)
C _{tau} (ng/mL)	154.66	185.08	119.7 (74.94, 191.1)
Cohort 5	RAL+TVD (N = 5)	SOF+RAL+TVD (N = 5)	SOF+ RAL+TVD / RAL+TVD
AUC _{tau} (h·ng/mL)	10,576.48	10,666.58	100.9 (97.70, 104.1)
C _{max} (ng/mL)	1917.65	2183.59	113.9 (103.1, 125.6)
C _{tau} (ng/mL)	78.79	87.87	111.5 (89.5, 139.0)

Zidovudine

Figure 11 shows mean (SD) plasma concentration-time profiles for ZDV following administration of EFV+ZDV/3TC alone and in combination with SOF. Slight differences in the plasma concentration-time profile of ZDV following administration of EFV+ZDV/3TC (Day 0) or SOF+EFV+ZDV/3TC (Day 7) were observed and may be attributed to the small size of the cohort.

Figure 11 Mean (SD) Zidovudine Plasma Concentration-Time Profiles Following Administration of Efavirenz+Zidovudine/Lamivudine Alone and in Combination with Sofosbuvir

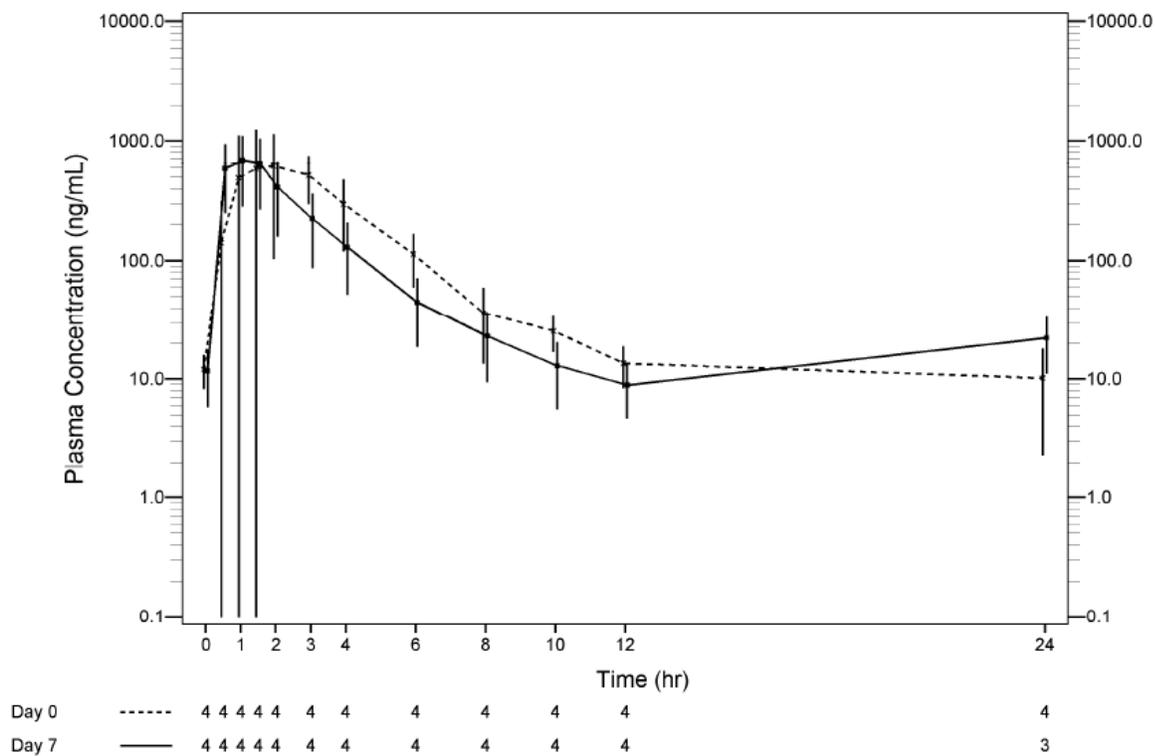


Table 8 presents ZDV PK parameters following administration of EFV+ZDV/3TC alone and in combination with SOF.

Table 8 Zidovudine Plasma Pharmacokinetic Parameters Following Administration of Efavirenz+Zidovudine/Lamivudine Alone and in Combination with Sofosbuvir

ZDV PK Parameter	Mean (%CV)	
	Cohort 2 (N = 4)	
	Day 0 EFV+ZDV/3TC	Day 7 SOF+EFV+ZDV/3TC
AUC _{tau} (h·ng/mL)	2335.1 (56.6)	1850.4 (38.3)
C _{max} (ng/mL)	810.2 (55.3)	940.8 (22.2)
C _{tau} (ng/mL)	13.5 (38.3)	8.9 (47.5)
T _{max} (h) ^a	2.25 (1.50, 3.00)	0.83 (0.58, 1.33)
t _{1/2} (h) ^a	2.09 (1.87, 2.66)	2.54 (2.44, 2.92)
T _{last} (h) ^a	12.00 (12.00, 12.00)	12.00 (12.00, 12.00)

^a Median (Q1, Q3)

Table 9 presents statistical comparisons of the primary ZDV PK parameters following administration of EFV+ZDV/3TC alone and in combination with SOF. Mean AUC_{tau} and C_{tau} of

ZDV were decreased by 16% and 37%, respectively, but C_{max} of ZDV was increased by 27%. The study has wide 90% CIs due to the small sample size.

Table 9 Statistical Comparisons of Zidovudine Plasma Pharmacokinetic Parameters Following Administration of Efavirenz+Zidovudine/Lamivudine Alone and in Combination with Sofosbuvir

ZDV PK Parameter	GLSM		%GLSM Ratio (90% CI)
	Day 0	Day 7	
Cohort 2	EFV+ZDV/3TC (N = 4)	SOF+EFV+ZDV/3TC (N = 4)	SOF+EFV+ZDV/3TC / EFV+ZDV/3TC
AUC _{tau} (h·ng/mL)	2060.11	1740.03	84.46 (66.10, 107.9)
C _{max} (ng/mL)	726.22	924.75	127.3 (76.88, 210.9)
C _{tau} (h·ng/mL)	12.78	8.01	62.69 (48.17, 81.58)

Lamivudine:

Figure 12 shows mean (SD) plasma concentration-time profiles for 3TC following administration of EFV+ZDV/3TC alone and in combination with SOF. The differences observed in the plasma concentration-time profiles for 3TC following administration of EFV+ZDV/3TC (Day 0) or SOF+EFV+ZDV/3TC (Day 7) may be attributed to the small sample size in Cohort 2.

Figure 12 Mean (SD) Lamivudine Plasma Concentration-Time Profiles Following Administration of Efavirenz+Zidovudine/Lamivudine Alone and in Combination with Sofosbuvir

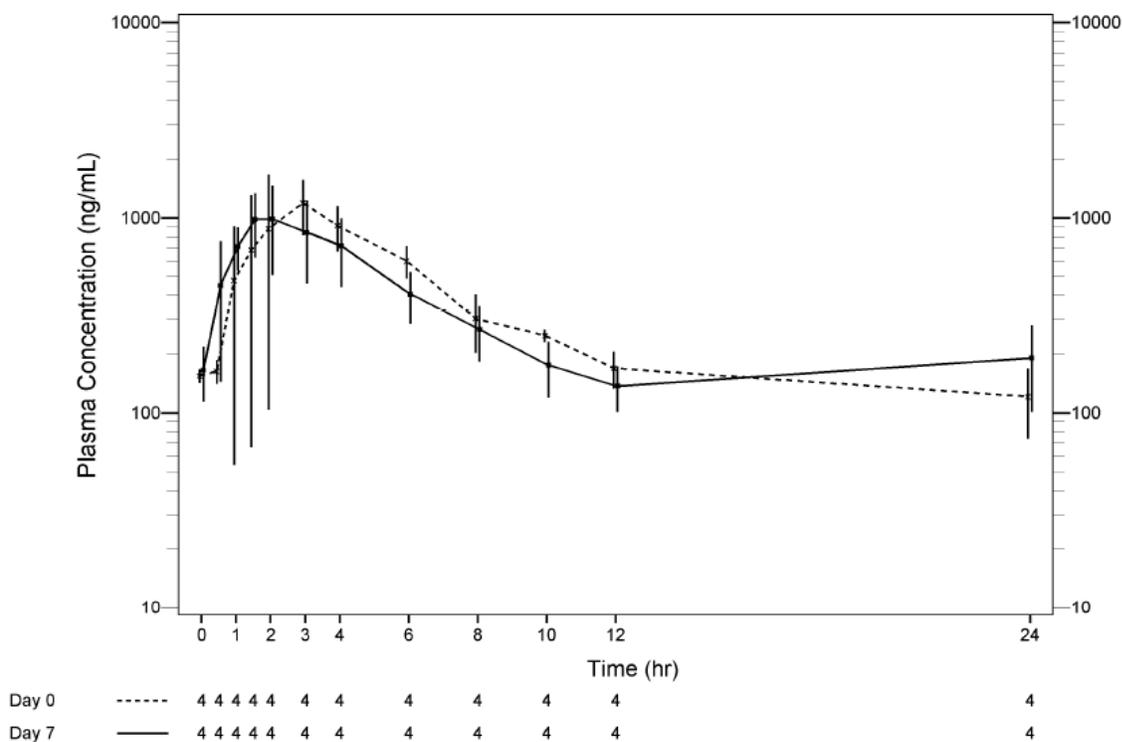


Table 10 presents 3TC PK parameters following administration of EFV+ZDV/3TC alone and in combination with SOF.

Table 10 Lamivudine Plasma Pharmacokinetic Parameters Following Administration of Efavirenz+Zidovudine/Lamivudine Alone and in Combination with Sofosbuvir

3TC PK Parameter	Mean (%CV)	
	Cohort 2 (N = 4)	
	Day 0 EFV+ZDV/3TC	Day 7 SOF+EFV+ZDV/3TC
AUC _{tau} (h·ng/mL)	6304.3 (30.0)	5562.6 (27.6)
C _{max} (ng/mL)	1250.8 (39.1)	1071.9 (38.6)
C _{tau} (ng/mL)	169.2 (21.4)	137.2 (26.1)
T _{max} (h) ^a	3.00 (2.50, 3.00)	1.58 (1.33, 1.83)
t _{1/2} (h) ^a	3.47 (3.17, 4.26)	3.61 (3.23, 4.62)
T _{last} (h) ^a	12.00 (12.00, 12.00)	12.00 (12.00, 12.00)

a Median (Q1, Q3)

Table 11 presents statistical comparisons of the primary 3TC PK parameters following administration of EFV+ZDV/3TC alone and in combination with SOF. Coadministration of SOF with EFV+ZDV/3TC resulted in 11–20% lower 3TC AUC_{tau}, C_{max}, and C_{tau}, and the upper limits of 90% CIs were all below 1. These magnitudes of decreases in 3TC exposure were not considered clinically significant; and the results suggest that SOF may be coadministered with EFV+ZDV/3TC without dose adjustments.

Table 11 Statistical Comparisons of Lamivudine Plasma Pharmacokinetic Parameters Following Administration of Efavirenz+Zidovudine/Lamivudine Alone and in Combination with Sofosbuvir

3TC PK Parameter	GLSM		%GLSM Ratio (90% CI)
	Day 0	Day 7	
Cohort 2	EFV+ZDV/3TC (N=4)	SOF+EFV+ZDV/3TC (N=4)	SOF+EFV+ZDV/3TC / EFV+ZDV/3TC
AUC _{tau} (h·ng/mL)	6121.82	5422.58	88.58 (79.40, 98.82)
C _{max} (ng/mL)	1189.53	1022.80	85.98 (77.71, 95.14)
C _{tau} (h·ng/mL)	165.72	133.24	80.40 (72.33, 89.37)

Atazanavir:

Figure 13 shows mean (SD) plasma concentration-time profiles for ATV following administration of ATV/r+TVD alone and in combination with SOF.

Figure 13. P7977-1910 Part A: Mean (SD) Atazanavir Plasma Concentration-Time Profiles Following Administration of Ritonavir-Boosted Atazanavir+Truvada Alone and in Combination with Sofosbuvir

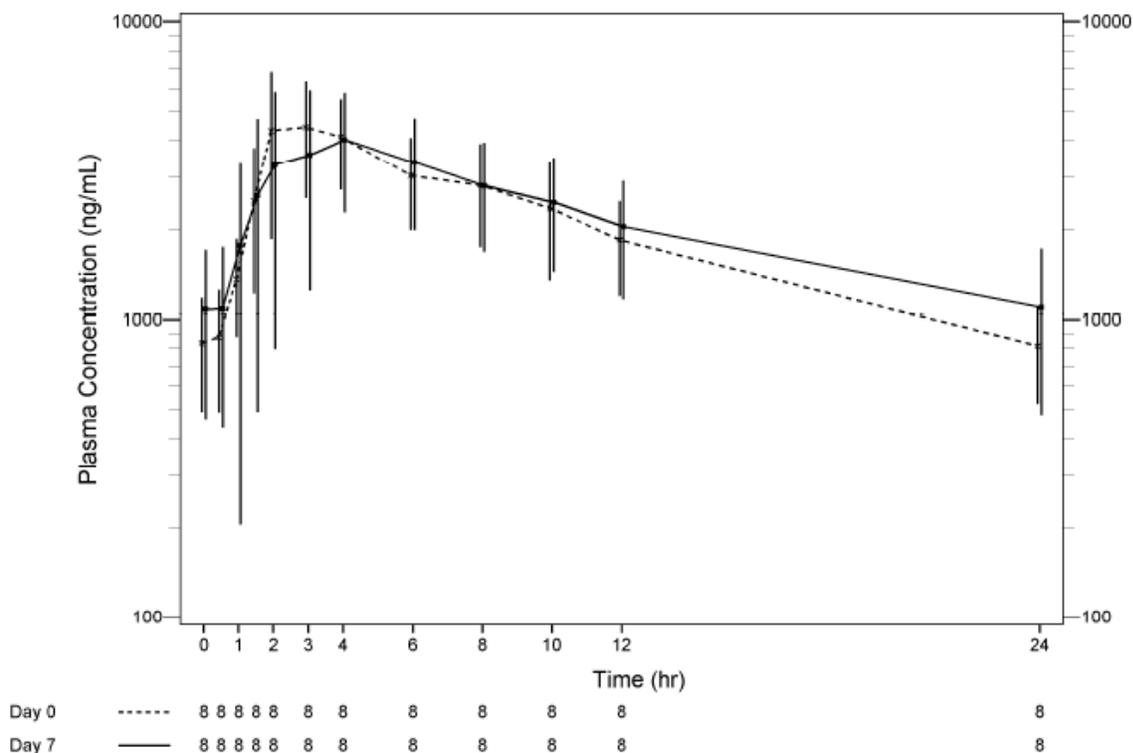


Table 12 presents ATV PK parameters following administration of ATV/r+TVD alone and in combination with SOF.

Table 12. P7977-1910 Part A: Atazanavir Plasma Pharmacokinetic Parameters Following Administration of Ritonavir-Boosted Atazanavir+Truvada Alone and in Combination with Sofosbuvir

ATV PK Parameter	Mean (%CV)	
	Cohort 3 (N = 8)	
	Day 0 ATV/r+TVD	Day 7 SOF+ATV/r+TVD
AUC _{tau} (h·ng/mL)	49,628.5 (32.9)	52,445.4 (43.8)
C _{max} (ng/mL)	4931.5 (40.5)	4618.9 (43.9)
C _{tau} (ng/mL)	817.1 (35.9)	1103.4 (56.5)
T _{max} (h) ^a	2.00 (2.00, 3.50)	3.58 (2.08, 4.08)
t _{1/2} (h) ^a	9.83 (7.42, 11.78)	9.63 (8.31, 19.89)
T _{last} (h) ^a	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)

a Median (Q1, Q3)

Table 13 presents statistical comparisons of the primary ATV PK parameters following administration of ATV/r+TVD alone and in combination with SOF. The mean AUC_{tau} and C_{max}

of ATV are comparable following administration of ATV/r +TVD alone and in combination with SOF; while C_{tau} was increased by 22% when ATV/r +TVD was coadministered with SOF.

Table 13 Statistical Comparisons of Atazanavir Plasma Pharmacokinetic Parameters Following Administration of Ritonavir-Boosted Atazanavir+Truvada Alone and in Combination with Sofosbuvir

ATV PK Parameter	GLSM		%GLSM Ratio (90% CI)
	Day 0	Day 7	
Cohort 3	ATV/r+TVD (N = 8)	SOF+ATV/r+TVD (N = 8)	SOF+ATV/r+TVD / ATV/r+TVD
AUC _{tau} (h·ng/mL)	47,053.52	47,245.17	100.4 (80.43, 125.3)
C _{max} (ng/mL)	4569.26	4186.78	91.63 (76.39, 109.9)
C _{tau} (h·ng/mL)	775.09	949.41	122.49 (90.57, 165.7)

Darunavir:

Figure 14 shows mean (SD) plasma concentration-time profiles for DRV following administration of DRV/r+TVD alone and in combination with SOF. The differences in the plasma concentration-time profiles observed following administration of DRV/r+TVD (Day 0) or SOF+DRV/r+TVD (Day 7) may be attributed to the small sample size in Cohort 4.

Figure 14. P7977-1910 Part A: Mean (SD) Darunavir Plasma Concentration-Time Profiles Following Administration of Ritonavir-Boosted Darunavir+Truvada Alone and in Combination with Sofosbuvir

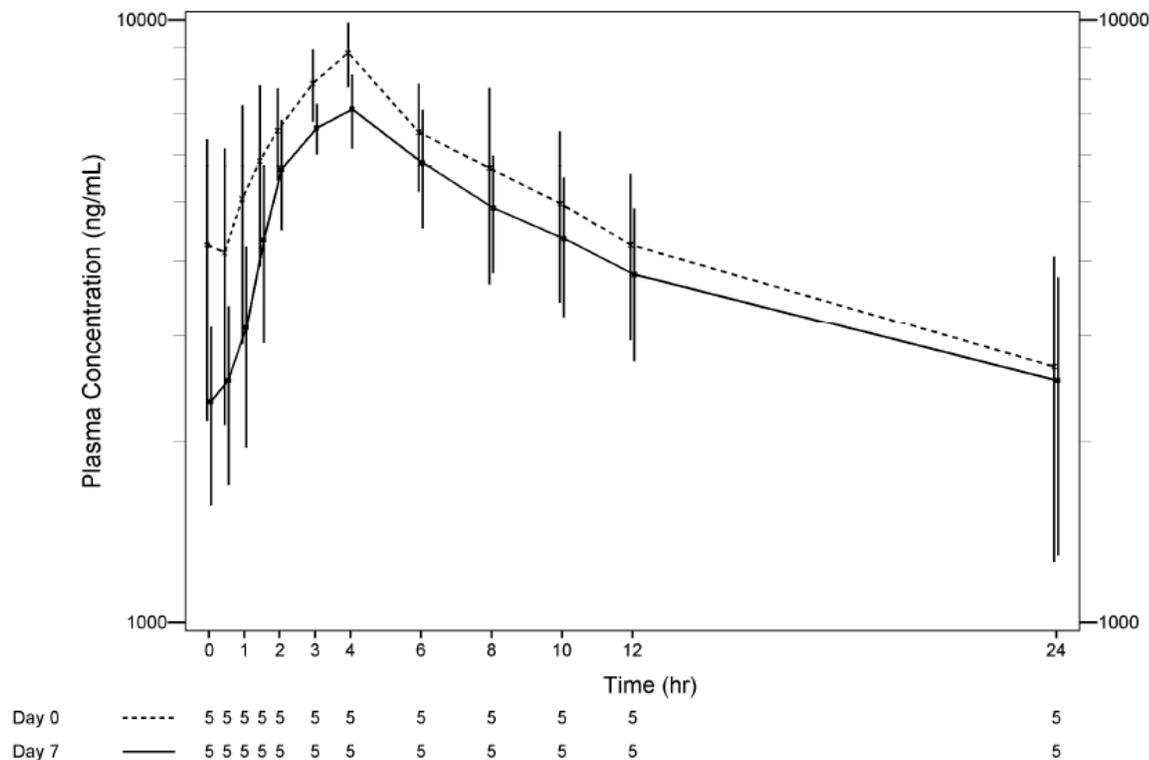


Table 14 presents DRV PK parameters following administration of DRV/r+TVD alone and in combination with SOF.

Table 14. P7977-1910 Part A: Darunavir Plasma Pharmacokinetic Parameters Following Administration of Ritonavir-Boosted Darunavir+Truvada Alone and in Combination with Sofosbuvir

DRV PK Parameter	Mean (%CV)	
	Cohort 4 (N = 5)	
	Day 0 DRV/r+TVD	Day 7 SOF+DRV/r+TVD
AUC _{tau} (h·ng/mL)	113,427.1 (26.7)	98,055.5 (25.5)
C _{max} (ng/mL)	8935.7 (10.5)	7133.3 (13.9)
C _{tau} (ng/mL)	2661.6 (52.7)	2523.7 (48.8)
T _{max} (h) ^a	4.00 (3.00, 4.00)	4.08 (4.08, 4.08)
t _{1/2} (h) ^a	13.75 (9.39, 20.59)	14.71 (11.93, 25.64)
T _{last} (h) ^a	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)

a Median (Q1, Q3)

Table 15 presents statistical comparisons of the primary DRV PK parameters following administration of DRV/r+TVD alone and in combination with SOF. Darunavir Mean AUC_{tau} and C_{tau} were comparable for each treatment, with wide 90%CI due to the small sample size. DRV C_{max} was reduced by 20% when DRV/r+TVD were administered in combination with SOF. The results were consistent with the results from Study GS-US-334-0131. These data suggest that coadministration with SOF does not clinically significantly affect PK of DRV/r+TVD.

Table 15 Statistical Comparisons of Darunavir Plasma Pharmacokinetic Parameters Following Administration of Ritonavir-Boosted Darunavir+Truvada Alone and in Combination with Sofosbuvir

DRV PK Parameter	GLSM		%GLSM Ratio (90% CI)
	Day 0	Day 7	
Cohort 4	DRV/r+TVD (N = 5)	SOF+DRV/r+TVD (N = 5)	SOF+DRV/r+TVD / DRV/r+TVD
AUC _{tau} (h·ng/mL)	110,298.54	95,703.99	86.77 (69.63, 108.1)
C _{max} (ng/mL)	8895.20	7083.14	79.63 (67.83, 93.5)
C _{tau} (ng/mL)	2330.34	2315.32	99.36 (64.95, 152.0)

Ritonavir:

Figure 15 and Figure 16 show mean (SD) plasma concentration-time profiles for RTV following administration of ATV/r+TVD or DRV/r+TVD alone and in combination with SOF.

Figure 15 Mean (SD) Ritonavir Plasma Concentration-Time Profiles Following Administration of Ritonavir-Boosted Atazanavir+Truvada Alone and in Combination with Sofosbuvir

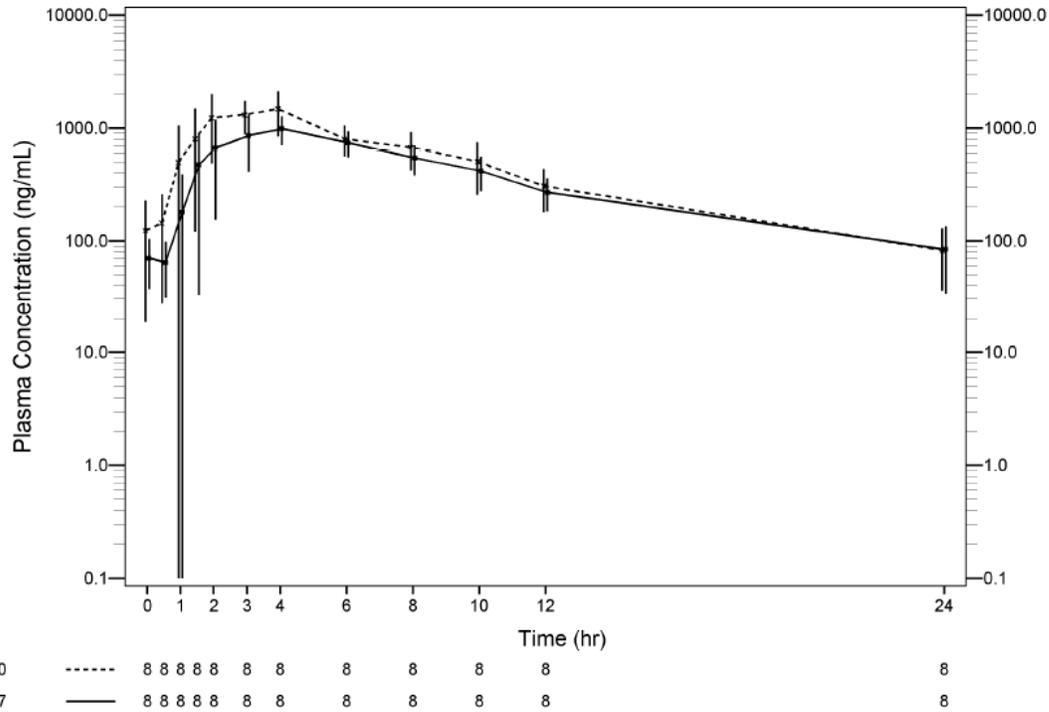


Figure 16 Mean (SD) Ritonavir Plasma Concentration-Time Profiles Following Administration of Ritonavir-Boosted Darunavir+Truvada Alone and in Combination with Sofosbuvir

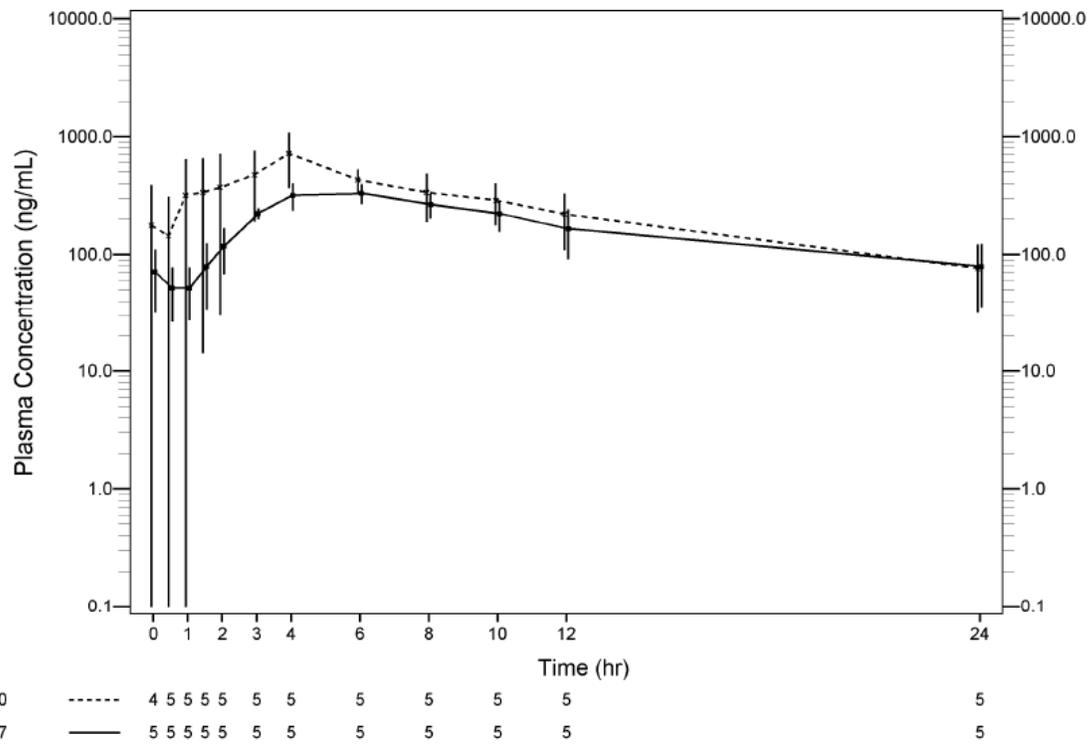


Table 16 presents RTV PK parameters following administration of ARVs alone and in combination with SOF.

Table 16 Ritonavir Plasma Pharmacokinetic Parameters Following Administration of Antiretroviral Agents Alone and in Combination with Sofosbuvir

RTV PK Parameter	Mean (%CV)			
	Cohort 3 (N = 8)		Cohort 4 (N = 5)	
	Day 0 ATV/r+TV D	Day 7 SOF+ATV/r+TV D	Day 0 DRV/r+TV D	Day 7 SOF+DRV/r+TV D
AUC _{tau} (h·ng/mL)	11,425.2 (41.2)	8739.2 (30.6)	6176.8 (38.3)	4087.9 (29.6)
C _{max} (ng/mL)	1601.5 (42.7)	1045.1 (31.5)	788.1 (44.0)	348.3 (23.4)
C _{tau} (ng/mL)	82.7 (56.0)	84.3 (59.9)	76.4 (58.8)	79.3 (55.0)
T _{max} (h) ^a	4.00 (2.50, 4.00)	3.58 (3.08, 4.08)	4.00 (3.00, 4.00)	6.08 (4.08, 6.08)
t _{1/2} (h) ^a	5.10 (4.68, 6.31)	5.31 (4.70, 7.27)	7.85 (7.83, 8.30)	9.64 (7.30, 11.98)
T _{last} (h) ^a	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)	24.00 (24.00, 24.00)

a Median (Q1, Q3)

Table 17 presents statistical comparisons of the primary RTV PK parameters following administration of ARVs alone and in combination with SOF. Coadministration of SOF with ATV/r+TV D or DRV/r+TV D resulted in comparable RTV C_{tau} estimates, and 21–53% lower RTV AUC_{tau} and C_{max} compared with ATV/r+TV D or DRV/r+TV D alone.

Table 17 Statistical Comparisons of Ritonavir Plasma Pharmacokinetic Parameters Following Administration of Antiretroviral Agents Alone and in Combination with Sofosbuvir

RTV PK Parameter	GLSM		%GLSM Ratio (90% CI)
	Day 0	Day 7	
Cohort 3	ATV/r+TV D (N = 8)	SOF+ATV/r+TV D (N = 8)	SOF+ATV/r+TV D / ATV/r+TV D
AUC _{tau} (h·ng/mL)	10,617.03	8385.96	78.99 (64.57, 96.62)
C _{max} (ng/mL)	1476.04	1002.65	67.93 (52.81, 87.37)
C _{tau} (ng/mL)	72.93	71.57	98.13 (75.36, 127.8)
Cohort 4	DRV/r+TV D (N = 5)	SOF+DRV/r+TV D (N = 5)	SOF+DRV/r+TV D / DRV/r+TV D
AUC _{tau} (h·ng/mL)	5895.03	3938.39	66.81 (46.00, 97.03)
C _{max} (ng/mL)	728.18	341.01	46.83 (29.00, 75.63)
C _{tau} (ng/mL)	62.38	65.13	104.4 (75.57, 144.3)

Raltegravir:

Figure 17 shows mean (SD) plasma concentration-time profiles for RAL following administration of RAL+TV D alone and in combination with SOF. The plasma concentration-time profiles of RAL were comparable following administration of RAL+TV D (Day 0) and SOF+RAL+TV D (Day 7).

Figure 17 Mean (SD) Raltegravir Plasma Concentration-Time Profiles Following Administration of Raltegravir+Truvada Alone and in Combination with Sofosbuvir

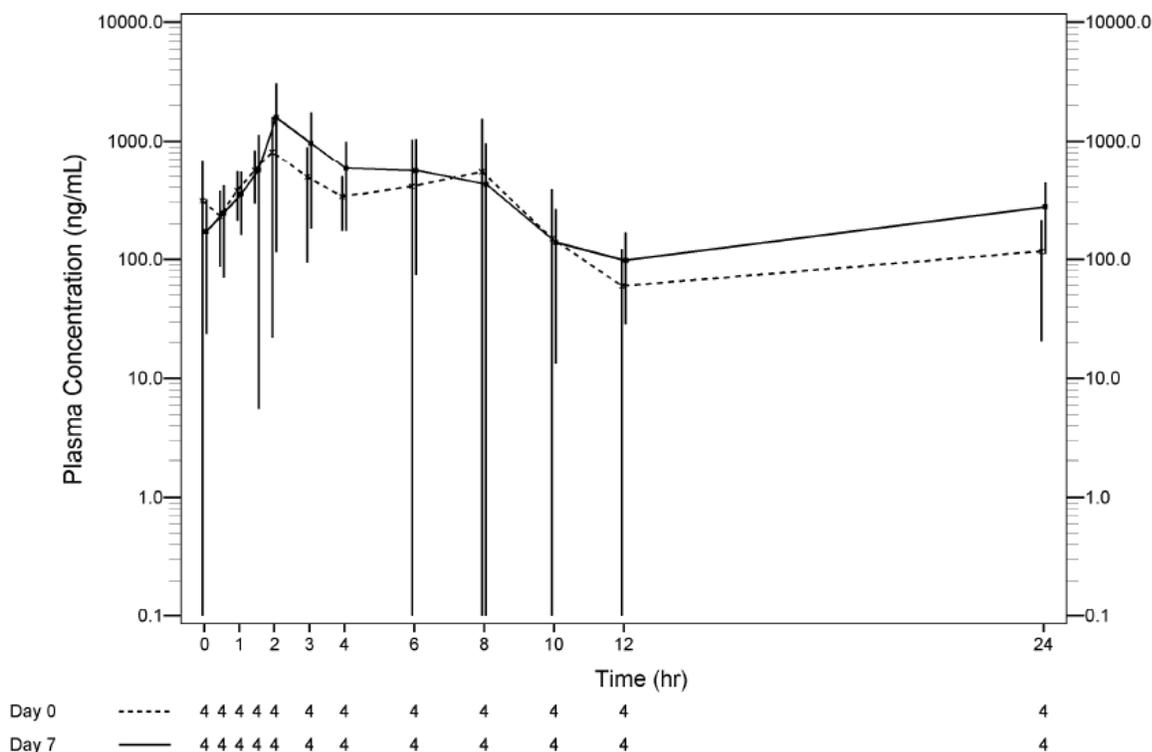


Table 18 presents RAL PK parameters following administration of RAL+TVD alone and in combination with SOF.

Table 18 Raltegravir Plasma Pharmacokinetic Parameters Following Administration of Raltegravir+Truvada Alone and in Combination with Sofosbuvir

RAL PK Parameter	Mean (%CV)	
	Cohort 5 (N = 4)	
	Day 0 RAL+TVD	Day 7 SOF+RAL+TVD
AUC _{tau} (h·ng/mL)	4361.6 (91.2)	5793.7 (45.3)
C _{max} (ng/mL)	1203.8 (78.0)	1845.8 (66.4)
C _{tau} (ng/mL)	59.7 (104.1)	98.1 (70.9)
T _{max} (h) ^a	2.00 (1.75, 5.00)	2.08 (1.58, 5.08)
t _{1/2} (h) ^a	3.37 (2.10, 4.55)	2.33 (1.95, 6.98)
T _{last} (h) ^a	12.00 (12.00, 12.00)	12.00 (12.00, 12.00)

a Median (Q1, Q3)

Table 19 presents statistical comparisons of the primary RAL PK parameters following administration of RAL+TVD alone and in combination with SOF.

Table 19 Statistical Comparisons of Raltegravir Plasma Pharmacokinetic Parameters Following Administration of Raltegravir+Truvada Alone and in Combination with Sofosbuvir

RAL PK Parameter	GLSM		%GLSM Ratio (90% CI)
	Day 0	Day 7	
Cohort 5	RAL+TVD (N = 4)	SOF+RAL+TVD (N = 4)	SOF+RAL+TVD / RAL+TVD
AUC _{tau} (h·ng/mL)	3161.80	5219.87	165.1 (47.02, 579.6)
C _{max} (ng/mL)	882.80	1453.71	164.7 (35.56, 762.5)
C _{tau} (ng/mL)	43.51	81.24	186.7 (54.04, 645.2)

Sofosbuvir:

The PK parameters of SOF, GS-566500, and GS-331007 in the presence of a background HIV regimen was compared to historical data from HCV-monoinfected subjects who received SOF alone for 7 days in Study P2938-0212. In P2938-0212 SOF was administered under fasting conditions; while in the current study SOF was administered under fasting conditions in Cohorts 1 and 2, but with food in Cohorts 3, 4, and 5, according to prescribing information for the coadministered HIV medications. studies P7977-0111 and P7977-1318 demonstrated that SOF administration in the fed state results in 67% -91% increase on AUC_{inf} and prolonged T_{max} (increased from 2-3 hours to 4 hours) as compared to the values in the fasting conditions. Due to the differences in dosing conditions in the current study and Study P2938-0212, and the cross-study comparisons of SOF, GS-566500, and GS-331007 PK profiles should be interpreted with caution.

Table 20 presents SOF PK parameters following coadministration with ARVs in Cohorts 1 through 5 and administered as a single agent in Study P2938-0212.

Table 20 Sofosbuvir Plasma Pharmacokinetic Parameters Following Coadministration with Antiretroviral Agents and Administered and as a Single Agent in Study P2938-0212

SOF PK Parameter	Mean (%CV) (Day 7)					
	Cohort					
	Study P2938-0212	1	2	3	4	5
	SOF (N = 8)	SOF+ATR (N = 8)	SOF+EFV+ ZDV/3TC (N = 4)	SOF+ATVr+ TVD (N = 8)	SOF+DRVr+ TVD (N = 5)	SOF+RAL+ TVD (N = 5)
AUC _{tau} (h·ng/mL)	538.1 (39.0)	867.5 (53.1)	627.6 (50.3)	2269.4 (25.0)	1338.0 (30.5)	1784.9 (37.6)
C _{max} (ng/mL)	602.6 (47.2)	635.0 (54.2)	285.1 (21.8)	1228.9 (38.6)	672.7 (40.0)	1305.6 (38.3)
C _{tau} (ng/mL)	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
T _{max} (h) ^a	0.50 (0.50, 0.77)	1.25 (1.00, 2.25)	1.50 (1.00, 2.50)	2.00 (1.25, 2.00)	2.00 (1.50, 2.00)	1.00 (1.00, 1.00)
t _{1/2} (h) ^a	0.48 (0.44, 0.52)	0.54 (0.41, 0.68)	0.78 (0.51, 1.01)	0.71 (0.58, 0.86)	0.67 (0.58, 0.88)	0.52 (0.51, 0.63)
T _{last} (h) ^a	3.00 (3.00, 4.00)	4.00 (4.00, 6.00)	5.00 (3.50, 7.00)	6.00 (5.00, 8.00)	6.00 (6.00, 6.00)	4.00 (4.00, 4.00)

a Median (Q1, Q3)

Table 21 presents statistical comparisons of the primary SOF PK parameters following coadministration with ARVs in Cohorts 1 through 5 and administered as a single agent in Study P2938-0212.

Table 21 Statistical Comparisons of Sofosbuvir Plasma Pharmacokinetic Parameters Following Coadministration with Antiretroviral Agents and Administered as a Single Agent in Study P2938-0212

SOF PK Parameter	GLSM (Day 7)		%GLSM Ratio (90% CI)
	P2938-0212 (N = 8)	SOF+ATR (N = 8)	
Cohort 1	P2938-0212 (N = 8)	SOF+ATR (N = 8)	SOF+ATR / P2938-0212
AUC _{tau} (h·ng/mL)	499.21	750.50	150.3 (94.17, 240.0)
C _{max} (ng/mL)	548.49	544.48	101.1 (63.73, 160.4)
Cohort 2	P2938-0212 (N = 8)	SOF+EFV+ZDV/3TC (N = 4)	SOF+EFV+ZDV/3TC / P2938-0212
AUC _{tau} (h·ng/mL)	499.21	564.73	113.1 (67.49, 189.6)
C _{max} (ng/mL)	548.49	279.89	51.03 (32.54, 80.02)
Cohort 3	P2938-0212 (N = 8)	SOF+ATV/r+TVD (N = 8)	SOF+ATV/r+TVD / P2938-0212
AUC _{tau} (h·ng/mL)	499.21	2204.95	441.7 (323.0, 604.0)
C _{max} (ng/mL)	548.49	1147.99	209.3 (143.0, 306.3)
Cohort 4	P2938-0212 (N = 8)	SOF+DRV/r+TVD (N = 5)	SOF+DRV/r+TVD / P2938-0212
AUC _{tau} (h·ng/mL)	499.21	1283.71	257.2 (171.4, 385.7)
C _{max} (ng/mL)	548.49	630.17	114.9 (72.96, 180.9)
Cohort 5	P2938-0212 (N = 8)	SOF+RAL+TVD (N = 5)	SOF+RAL+TVD / P2938-0212
AUC _{tau} (h·ng/mL)	499.21	1668.97	334.3 (215.3, 519.1)
C _{max} (ng/mL)	548.49	1200.22	218.8 (134.2, 356.9)

GS-566500:

Table 22 presents GS-566500 PK parameters following coadministration of SOF with ARVs in Cohorts 1 through 5 and administered as a single agent in Study P2938-0212. In general, the changes observed in SOF exposure following coadministration with the ARVs were reflected in GS-566500 exposure.

Table 10-22 GS-566500 Plasma Pharmacokinetic Parameters Following Administration of Sofosbuvir in Combination with Antiretroviral Agents and as a Single Agent in Study P2938-0212

GS-566500 PK Parameter	Mean (%CV) (Day 7)					
	Cohort					
	Study P2938-0212	1	2	3	4	5
	SOF (N = 8)	SOF+ATR (N = 8)	SOF+EFV+ ZDV/3TC (N = 4)	SOF+ATVr+ TVD (N = 8)	SOF+DRVr+ TVD (N = 5)	SOF+RAL+ IDV (N = 5)
AUC _{tau} (h·ng/mL)	853.1 (45.7)	3265.2 (149.1) ^d	1885.0 (48.1)	4899.5 (68.1) ^e	3311.8 (33.0)	2209.5 (32.5)
C _{max} (ng/mL)	235.2 (38.4)	625.7 (146.7) ^f	393.8 (80.2)	901.9 (64.5) ^g	621.9 (25.1)	543.2 (44.8)
C _{tau} (ng/mL)	BLQ	4.8 (186.2) ^b	BLQ	5.8 (200.1) ^b	2.4 (223.6) ^c	BLQ
T _{max} (h) ^a	1.50 (1.00, 1.75)	2.00 (1.50, 3.50)	3.00 (1.75, 5.00)	2.50 (2.00, 3.50)	3.00 (3.00, 3.00)	1.50 (1.50, 2.00)
t _{1/2} (h) ^a	2.17 (2.00, 2.31)	2.98 (2.66, 3.16)	2.55 (2.29, 2.59)	2.81 (2.50, 3.60)	2.48 (2.48, 2.86)	2.56 (2.46, 2.57)
T _{last} (h) ^a	9.00 (9.00, 10.50)	12.00 (12.00, 20.00)	16.00 (14.00, 16.00)	16.00 (16.00, 20.00)	16.00 (16.00, 16.00)	12.00 (12.00, 16.00)

a Median (Q1, Q3)

b N = 2

c N = 1

d Skewed by one subject at 15,061

e Skewed by one subject at 12,802

f Skewed by one subject at 2879

g Skewed by one subject at 2236.3

Table 23 presents statistical comparisons of the primary GS-566500 PK parameters following coadministration of SOF with ARVs in Cohorts 1 through 5 and administered as a single agent in Study P2938-0212.

Table 23. P7977-1910 Part A: Statistical Comparisons of GS-566500 Plasma Pharmacokinetic Parameters Following Administration of Sofosbuvir in Combination with Antiretroviral Agents and as a Single Agent in Study P2938-0212

GS-566500 PK Parameter	GLSM (Day 7)		%GLSM Ratio (90% CI)
Cohort 1	Study P2938-0212 (N = 8)	SOF+ATR (N = 8)	SOF+ATR / P2938-0212
AUC _{tau} (h·ng/mL)	778.71	1831.09	235.1 (118.4, 467.1)
C _{max} (ng/mL)	219.79	375.27	170.7 (91.45, 318.8)
C _{tau} (ng/mL)	–	19.0	–
Cohort 2	Study P2938-0212 (N = 8)	SOF+EFV+ZDV/3TC (N = 4)	SOF+EFV+ZDV/3TC / P2938-0212
AUC _{tau} (h·ng/mL)	778.71	1725.49	221.6 (131.6, 373.2)
C _{max} (ng/mL)	219.79	323.73	147.3 (84.46, 256.9)
C _{tau} (ng/mL)	–	–	–
Cohort 3	Study P2938-0212 (N = 8)	SOF+ATV/r+TVD (N = 8)	SOF+ATV/r+TVD / P2938-0212
AUC _{tau} (h·ng/mL)	778.71	4270.37	548.4 (357.0, 842.4)
C _{max} (ng/mL)	219.79	788.68	358.8 (238.7, 539.5)
C _{tau} (ng/mL)	–	21.8	–
Cohort 4	Study P2938-0212 (N = 8)	SOF+DRV/r+TVD (N = 5)	SOF+DRV/r+TVD / P2938-0212
AUC _{tau} (h·ng/mL)	778.71	3172.24	407.4 (265.5, 625.1)
C _{max} (ng/mL)	219.79	605.01	275.3 (190.6, 397.6)
C _{tau} (ng/mL)	–	12.1	–
Cohort 5	Study P2938-0212 (N = 8)	SOF+RAL+TVD (N = 5)	SOF+ATV/r+TVD / P2938-0212
AUC _{tau} (h·ng/mL)	778.71	2133.83	274.0 (180.9, 415.1)
C _{max} (ng/mL)	219.79	509.10	231.6 (154.7, 346.9)
C _{tau} (ng/mL)	–	–	–

GS-331007:

Table 24 presents GS-331007 PK parameters following coadministration of SOF with ARVs in Cohorts 1 through 5 and administered as a single agent in Study P2938-0212.

Table 24 GS-331007 Plasma Pharmacokinetic Parameters Following Administration of Sofosbuvir in Combination with Antiretroviral Agents and as a Single Agent in Study P2938-0212

GS-331007 PK Parameter	Mean (%CV) (Day 7)					
	Cohort					
	Study P2938-0212	1	2	3	4	5
	SOF (N = 8)	SOF+ATR (N = 8)	SOF+EFV+ ZDV/3TC (N = 4)	SOF+ATVr+ TVD (N = 8)	SOF+DRVr+ TVD (N = 5)	SOF+RAL+ TVD (N = 5)
AUC _{tau} (h·ng/mL)	9638.9 (18.7)	10,805.7 (31.7)	10,542.1 (14.6)	13,561.0 (14.3)	19,068.8 (31.1) ^b	8465.8 (19.0)
C _{max} (ng/mL)	1378.3 (19.2)	1215.2 (37.6)	1465.2 (32.5)	1062.6 (16.6)	1381.2 (23.7)	878.0 (29.9)
C _{tau} (ng/mL)	98.2 (23.4)	146.9 (37.1)	96.9 (46.8)	363.6 (24.8)	463.3 (34.3)	176.9 (28.9)
T _{max} (h) ^a	2.00 (1.75, 3.00)	4.00 (3.00, 6.00)	4.50 (2.50, 6.00)	4.00 (3.00, 4.00)	4.00 (4.00, 6.00)	3.00 (2.00, 3.00)
t _{1/2} (h) ^a	10.09 (9.05, 11.75)	7.83 (6.99, 10.09)	6.28 (5.54, 7.32)	20.26 (16.47, 25.81)	15.14 (13.91, 17.03)	16.75 (13.12, 21.93)
T _{last} (h) ^a	23.58 (22.73, 23.97)	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)

a Median (Q1, Q3)

b Three subjects had values above 20,000; 1 subject had a value of 16,000 and 1 subject had a value of at 10,000.

Table 25 presents statistical comparisons of the primary GS-331007 PK parameters following coadministration of SOF with ARVs in Cohorts 1 through 5 and administered as a single agent in Study P2938-0212.

Table 25 Statistical Comparisons of GS-331007 Plasma Pharmacokinetic Parameters Following Administration of Sofosbuvir in Combination with Antiretroviral Agents and as a Single Agent in Study P2938-0212

GS-331007 PK Parameter	GLSM (Day 7)		%GLSM Ratio (90% CI)
Cohort 1	Study P2938-0212 (N = 8)	SOF+ATR (N = 8)	SOF+ATR / P2938-0212
AUC _{tau} (h·ng/mL)	9497.52	10,406.95	109.6 (88.83, 135.2)
C _{max} (ng/mL)	1358.68	1144.81	84.26 (65.38, 108.59) ^a
C _{tau} (ng/mL)	95.2	138.26	144.2 (109.6, 189.6)
Cohort 2	Study P2938-0212 (N = 8)	SOF+EFV+ZDV/3TC (N = 4)	SOF+EFV+ZDV/3TC / P2938-0212
AUC _{tau} (h·ng/mL)	9497.52	10,454.43	110.1 (90.81, 133.4)
C _{max} (ng/mL)	1358.68	1406.26	103.5 (79.77, 134.3)
C _{tau} (ng/mL)	95.92	88.96	92.75 (64.71, 133.0)
Cohort 3	Study P2938-0212 (N = 8)	SOF+ATV/r+TVD (N = 8)	SOF+ATV/r+TVD / P2938-0212
AUC _{tau} (h·ng/mL)	9497.52	13,443.86	141.6 (122.7, 163.3)
C _{max} (ng/mL)	1358.68	1051.46	77.39 (66.99, 89.40)
C _{tau} (ng/mL)	95.92	352.57	367.6 (294.3, 459.1)
Cohort 4	Study P2938-0212 (N = 8)	SOF+DRV/r+TVD (N = 5)	SOF+DRV/r+TVD / P2938-0212
AUC _{tau} (h·ng/mL)	9497.52	18,188.26	191.5 (146.4, 250.5)
C _{max} (ng/mL)	1358.68	1346.71	99.12 (79.89, 123.0)
C _{tau} (ng/mL)	95.92	437.88	456.5 (336.4, 619.6)
Cohort 5	Study P2938-0212 (N = 8)	SOF+RAL+TVD (N = 5)	SOF+RAL+TVD / P2938-0212
AUC _{tau} (h·ng/mL)	9497.52	8348.48	87.90 (72.87, 106.0)
C _{max} (ng/mL)	1358.68	848.50	62.45 (49.64, 78.56)
C _{tau} (ng/mL)	95.92	170.32	177.6 (135.5, 232.7)

^a One subject who received ATR+SOF had high GS-566500 C_{max}, and high GS-331007 C_{max} (2050 ng/mL) and AUC (18,332 h·ng/mL).

Conclusion: The results from this study were generally in agreement with the results from Study GS-US-334-0131, and show no clinically significant drug interactions between antiretrovirals and SOF.

4.2.4.3 P7977-1819: An Open-Label, Randomized, Three Period, Cross-Over, Drug Interaction Study to Assess the Effect on Pharmacokinetics of Co-administration of PSI-7977 and Cyclosporine or Tacrolimus in Healthy Subjects

Objectives:

- To evaluate the effect of sofosbuvir (SOF; GS-7977; formerly PSI-7977) coadministration on the single-dose pharmacokinetics (PK) of cyclosporine or tacrolimus in healthy subjects
- To evaluate the effect of cyclosporine or tacrolimus coadministration on the single-dose PK of sofosbuvir and metabolites in healthy subjects
- To evaluate the safety and tolerability of sofosbuvir coadministration with cyclosporine or tacrolimus in healthy subject

Study Design: Subjects were enrolled in 1 of the following 2 groups:

- **Group 1:** Subjects received a single dose of sofosbuvir 400 mg (Treatment 1A), a single dose of cyclosporine 600 mg (Treatment 1B), and a combination of sofosbuvir 400 mg and cyclosporine 600 mg (Treatment 1C).
- **Group 2:** Subjects received a single dose of sofosbuvir 400 mg (Treatment 2A), a single dose of tacrolimus 5 mg (Treatment 2B), and a combination of sofosbuvir 400 mg and tacrolimus 5 mg (Treatment 2C).

Within each group, subjects were randomized to a 3-way crossover of 3 treatment periods with a 2-week washout period between doses.

Subjects: 35 subjects for PK analysis set

Formulation: Sofosbuvir 200-mg tablets (Lot # 11G086-P1); Cyclosporine: Neoral® 100-mg capsules (Lot No. F4146A); Tacrolimus: Prograf® 5-mg capsules (Lot # 040722)

PK Sampling: For Groups 1 and 2, serial blood samples were collected relative to the dosing of sofosbuvir, cyclosporine, and tacrolimus at the following time points: 0 (baseline), 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 24, 48, and 72 hours postdose. For Group 2, an additional blood sample was collected at 96 hours postdose.

Analytical methods: Plasma concentrations of sofosbuvir, GS-566500, GS-331007, and blood concentrations of cyclosporine and tacrolimus were determined using fully validated high-performance liquid chromatography-tandem mass spectroscopy (LC/MS/MS) bioanalytical methods. All samples were analyzed in the timeframe supported by frozen stability storage data. The assays for sofosbuvir, GS-566500, GS-331007, and tacrolimus were performed and validated by (b) (4). The assay for cyclosporine was performed and validated by (b) (4).

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

Table 1 Summary of Quality Control (QC) Results –Study P7977-0814

Analyte	Linear range (ng/mL)	Between Run Precision (%CV)	Between Run Bias (% Deviation)	QC samples (ng/mL)	Validation sample for stability and conditions
SOF	5 – 5000 R ² > 0.990	≤ 5.8	0.7 to 5.9	15, 30, 500 and 4000	Stable for 99 days and ≥ 5 freeze/thaw cycles in plasma at -70°C
GS-566500	10 – 5000 R ² > 0.991	≤ 6.7	1.1 to 2.6	30, 500 and 4000	Stable for 125 days and ≥ 5 freeze/thaw cycles in plasma at -70°C
GS-331007	10 – 5000 R ² > 0.991	≤ 7.5	-0.8 to 2.5	30, 500 and 4000	Stable for 184 days and ≥ 5 freeze/thaw cycles in plasma at -70°C
Cyclosporine	5 – 5000 R ² > 0.998	≤ 4.1	-12.7 to -8.3	15, 300, and 4000	Stable for 10 weeks at -70 °C
tacrolimus	0.2 – 100 R ² > 0.994	≤ 26.8*	-0.3 to 9.3	0.4, 20, and 80	Stable for 218 days at -20 °C

*high between run CV% was from the lowest concentration 0.4 ng/mL, and is not expect to affect the final conclusions.

Pharmacokinetic Results:

Cyclosporine:

The mean (SD) blood concentration-time profiles for cyclosporine, following administration of cyclosporine alone and in the presence of a single dose of sofosbuvir, are shown in [Figure 1](#). The concentration profiles of cyclosporine, when administered with and without sofosbuvir, were similar.

Figure 1. P7977-1819: Mean (SD) Cyclosporine Blood Concentration-Time Profiles Following Administration of Cyclosporine Alone or in Combination with Sofosbuvir

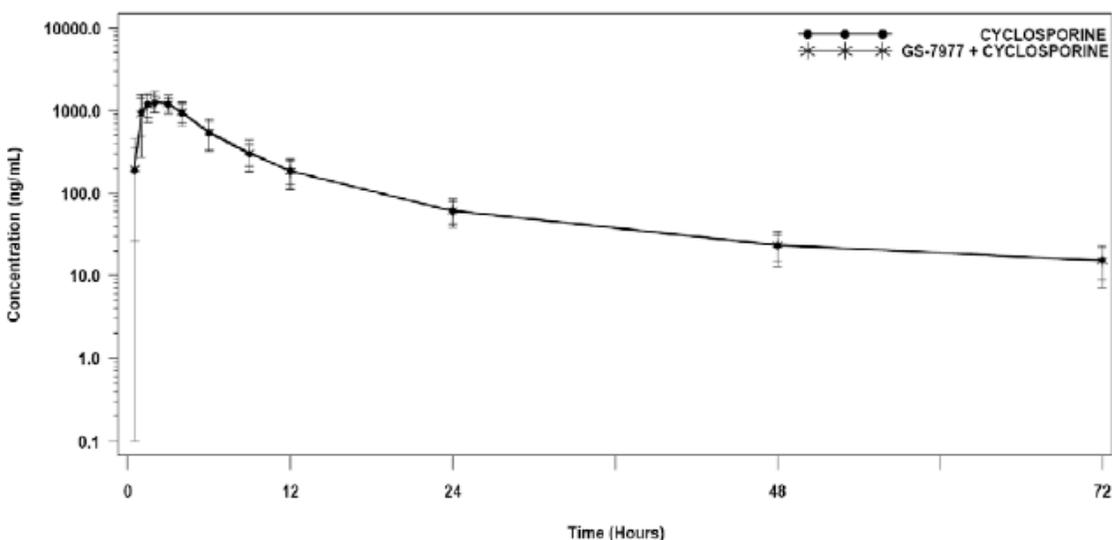


Table 2 presents the PK parameters for cyclosporine calculated following administration of cyclosporine alone or in combination with sofosbuvir. Coadministration of sofosbuvir with cyclosporine had minimal effect on cyclosporine blood PK parameters.

Table 2 P7977-1819: Cyclosporine Blood Pharmacokinetic Parameters Following Administration of Cyclosporine Alone or in Combination with Sofosbuvir

Cyclosporine PK Parameter	Mean (%CV)	
	Cyclosporine Alone (N = 19)	Cyclosporine +SOF (N = 19)
AUC _{0-last} (h·ng/mL)	9843.13 (18.55)	10,004.19 (29.63)
AUC _{0-inf} (h·ng/mL)	10,425.72 (19.78)	10,566.57 (30.69)
%ExtrapAUC (%)	5.36 (47.25)	5.06 (49.32)
C _{max} (ng/mL)	1377.37 (17.05)	1471.21 (22.35)
T _{max} (h) ^a	3.00 (1.50, 3.02)	2.00 (1.98, 3.00)
t _{1/2} (h) ^a	24.42 (21.45, 26.46)	23.63 (19.14, 26.77)
CL/F (mL/h)	59,776.62 (20.53)	62,939.66 (37.66)

a Median (Q1, Q3)

Table 3 presents the statistical comparisons of the primary PK parameters for cyclosporine following administration of cyclosporine alone and in combination with sofosbuvir. The 90% CIs of the GLSM ratios for the cyclosporine PK parameters AUC_{0-last}, AUC_{0-inf}, and C_{max} were within the predetermined boundaries of 80% to 125%.

Table 3. P7977-1819: Statistical Evaluations of Cyclosporine Blood Pharmacokinetic Parameters Following Administration of Cyclosporine Alone or in Combination with Sofosbuvir

Cyclosporine PK Parameter	GLSM		%GLSM Ratio (90% CI) Cyclosporine+SOF/ Cyclosporine Alone
	Cyclosporine Alone (N = 19)	Cyclosporine+SOF (N = 19)	
AUC _{0-last} (h·ng/mL)	9680.5	9558.3	98.7 (85.4, 114.1)
AUC _{0-inf} (h·ng/mL)	10,232.1	10,071.3	98.4 (84.8, 114.3)
C _{max} (ng/mL)	1359.3	1433.7	105.5 (94.2, 118.1)

Tacrolimus:

The mean (SD) blood concentration-time profiles for tacrolimus, following administration of tacrolimus alone and in the presence of a single dose of sofosbuvir, are shown in Figure 2. The maximum concentration of tacrolimus (C_{max}) was marginally lower and the time to C_{max}, (T_{max}), was slightly prolonged upon coadministration of tacrolimus with sofosbuvir compared to following administration of tacrolimus alone, with no apparent change in the terminal phase of the profiles.

Figure 2. P7977-1819: Mean (SD) Tacrolimus Blood Concentration-Time Profiles Following Administration of Tacrolimus Alone or in Combination with Sofosbuvir

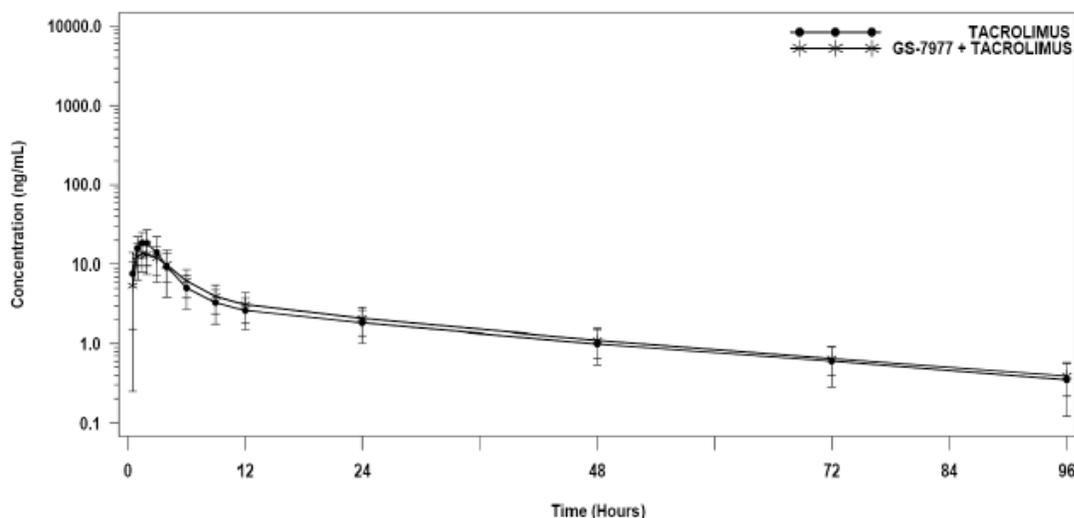


Table 4 presents the PK parameters for tacrolimus calculated following administration of tacrolimus alone or in combination with sofosbuvir.

Table 4. P7977-1819: Tacrolimus Blood Pharmacokinetic Parameters Following Administration of Tacrolimus Alone or in Combination With Sofosbuvir

Tacrolimus PK Parameters	Mean (% CV)	
	Tacrolimus Alone (N = 16)	Tacrolimus+SOF (N = 16)
AUC _{0-last} (h·ng/mL)	176.40 (43.18)	183.04 (33.83)
AUC _{0-inf} (h·ng/mL)	194.32 (43.03)	201.24 (33.50)
% ExtrapAUC (%)	9.42 (26.13)	9.14 (24.31)
C _{max} (ng/mL)	21.92 (33.51)	15.95 (31.38)
T _{max} (h) ^a	1.50 (1.00, 2.00)	2.00 (1.48, 2.99)
t _{1/2} (h) ^a	29.81 (28.40, 34.03)	29.53 (27.83, 32.14)
CL/F (mL/h)	31,936.87 (53.15)	27,660.46 (33.48)

a Median (Q1, Q3)

Table 5 presents the statistical comparisons of the primary PK parameters for tacrolimus following administration of tacrolimus alone and in combination with sofosbuvir. The upper bounds of the 90% CIs of the GLSM ratios for the tacrolimus PK parameters AUC_{0-last} and AUC_{0-inf} exceeded the prespecified bounds of 80% to 125% and the GLSM C_{max} was 27.4% lower when tacrolimus was coadministered with sofosbuvir compared with administration of tacrolimus alone. However, these changes in tacrolimus exposure are unlikely to be clinically significant.

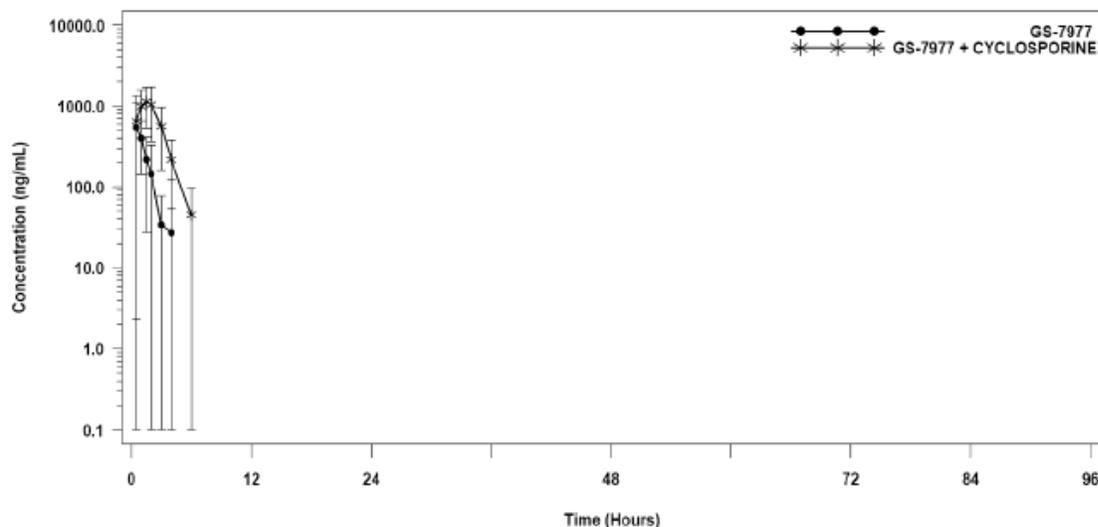
Table 5. P7977-1819: Statistical Evaluations of Tacrolimus Blood Pharmacokinetic Parameters Following Administration of Tacrolimus Alone or with Sofosbuvir

Tacrolimus PK Parameter	GLSM		%GLSM Ratio (90% CI) Tacrolimus +SOF/Tacrolimus Alone
	Tacrolimus Alone (N = 16)	Tacrolimus+SOF (N = 16)	
AUC _{0-last} (h·ng/mL)	159.2	173.2	108.9 (84.3, 140.5)
AUC _{0-inf} (h·ng/mL)	175.8	190.7	108.5 (84.4, 139.5)
C _{max} (ng/mL)	20.8	15.1	72.6 (59.0, 89.5)

Sofosbuvir:

The mean (SD) plasma concentration-time profiles for sofosbuvir, following administration of sofosbuvir alone and in combination with a single dose of cyclosporine, are shown in [Figure 3](#). The sofosbuvir C_{max} was substantially higher and the T_{max} and t_{1/2} were prolonged after coadministration of sofosbuvir and cyclosporine compared with administration of sofosbuvir alone.

Figure 3. P7977-1819: Mean (SD) Sofosbuvir Plasma Concentration-Time Profiles Following Administration of Sofosbuvir Alone or in Combination With a Single Dose of Cyclosporine



The mean (SD) plasma concentration-time profiles for sofosbuvir, following administration of sofosbuvir alone and in combination with a single dose of tacrolimus, are shown in [Figure 4](#).

Figure 4. P7977-1819: Mean (SD) Sofosbuvir Plasma Concentration-Time Profiles Following Administration of Sofosbuvir Alone or in Combination with a Single Dose of Tacrolimus

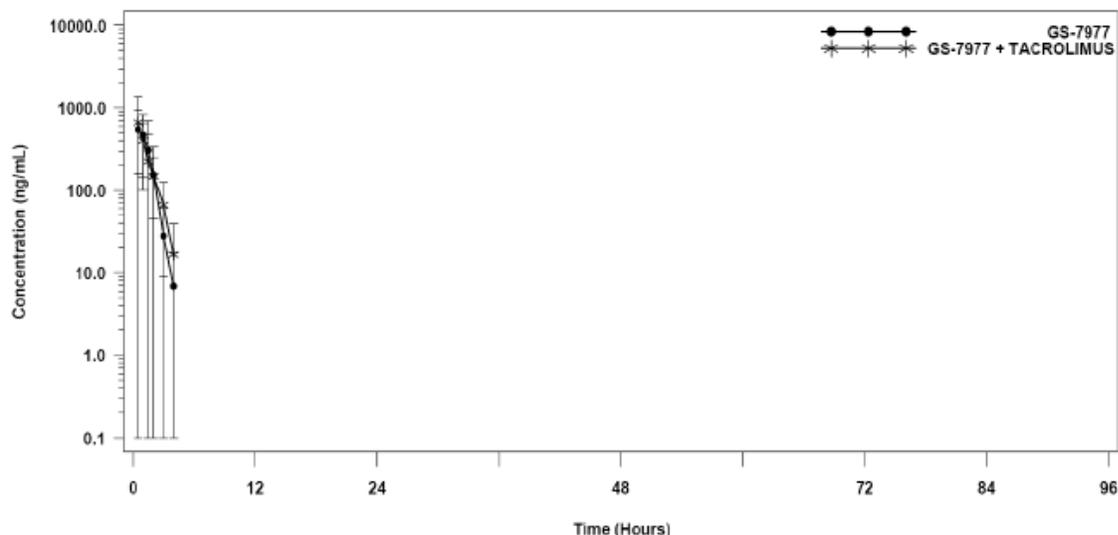


Table 6 presents the PK parameters for sofosbuvir calculated following administration of sofosbuvir alone or in combination with cyclosporine or tacrolimus. Cyclosporine substantially increased the exposure of sofosbuvir (3.6-fold increase in AUC_{0-inf} and 2.2-fold increase in C_{max}). The median T_{max} and t_{1/2} of sofosbuvir were increased when sofosbuvir was coadministered with cyclosporine. These data indicate an increase in bioavailability and/or a decrease in systemic elimination of sofosbuvir in the presence of cyclosporine. Tacrolimus did not substantially alter the exposure of sofosbuvir. The median T_{max} and t_{1/2} for sofosbuvir were similar following administration of sofosbuvir alone or in combination with tacrolimus.

Table 6. P7977-1819: Sofosbuvir Plasma Pharmacokinetic Parameters Following Administration of Sofosbuvir Alone or in Combination with Cyclosporine or Tacrolimus

SOF PK Parameter	Mean (%CV)		Mean (%CV)	
	SOF Alone (Group 1) (N = 18)	SOF +Cyclosporine (Group 1) (N = 19)	SOF Alone (Group 2) (N = 15)	SOF+Tacrolimus (Group 2) (N = 16)
AUC _{0-last} (h·ng/mL)	696.52 (59.56)	3012.56 (35.51)	761.56 (62.41)	777.13 (46.68)
AUC _{0-inf} (h·ng/mL)	849.15 (101.70)	3033.84 (35.36)	769.63 (62.25)	820.05 (43.52)
% ExtrapAUC (%)	5.12 (298.81)	0.75 (79.99)	1.23 (76.31)	5.05 (222.42)
C _{max} (ng/mL)	675.69 (75.78)	1497.99 (39.70)	748.87 (51.88)	819.91 (76.44)
T _{max} (h) ^a	0.50 (0.50, 1.00)	1.00 (0.50, 1.50)	0.50 (0.50, 1.00)	0.50 (0.50, 1.00)
t _{1/2} (h) ^a	0.44 (0.39, 0.53)	0.65 (0.56, 1.06)	0.44 (0.39, 0.50)	0.57 (0.47, 0.75)
CL/F (mL/h)	762,740.48 (57.85)	157,138.93 (52.34)	760,381.28 (71.74)	572,862.95 (41.89)

^a Median (Q1, Q3)

Table 7 presents the statistical evaluations of the sofosbuvir PK parameters determined following administration of sofosbuvir alone or in combination with cyclosporine or tacrolimus.

With respect to the impact of cyclosporine on the PK of sofosbuvir, the 90% CIs of the GLSM ratios for the sofosbuvir PK parameters AUC_{0-last}, AUC_{0-inf}, and C_{max} were substantially increased by coadministration with cyclosporine (154% to 385% increase) and were not contained within the predetermined boundaries of 80% to 125%

With respect to the impact of tacrolimus on the PK of sofosbuvir, the 90% CIs of the GLSM ratios for the sofosbuvir PK parameters were altered: AUC_{0-last} were increased by 7.1%, AUC_{0-inf} were increased by 12.7%, C_{max} were decreased by 3.5%, and they were not contained within the predetermined boundaries of 80% to 125% (upper bound of 90% CI for all PK parameters exceeded 125%). However, the effect of tacrolimus on sofosbuvir exposure was modest and is not considered clinically significant.

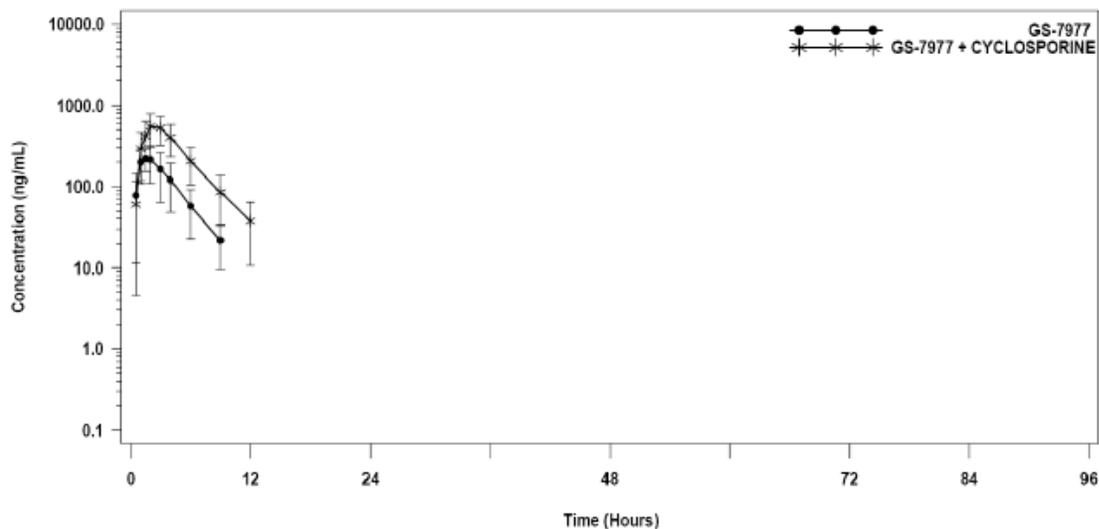
Table 7. P7977-1819: Statistical Evaluations of Sofosbuvir Plasma Pharmacokinetic Parameters Following Administration of Sofosbuvir Alone or in Combination with Cyclosporine or Tacrolimus

SOF PK Parameter	GLSM		%GLSM Ratio (90% CI) SOF +Cyclosporine/SOF Alone	GLSM		%GLSM Ratio (90% CI) SOF +Tacrolimus/SOF Alone
	SOF Alone (N = 18)	SOF +Cyclosporine (N = 19)		SOF Alone (N = 15)	SOF +Tacrolimus (N = 16)	
AUC _{0-last} (h·ng/mL)	592.2	2871.7	484.9 (363.1, 647.5)	635.5	680.4	107.1 (76.6, 149.6)
AUC _{0-inf} (h·ng/mL)	638.3	2894.2	453.4 (326.2, 630.1)	643.4	725.3	112.7 (81.0, 156.9)
C _{max} (ng/mL)	550.9	1398.2	253.8 (186.7, 345.0)	651.0	628.3	96.5 (65.0, 143.4)

GS-566500

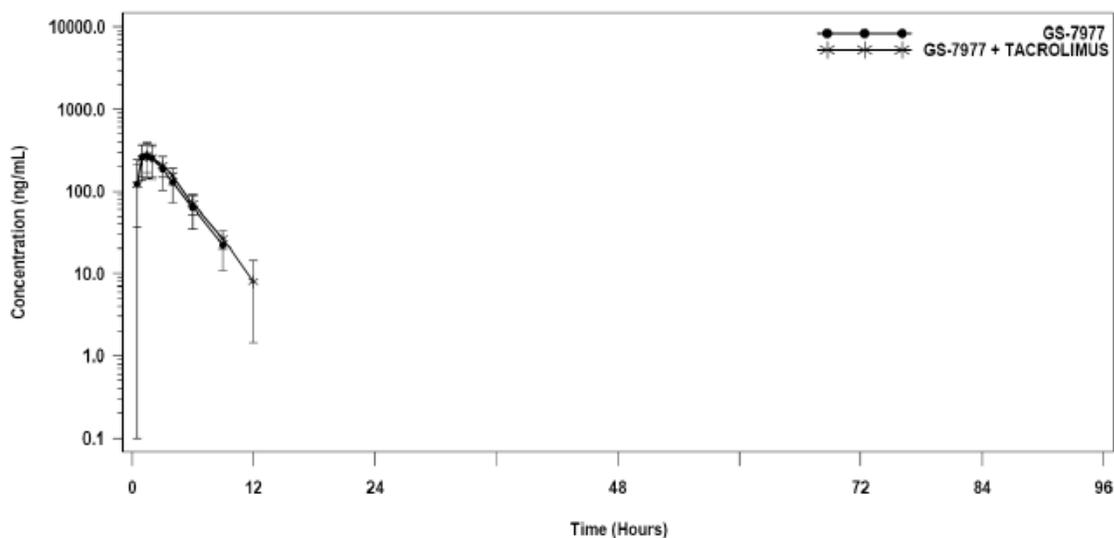
The mean (SD) plasma concentration-time profiles for GS-566500, following administration of sofosbuvir alone and in the presence of a single dose of cyclosporine, are shown in [Figure 5](#). The GS-566500 C_{max} was significantly higher and the T_{max} and t_{1/2} were slightly longer after coadministration of sofosbuvir and cyclosporine compared with administration of sofosbuvir alone. Cyclosporine altered the PK concentration-time profile of GS-566500.

Figure 5. P7977-1819: Mean (SD) GS-566500 Plasma Concentration-Time Profiles When Sofosbuvir was Administered Alone or in Combination With a Single Dose of Cyclosporine



The mean (SD) plasma concentration-time profiles for GS-566500, following administration of sofosbuvir alone and in combination with a single dose of tacrolimus, are shown in [Figure 6](#). Tacrolimus did not alter the PK concentration-time profile of GS-566500.

Figure 6. P7977-1819: Mean (SD) GS-566500 Plasma Concentration-Time Profiles When Sofosbuvir was Administered Alone or in Combination with a Single Dose of Tacrolimus



[Table 8](#) presents the PK parameters for GS-566500 following administration of sofosbuvir alone or in combination with cyclosporine or tacrolimus. The increase in the exposure of sofosbuvir following coadministration with cyclosporine was reflected in the PK profile of its metabolite GS-566500. Consistent with the increase in sofosbuvir AUC and C_{max}, the exposure of GS-566500 was substantially increased when sofosbuvir was coadministered with cyclosporine (3.0-fold increase in AUC_{0-inf} and 2.4-fold increase in C_{max}). The median T_{max} and t_{1/2} for GS-566500 were comparable when SOF was administered alone and in combination with cyclosporine.

Consistent with the observations for sofosbuvir, tacrolimus did not substantially alter the exposure of GS-566500. The AUC_{0-inf} and C_{max} of GS-566500 were similar when sofosbuvir was administered alone and with tacrolimus. The median T_{max} and t_{1/2} for GS-566500 were also similar when sofosbuvir was administered alone and in combination with tacrolimus. These data suggest no significant effect of tacrolimus on the PK of GS-566500.

Table 8. P7977-1819: GS-566500 Plasma Pharmacokinetic Parameters Following Administration of Sofosbuvir Alone or in Combination with Cyclosporine or Tacrolimus

GS-566500 PK Parameters	Mean (%CV)		Mean (%CV)	
	SOF Alone (Group 1) (N = 18)	SOF +Cyclosporine (Group 1) (N = 19)	SOF Alone (Group 2) (N = 15)	SOF +Tacrolimus (Group 2) (N = 16)
AUC _{0-last} (h·ng/mL)	936.64 (46.42)	2698.92 (36.34)	1088.07 (38.33)	1183.78 (27.24)
AUC _{0-inf} (h·ng/mL)	985.53 (44.35)	2832.05 (38.13)	1137.18 (37.00)	1231.26 (25.32)
% ExtrapAUC (%)	5.62 (53.31)	4.24 (51.24)	4.82 (45.21)	4.36 (78.56)
C _{max} (ng/mL)	255.66 (43.72)	618.86 (34.33)	310.51 (33.46)	310.44 (38.07)
T _{max} (h) ^a	1.50 (1.00, 1.53)	2.00 (2.00, 3.00)	1.05 (1.00, 2.00)	1.80 (1.00, 2.03)
t _{1/2} (h) ^a	2.04 (2.00, 2.16)	2.16 (1.96, 2.51)	2.03 (1.84, 2.19)	2.10 (1.92, 2.19)

a Median (Q1, Q3)

The statistical evaluations of the GS-566500 PK parameters determined following administration of sofosbuvir alone or in combination with cyclosporine or tacrolimus are shown in [Table 9](#). With respect to the impact of cyclosporine on the PK of sofosbuvir, the 90% CIs of the GLSM ratios for the GS-566500 PK parameters AUC_{0-last}, AUC_{0-inf}, and C_{max} were not contained within the predetermined boundaries of 80% to 125%. GS-566500 AUC_{0-last}, AUC_{0-inf}, and C_{max} were substantially increased (154% to 203% increase) by coadministration of sofosbuvir with cyclosporine.

With respect to the impact of tacrolimus on the PK of sofosbuvir, the 90% CIs of the GLSM ratios for the GS-566500 PK parameters AUC_{0-last}, AUC_{0-inf}, and C_{max} were not contained within the predetermined boundaries of 80% to 125%. Compared to the increase in GS-566500 exposure observed following coadministration with cyclosporine, the effect of tacrolimus on GS-566500 exposure was modest and not considered clinically relevant.

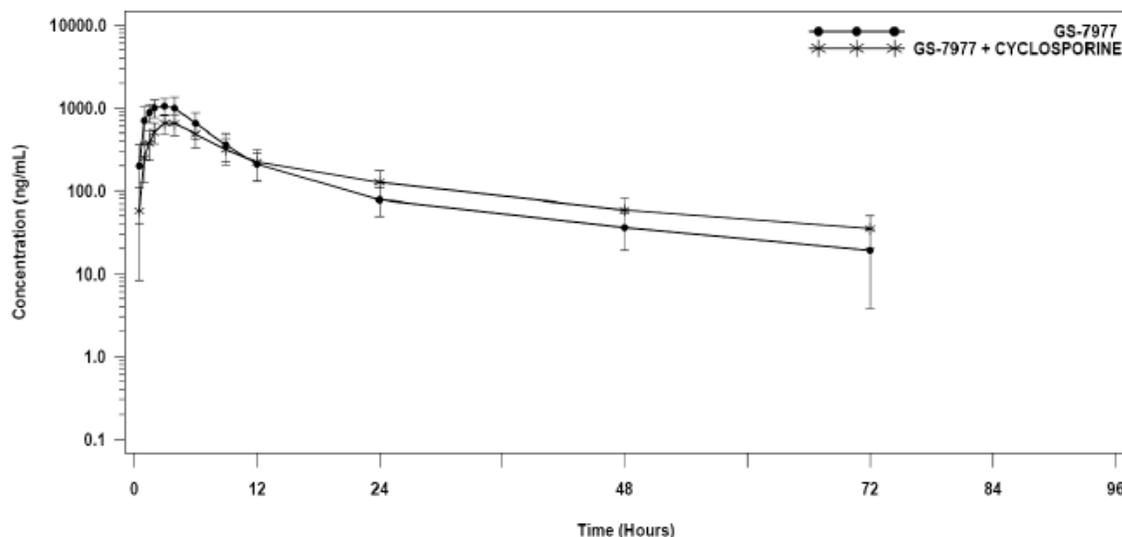
Table 9. P7977-1819: Statistical Evaluations of GS-566500 Plasma Pharmacokinetic Parameters Following Administration of Sofosbuvir Alone or in Combination with Cyclosporine or Tacrolimus

GS-566500 PK Parameter	GLSM		%GLSM Ratio (90% CI) SOF+ Cyclosporine/ SOF Alone	GLSM		%GLSM Ratio (90% CI) SOF+ Tacrolimus/ SOF Alone
	SOF Alone (N = 18)	SOF +Cyclosporine (N = 19)		SOF Alone (N = 15)	SOF +Tacrolimus (N = 16)	
AUC _{0-last} (h·ng/mL)	866.7	2623.9	302.8 (247.5, 370.3)	1007.9	1106.7	109.8 (87.9, 137.1)
AUC _{0-inf} (h·ng/mL)	918.8	2744.3	298.7 (245.0, 364.2)	1059.2	1160.0	109.5 (88.8, 135.1)
C _{max} (ng/mL)	236.1	600.5	254.3 (206.9, 312.5)	293.4	277.6	94.6 (76.4, 117.2)

GS-331007

The mean (SD) plasma concentration-time profiles for GS-331007, following administration of sofosbuvir alone and in combination with a single dose of cyclosporine, are shown in [Figure 7](#). Cyclosporine altered the plasma concentration-time profile, specifically the C_{max}, of GS-331007.

Figure 7. P7977-1819: Mean (SD) GS-331007 Plasma Concentration-Time Profiles When Sofosbuvir was Administered Alone or in Combination With a Single Dose of Cyclosporine



The mean (SD) plasma concentration-time profiles for GS-331007, following administration of sofosbuvir alone and in combination with a single dose of tacrolimus, are shown in [Figure 9](#). The plasma concentration-time profiles for GS-331007 determined following administration of sofosbuvir alone or administration of sofosbuvir+tacrolimus were comparable, suggesting that tacrolimus did not alter the PK concentration-time profile of GS-331007.

Figure 9. P7977-1819: Mean (SD) GS-331007 Plasma Concentration-Time Profiles When Sofosbuvir was Administered Alone or in Combination With a Single Dose of Tacrolimus

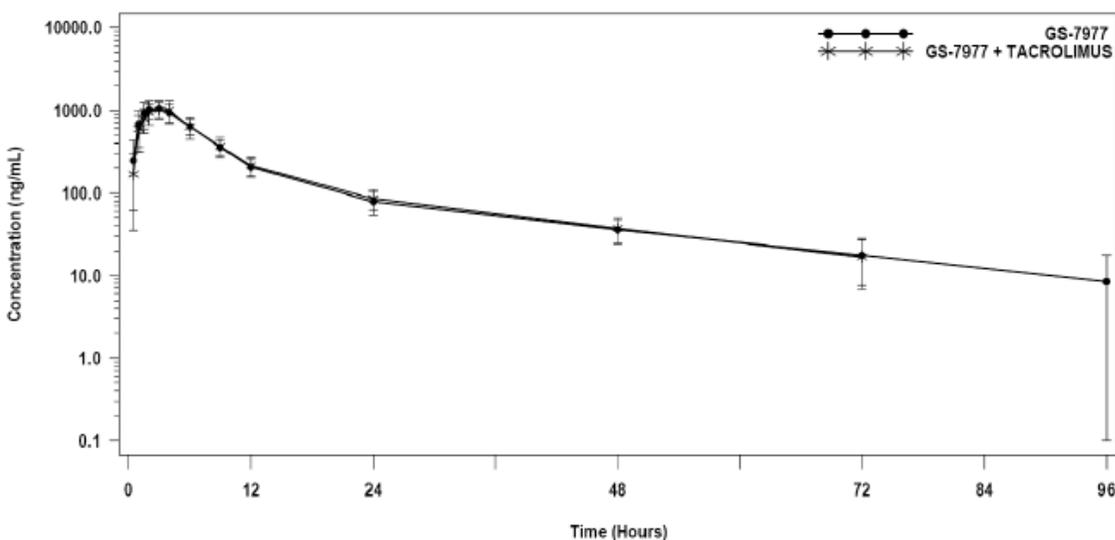


Table 10 presents the PK parameters for GS-331007 determined following administration of sofosbuvir alone or in combination with cyclosporine or tacrolimus. In contrast to the effect of cyclosporine on the exposure of sofosbuvir and GS-566500, coadministration of sofosbuvir with cyclosporine did not alter the AUC_{0-inf} or AUC_{0-last} for GS-331007. The C_{max} for GS-331007 was modestly decreased (42%) in the presence of cyclosporine, but the median T_{max} and t_{1/2} were unaffected by cyclosporine coadministration. Similar to the effect of tacrolimus on the exposure of sofosbuvir and GS-566500, tacrolimus did not substantially alter the exposure of GS-331007. The GS-331007 median T_{max} and t_{1/2} were similar when sofosbuvir was administered alone and with tacrolimus. These data suggest no significant effect of tacrolimus on the PK of GS-331007.

Table 10. P7977-1819: GS-331007 Plasma Pharmacokinetic Parameters Following Administration of Sofosbuvir Alone or with Cyclosporine or Tacrolimus

GS-331007 PK Parameter	Mean (%CV)		Mean (%CV)	
	SOF Alone (Group 1) (N = 18)	SOF +Cyclosporine (Group 1) (N = 19)	SOF Alone (Group 2) (N = 15)	SOF+Tacrolimus (Group 2) (N = 16)
AUC _{0-last} (h·ng/mL)	10,597.38 (25.87)	10,063.16 (28.49)	10,774.81 (18.05)	10,775.14 (21.31)
AUC _{0-inf} (h·ng/mL)	11,397.29 (26.73)	11,369.93 (29.59)	11,340.41 (18.68)	11,419.44 (20.59)
% ExtrapAUC (%)	6.64 (75.90)	11.08 (33.37)	4.82 (56.46)	5.73 (63.88)
C _{max} (ng/mL)	1172.54 (26.67)	679.29 (27.18)	1132.25 (22.62)	1108.65 (23.62)
T _{max} (h) ^a	3.00 (2.00, 3.98)	3.00 (3.00, 4.00)	2.00 (1.50, 3.00)	3.00 (2.00, 4.00)
t _{1/2} (h) ^a	23.11 (20.16, 28.66)	24.85 (20.46, 27.69)	22.21 (19.62, 31.61)	22.55 (19.00, 25.22)

^a Median (Q1, Q3)

The statistical evaluations of the GS-331007 PK parameters determined following administration of sofosbuvir alone or in combination with cyclosporine or tacrolimus are shown in Table 11.

The 90% CIs of the GLSM ratios for the GS-331007 PK parameters AUC_{0-last} and AUC_{0-inf} were contained within the predetermined boundaries of 80% to 125%, but the C_{max} was not. The modest decrease in GS-331007 C_{max} is not considered clinically significant. The 90% CIs of the GLSM ratio for GS-331007 PK parameters AUC_{0-last}, AUC_{0-inf}, and C_{max} were contained within the predetermined boundaries of 80% to 125%, suggesting that tacrolimus did not significantly affect the PK of GS-331007.

Table 11. P7977-1819: Statistical Evaluations of GS-331007 Plasma Pharmacokinetic Parameters Following Administration of Sofosbuvir Alone or in Combination With Cyclosporine or Tacrolimus

GS-331007 PK Parameter	GLSM		%GLSM Ratio (90% CI) SOF +Cyclosporine/ SOF Alone	GLSM		%GLSM Ratio (90% CI) SOF +Tacrolimus/ SOF Alone
	SOF Alone (N = 18)	SOF +Cyclosporine (N = 19)		SOF Alone (N = 15)	SOF +Tacrolimus (N = 16)	
AUC _{0-last} (h·ng/mL)	10,267.9	10,137.3	98.7 (86.0, 113.4)	10,615.6	10,471.0	98.6 (86.5, 112.4)
AUC _{0-inf} (h·ng/mL)	11,014.7	11,429.8	103.8 (89.8, 120.0)	11,157.5	11,101.0	99.5 (87.4, 113.3)
C _{max} (ng/mL)	1136.0	683.8	60.2 (52.5, 69.1)	1102.9	1074.2	97.4 (83.0, 114.2)

Discussion:

Although Sofosbuvir and GS-566500 exposures were significantly increased by cyclosporine, the safety margins for sofosbuvir (and metabolites), after coadministration with cyclosporine, continue to remain adequate (Table 13-1; AUC safety margin ranges from 1.9 to 16.0) compared with exposures obtained in toxicology studies and, therefore, dose modification of sofosbuvir is not warranted.

Table 13-1. Estimated Safety Margins for Sofosbuvir, GS-566500 and GS-331007 Based on AUC after Oral Administration With Cyclosporine

Species	Duration	NOAEL (mg/kg/day)	AUC _{0-t} (µg·h/mL) (M/F)			AUC Safety Margin (M/F)		
			SOF ^a	GS-566500	GS-331007	SOF ^b	GS-566500 ^c	GS-331007 ^d
Rat	Once Daily for 4 weeks	500	not evaluable ^e	42.5/43.2	55.0/59.3	not estimated	15.7/16.0	4.8/5.2
	Once Daily for 26 weeks	500	not determined	not determined	66.5/65.5	not estimated	not estimated	5.8/5.7
Dog	Once Daily for 4 weeks	100	7.0/11.2	5.2/8.7	39.7/68.6	2.3/3.7	1.9/3.2	3.5/6.0
	Once Daily for 39 weeks	100	not determined	not determined	76.3/103.7	not estimated	not estimated	6.7/9.1

- a SOF AUC estimated from the GS-9851 AUC by assuming that SOF accounts for approximately half of the GS-9851 AUC.
- b Safety margins were obtained by dividing the mean SOF AUC_{0-t} from the no observed adverse effect level (NOAEL) of a 4-week dog study at 100 mg/kg/day with the AUC_{0-inf} (3.0 µg·h/mL) determined in humans after administration of SOF 400 mg with cyclosporine. Safety margins were not estimated for SOF in the rat.
- c Safety margins were obtained by dividing the mean GS-566500 AUC_{0-t} from the NOAELs of a 4-week rat study at 500 mg/kg/day, and a 4-week dog study at 100 mg/kg/day with the AUC_{0-inf} (2.7 µg·h/mL) determined in humans after administration of SOF 400 mg with cyclosporine.
- d Safety margins were obtained by dividing the mean GS-331007 AUC_{0-t} from the NOAELs of a 4- and a 26-week rat study at 500 mg/kg/day, and 4- and 39-week dog study at 100 mg/kg/day with the AUC_{0-inf} (11.4 µg·h/mL) determined in humans after administration of SOF 400 mg with cyclosporine.
- e Due to instability of GS-9851 and SOF in rat plasma (high esterase activity), plasma exposure of GS-9851 and SOF in rats cannot be accurately measured.

Conclusion: No dose adjustment is required when SOF is coadministered with cyclosporine or tacrolimus.

4.2.4.4 P7977-0814: A Phase I, Open-Label, Single-Sequence Drug-Drug Interaction Trial in Healthy Subjects Receiving Stable Methadone Maintenance Therapy to Investigate the Potential Interaction at Steady State between PSI-7977 400 mg QD and Methadone

Objectives:

- To evaluate the effect of steady state sofosbuvir (GS-7977; formerly PSI-7977) on the steady-state pharmacokinetics (PK) of R- and S-methadone
- To evaluate the potential effect of steady-state sofosbuvir and metabolites on the pharmacodynamic effects of stable methadone maintenance therapy
- To evaluate the short-term safety and tolerability during coadministration of steady-state sofosbuvir with methadone during stable methadone maintenance therapy
- To compare the PK of sofosbuvir and metabolites to historical controls

Study Design: This was an open-label, single-sequence, drug-drug interaction study in healthy subjects on stable methadone maintenance therapy for opiate addiction. Subjects received

sofosbuvir 400 mg once daily on Days 1 through 7. Subjects received individualized doses of methadone ranging from 30 mg to 130 mg.

Subjects: Fifteen healthy subjects who are receiving Stable Methadone Maintenance Therapy, males or females aged 18 to 60 years, inclusive, were enrolled into the study. Subject 114 had methadone plasma concentrations less than LLOQ at all time points on Day -1 and, therefore, was excluded from the PK analysis.

Formulation: Sofosbuvir 200-mg tablets (Lot #: 0G069-P1); methadone 30-130 mg liquid or tablet formulation.

PK Sampling: Blood samples for sofosbuvir, GS-566500, and GS-331007 plasma concentrations were collected predose (≤ 15 minutes before dosing) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours postdose on Day 1; predose (≤ 15 minutes before dosing) on Days 2, 3, and 5; and predose (≤ 15 minutes before dosing) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72, and 96 hours postdose on Day 7.

Blood samples for R- and S-methadone plasma concentrations were collected predose (≤ 15 minutes before dosing) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours postdose on Day -1; predose (≤ 15 minutes before dosing) on Day 1, 3, and 5; and predose (≤ 15 minutes before dosing) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 hours postdose on Day 7.

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

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

Table 1 Summary of Quality Control (QC) Results –Study P7977-0814

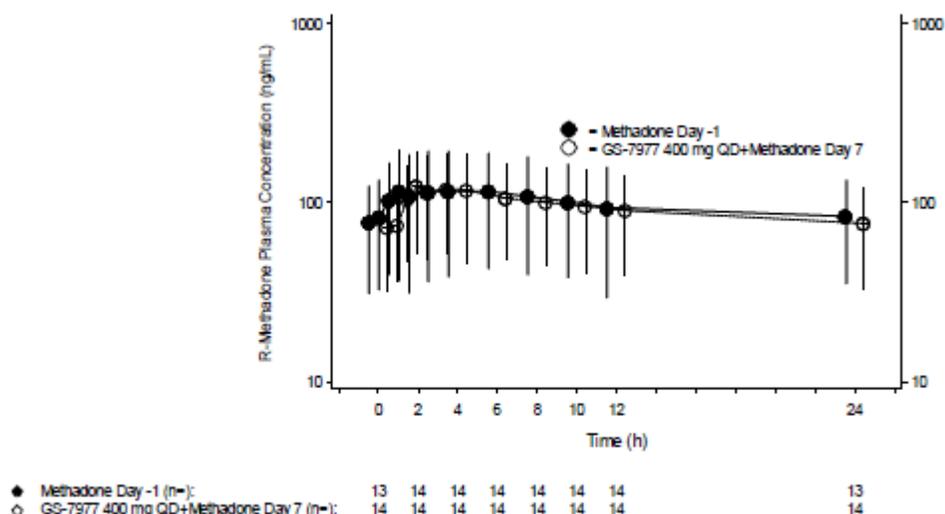
Analyte	Linear range (ng/mL)	Between Run Precision (%CV)	Between Run Bias (% Deviation)	QC samples (ng/mL)	Validation sample for stability and conditions
SOF	5 – 5000 $R^2 > 0.994$	≤ 6.6	0.4 to 6.1	5, 15, 30, 500 and 4000	Stable for 99 days and ≥ 5 freeze/thaw cycles in plasma at -70°C
GS-566500	10 – 5000 $R^2 > 0.995$	≤ 8.5	-0.7 to 6.9	30, 500 and 4000	Stable for 125 days and ≥ 5 freeze/thaw cycles in plasma at -70°C
GS-331007	10 – 5000 $R^2 > 0.996$	≤ 8.5	0.4 to 2.5	30, 500 and 4000	Stable for 184 days and ≥ 5 freeze/thaw cycles in plasma at -70°C
R--methadone	5 – 1000 $R^2 > 0.998$	≤ 6.8	0.7 to 6.1	15, 80, 400, and 800	Stable for 321 days at -20 °C
S--	5 – 1000	≤ 9.9	1.3 to 6.8	15, 80, 400,	Stable for 321 days at -20

methadone	$R^2 > 0.998$			and 800	°C
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Pharmacokinetic Results:

R-Methadone: The mean dose-normalized R-methadone steady-state plasma concentration-time profiles for subjects on a stable methadone therapy prior to (Day -1) and after administration of sofosbuvir (Day 7) are shown in a semi-logarithmic plot in Figure 1. The mean plasma profiles of R-methadone before and during sofosbuvir administration are similar.

Figure 1 Mean (SD) Dose-Normalized R-Methadone Plasma Concentration-Time Profiles for Subjects on a Stable Methadone Therapy Prior to (Day -1) and After Administration of Sofosbuvir (Day 7)



Note: Any plasma concentration value less limit of quantitation was set as missing.

Table 2 summarizes the dose-normalized PK parameters of R-methadone for subjects on stable methadone therapy prior to (Day -1) and after administration of sofosbuvir (Day 7). The median Tmax (3.50 vs. 2.02 h) of R-methadone occurred slightly earlier when methadone was coadministered with sofosbuvir.

Table 2. P7977-0814: R-Methadone Pharmacokinetic Parameters for Subjects on a Stable Methadone Therapy Prior to (Day -1) and After Administration of Sofosbuvir (Day 7)

R-Methadone PK Parameter	Mean (%CV)	
	Methadone Steady State (Day -1) (N = 14)	SOF+Methadone Steady State (Day 7) (N = 14)
AUC _{tau50} (h·ng/mL)	2294.5 (66.0)	2239.6 (55.7)
C _{max50} (ng/mL)	128.6 (58.9)	130.2 (56.3)
C _{tau50} (ng/mL)	83.87 (57.6)	76.26 (56.5)
T _{max} (h) ^a	3.50 (2.00, 6.00)	2.02 (1.52, 3.02)
t _{1/2} (h) ^a	36.51 (29.93, 51.81)	36.06 (30.33, 57.52)

a Median (Q1, Q3)

Note: AUC_{tau50}, C_{max50}, and C_{tau50} are AUC_{tau}, C_{max}, and C_{tau}, respectively and were dose normalized to 50-mg methadone dose.

Table 3 presents the GLSM and associated 90% CIs for AUC_{tau50}, C_{max50}, and C_{tau50} for R-methadone for subjects on stable methadone therapy prior to (Day -1) and after administration of sofosbuvir (Day 7). The 90% CIs of the GLSM ratio for R-methadone dose normalized AUC and C_{max} (AUC_{tau50}, and C_{max50}) were within 80% to 125%, while the 90% CIs of the GLSM ratio for R-methadone dose normalized C_{tau} (C_{tau50}) is slightly outside the boundaries of 80% to 125%, and considered not clinically significant.

Table 3. P7977-0814: Statistical Evaluations of R-Methadone Pharmacokinetic Parameters for Subjects on a Stable Methadone Therapy Prior to (Day -1) and After Administration of Sofosbuvir (Day 7)

R-Methadone PK Parameter	GLSM		%GLSM Ratio (90% Confidence Interval) SOF+Methadone/Methadone
	Methadone Steady State (Day -1) (N = 14)	SOF+Methadone Steady State (Day 7) (N = 14)	
AUC _{tau50} (h·ng/mL)	1789.89	1815.12	101.41 (84.67, 121.46)
C _{max50} (ng/mL)	104.96	104.06	99.13 (84.82, 115.87)
C _{tau50} (ng/mL)	65.43 ^a	61.21	93.55 (76.82, 113.93)

Note: AUC_{tau50}, C_{max50}, and C_{tau50} are AUC_{tau}, C_{max}, and C_{tau}, respectively, and were dose normalized to 50- mg methadone dose.

a n = 13

S-Methadone: Mean dose-normalized S-methadone steady-state plasma concentration-time profiles for subjects on stable methadone therapy prior to (Day -1) and after administration of sofosbuvir (Day 7) are shown in a semi-logarithmic plot in Figure 2. The mean plasma profiles of S-methadone before and during sofosbuvir administration are similar.

Figure 2. P7977-0814: Mean (SD) Dose-Normalized S-Methadone Plasma Concentration-Time Profiles for Subjects on a Stable Methadone Therapy Prior to (Day -1) and After Administration of Sofosbuvir (Day 7)

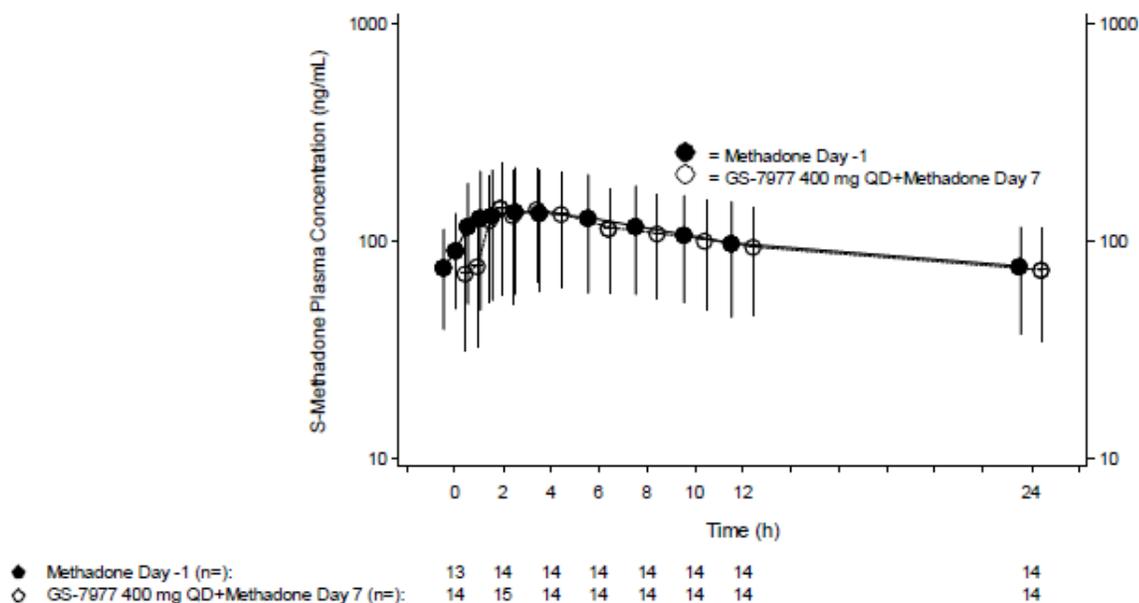


Table 4 summarizes the dose-normalized PK parameters of S-methadone for subjects on a stable methadone therapy prior to (Day -1) and after administration of sofosbuvir (Day 7). The median T_{max} (2.50 vs. 1.53 h) of S-methadone occurred slightly earlier when methadone was coadministered with sofosbuvir.

Table 4. P7977-0814: S-Methadone Pharmacokinetic Parameters for Subjects on a Stable Methadone Therapy Prior to (Day -1) and After Administration of Sofosbuvir (Day 7)

S-Methadone PK Parameter	Mean (%CV)	
	Methadone Steady State (Day -1) (N = 14)	SOF+Methadone Steady State (Day 7) (N = 14)
AUC_{tau50} (h·ng/mL)	2460.7 (52.7)	2388.5 (50.8)
C_{max50} (ng/mL)	154.3 (48.6)	155.0 (52.2)
C_{tau50} (ng/mL)	76.21 (50.9)	73.97 (53.2)
T_{max} (h) ^a	2.50 (2.00, 4.00)	1.53 (1.52, 2.02)
$t_{1/2}$ (h) ^a	31.98 (21.48, 50.01)	29.06 (20.09, 35.85)

^a Median (Q1, Q3)

Note: AUC_{tau50} , C_{max50} , and C_{tau50} are AUC_{tau} , C_{max} , and C_{tau} , respectively, and were dose normalized to 50-mg methadone dose.

Table 5 presents the GLSM and associated 90% CIs for AUC_{tau50} , C_{max50} , and C_{tau50} for S-methadone for subjects on stable methadone therapy prior to (Day -1) and after administration of sofosbuvir (Day 7). The 90% CIs of the GLSM ratio for S-methadone PK parameters (AUC_{tau50} , C_{max50} , and C_{tau50}) were slightly outside the boundaries of 80% to 125%, and considered not clinically significant.

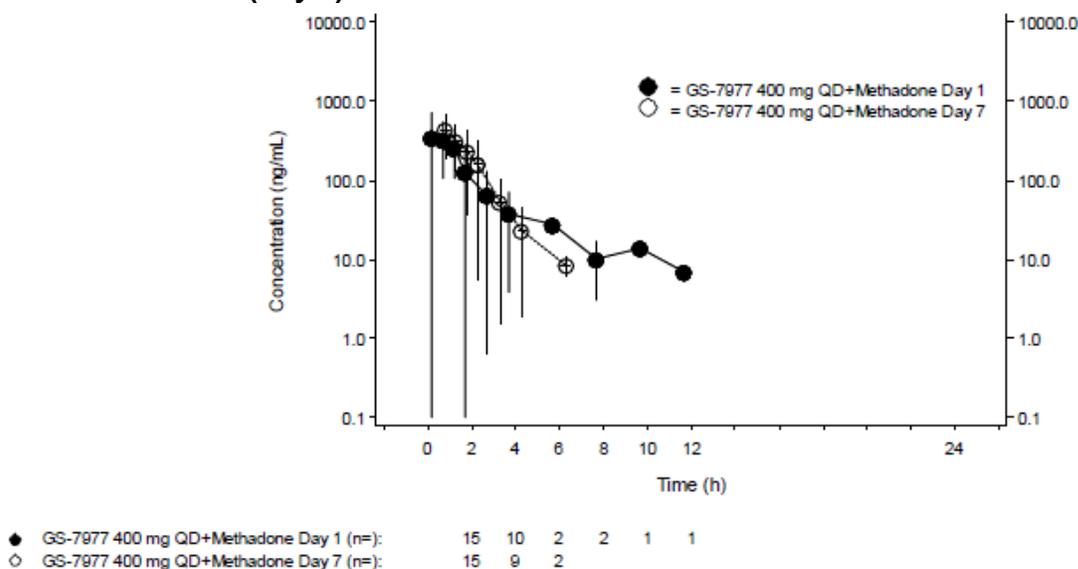
Table 5. P7977-0814: Statistical Evaluations of S-Methadone Pharmacokinetic Parameters for Subjects on a Stable Methadone Therapy Prior to (Day -1) and After Administration of Sofosbuvir (Day 7)

S-Methadone PK Parameter	GLSM		%GLSM Ratio (90% Confidence Interval) SOF+Methadone/ Methadone
	Methadone Steady State (Day -1) (N = 14)	SOF+Methadone Steady State (Day 7) (N = 14)	
AUC _{tau50} (h·ng/mL)	2085.81	1987.33	95.28 (77.32, 117.40)
C _{max50} (ng/mL)	134.24	127.04	94.64 (79.34, 112.88)
C _{tau50} (ng/mL)	63.02	59.74	94.79 (73.51, 122.23)

Note: AUC_{tau50}, C_{max50}, and C_{tau50} are AUC_{tau}, C_{max}, and C_{tau}, respectively, and were dose normalized to 50- mg methadone dose.

Sofosbuvir: Single- and multiple-dose mean (SD) sofosbuvir plasma concentration-time profiles of sofosbuvir after coadministration with methadone (Days 1 and 7) are shown in a semi-logarithmic plot in Figure 3. The plasma concentration-time profiles of sofosbuvir exhibited a rapid increase in concentration followed by a rapid decline. Consistent with the short elimination half-life and the 24-hour dosing interval, Day 1 and 7 plasma concentration profiles of sofosbuvir were comparable and did not exhibit accumulation following multiple dosing.

Figure 3. P7977-0814: Mean (SD) Sofosbuvir Plasma Concentration-Time Profiles for Subjects on a Stable Methadone Therapy Following a Single Dose (Day 1) and Multiple Doses of Sofosbuvir (Day 7)



Note: Any plasma concentration value less limit of quantitation was set as missing.

Table 6 summarizes the single- and multiple-dose sofosbuvir PK parameters (Days 1 and 7, respectively) following coadministration of sofosbuvir with methadone compared with single-

and multiple-dose sofosbuvir PK parameters from previous sofosbuvir monotherapy studies P7977-0613 (Day 1) and P2938-0212 (Day 7). Coadministration of sofosbuvir with methadone minimally altered single-dose or multiple-dose sofosbuvir PK parameters compared with historical data.

Table 6. P7977-0814: Sofosbuvir Pharmacokinetic Parameters Following a Single Dose (Day 1) and Multiple Doses of Sofosbuvir (Day 7) Coadministered with Methadone (Study P7977-0814) and as Monotherapy (Studies P7977-0613 and P2938-0212)

SOF PK Parameter	Mean (%CV)			
	Single Dose of SOF		Multiple Doses of SOF	
	SOF+ Methadone (Day 1) (N = 14)	SOF (Study P7977-0613) (Day 1) (N = 60)	SOF+Methadone Steady State (Day 7) (N = 14)	SOF Steady State (Study P2938-0212) (Day 7) (N = 8)
AUC _{inf} (h·ng/mL)	668.99 (32.7)	628.73 (44.9)	—	—
AUC _{last} (h·ng/mL)	642.37 (33.5)	622.20 (45.0)	661.54 (27.7)	—
AUC _{tau} (h·ng/mL)	—	—	671.91 (27.9)	538.12 (39.0)
C _{max} (ng/mL)	541.97 (59.5)	621.76 (56.1)	560.84 (36.2)	602.59 (47.2)
C _{last} (ng/mL)	19.42 (100.6)	8.79 (47.7)	12.44 (40.6)	—
T _{last} (h) ^a	4.00 (3.00, 4.00)	3.50 (3.00, 4.00)	4.00(3.00, 4.00)	—
T _{max} (h) ^a	1.00 (0.50, 1.50)	0.50 (0.50, 1.00)	0.75 (0.50, 1.50)	0.50 (0.50, 0.77)
t _{1/2} (h) ^a	0.57 (0.35, 1.10)	0.45 (0.39, 0.54)	0.42 (0.40, 0.68)	0.48 (0.44, 0.52)

a Median (Q1, Q3)

GS-566500

Single- and multiple-dose mean (SD) GS-566500 plasma concentration-time profiles of sofosbuvir after coadministration with methadone (Days 1 and 7) are shown in a semi-logarithmic plot in [Figure 4](#). The plasma concentration-time profiles of GS-566500 exhibited similar characteristics with sofosbuvir. Additionally, Day 1 and 7 plasma profiles of GS-566500 are comparable and did not exhibit accumulation following multiple dosing.

Figure 4. P7977-0814: Mean (SD) GS-566500 Plasma Concentration-Time Profiles on a Stable Methadone Therapy Following a Single Dose (Day 1) and Multiple Doses of Sofosbuvir (Day 7)

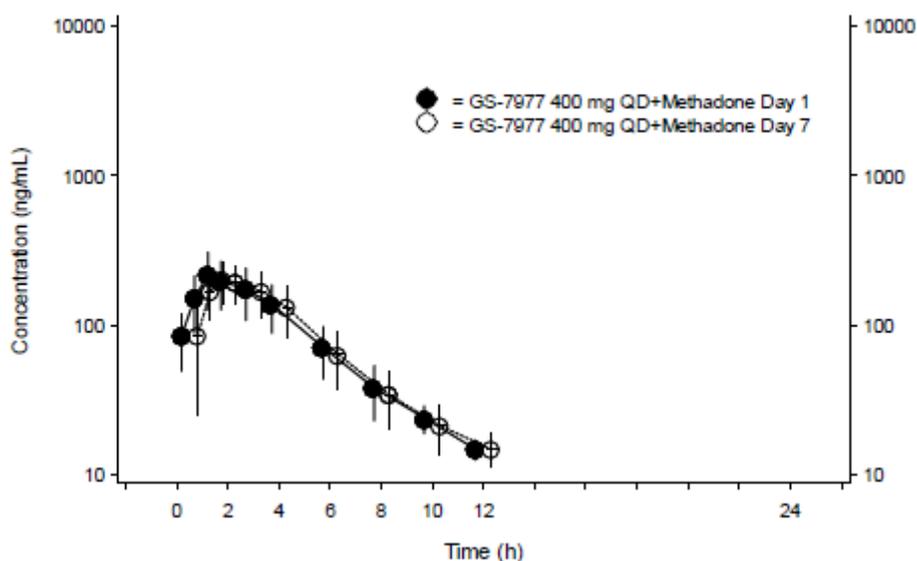


Table 7 summarizes the single- and multiple-dose GS-566500 PK parameters (Days 1 and 7) following coadministration of sofosbuvir with methadone compared with single- and multiple-dose sofosbuvir PK parameters from previous sofosbuvir monotherapy studies P7977-0613 (Day 1) and P2938-0212 (Day 7). Coadministration of sofosbuvir with methadone minimally altered single-dose or multiple-dose GS-566500 PK parameters compared with historical data.

Table 7. P7977-0814: GS-566500 Pharmacokinetic Parameters Following a Single Dose (Day 1) and Multiple Doses of Sofosbuvir (Day 7) Coadministered with Methadone (Study P7977-0814) and as Monotherapy (Studies P7977-0613 and P2938-0212)

GS-566500 PK Parameter	Mean (%CV)			
	Single Dose of SOF		Multiple Doses of SOF	
	SOF+Methadone (Day 1) (N = 14)	SOF (Study P7977-0613) (Day 1) (N = 60)	SOF+Methadone Steady State (Day 7) (N = 14)	SOF Steady State (Study P2938-0212) (Day 7) (N = 8)
AUC _{inf} (h·ng/mL)	1074.71 (28.4)	855.60 (31.0)	—	—
AUC _{last} (h·ng/mL)	1024.36 (29.8)	815.45 (32.4)	979.26 (27.1)	—
AUC ₀₋₁₂ (h·ng/mL)	—	—	1041.59 (28.5)	853.13 (45.7)
C _{max} (ng/mL)	244.31 (33.1)	232.31 (71.8)	228.85 (23.0)	235.16 (38.4)
C _{last} (ng/mL)	15.37 (16.2)	14.01 (22.5)	14.78 (21.5)	—
T _{last} (h) ^a	12.00 (12.00, 12.00)	10.00 (8.00, 10.00)	12.00 (10.00, 12.00)	—
T _{max} (h) ^a	1.50 (1.50, 3.00)	1.50 (1.00, 1.50)	1.75 (1.50, 2.00)	1.50 (1.00, 1.75)
t _{1/2} (h) ^a	2.27 (2.10, 2.45)	1.99 (1.87, 2.10)	2.29 (2.11, 2.47)	2.14 (1.99, 2.24)

a Median (Q1, Q3)

GS-331007

Single- and multiple-dose mean (SD) GS-331007 plasma concentration-time profiles of sofosbuvir after coadministration with methadone (Days 1 and 7, respectively) are shown in a semi-logarithmic plot in Figure 5. The plasma concentration-time profiles of GS-331007 reached

a maximum concentration at approximately 3 hours, followed by an apparent biphasic decrease over the 24-hour dosing interval. Consistent with the moderate elimination half-life and the 24-hour dosing interval, Day 1 and 7 plasma profiles of GS-331007 exhibited very modest accumulation following multiple dosing.

Figure 5. P7977-0814: Mean (SD) GS-331007 Plasma Concentration-Time Profiles for Subjects on a Stable Methadone Therapy Following a Single Dose (Day 1) and Multiple Doses of Sofosbuvir (Day 7)

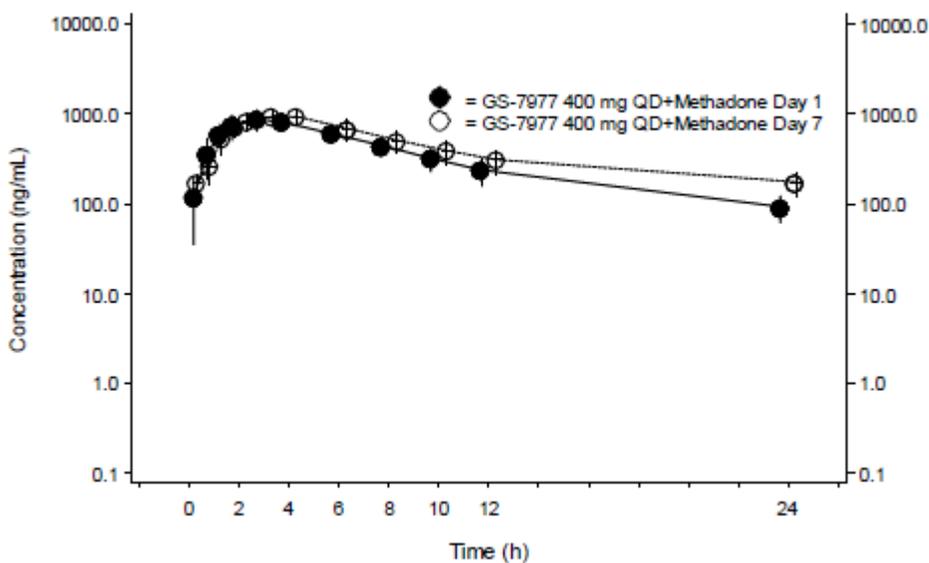


Table 8 summarizes the single- and multiple-dose GS-331007 PK parameters (Days 1 and 7) following coadministration of sofosbuvir with methadone compared with single- and multiple-dose sofosbuvir PK parameters from previous sofosbuvir monotherapy studies P7977-0613 (Day 1) and P2938-0212 (Day 7).

Table 8. P7977-0814: GS-331007 Pharmacokinetic Parameters Following a Single Dose (Day 1) and Multiple Doses of Sofosbuvir (Day 7) Coadministered with Methadone (Study P7977-0814) and as Monotherapy (Studies P7977-0613 and P2938-0212)

GS-331007 PK Parameter	Mean (%CV)			
	Single Dose of SOF		Multiple Doses of SOF	
	SOF+ Methadone (Day 1) (N = 14)	SOF (Study P7977-0613) (Day 1) (N = 60)	SOF+Methadone Steady State (Day 7) (N = 14)	SOF Steady State (Study P2938-0212) (Day 7) (N = 8)
AUC _{inf} (h·ng/mL)	9153.85 (15.6)	11,097.29 (22.4)	—	—
AUC _{last} (h·ng/mL)	7993.80 (14.8)	10,559.32 (23.2)	—	—
AUC _{tau} (h·ng/mL)	—	—	10,080.73 (22.4)	9638.94 (18.7)
C _{max} (ng/mL)	929.92 (20.6)	1113.34 (28.0)	1007.49 (15.4)	1378.33 (19.2)
C _{last} (ng/mL)	94.69 (29.5)	14.53 (22.9)	44.52 (68.1)	—
T _{last} (h) ^a	23.75 (23.75, 23.92)	72.00 (72.00, 96.00)	95.97 (95.95, 95.97)	—
T _{max} (h) ^a	3.00 (3.00, 3.00)	2.50 (2.00, 3.50)	3.00 (3.00, 4.00)	2.00 (1.75, 3.00)
t _{1/2} (h) ^a	8.20 (6.88, 8.94)	25.07 (20.35, 29.14)	33.35 (28.48, 37.15)	9.42 (8.84, 12.24)

a Median (Q1, Q3)

Coadministration of sofosbuvir with methadone had a minimal effect on the following single-dose GS-331007 PK parameters compared with the historical data in Study P7977-0613: mean AUC_{inf} (9153.85 vs. 11,097.29 h·ng/mL) and C_{max} (929.92 vs. 1113.34 ng/mL) and median T_{max} (3.00 vs. 2.50 h). Comparison of t_{1/2} estimates cannot be made because there were different sampling intervals for GS-331007 on Day 1 with methadone (first of the multiple doses of sofosbuvir) versus the historical data in Study P7977-0613 (single dose) (24 vs. 72 h). This difference contributed to the shorter estimate of t_{1/2} (8.20 h) on Day 1 when administered methadone compared with the t_{1/2} estimate in Study P7977-0613 (25.07 h) (confounded by the distribution phase), but did not substantially alter estimation of AUC_{inf}.

Coadministration of sofosbuvir with methadone had a minimal effect on the following multiple-dose GS-331007 PK parameters compared with historical data in Study P2938-0212: mean AUC_{tau} (10,080.73 vs. 9638.94 h·ng/mL) and C_{max} (1007.49 vs. 1378.33 ng/mL) and median T_{max} (3.00 vs. 2.00 h). Comparison of t_{1/2} estimates cannot be made because there were different sampling intervals for GS-331007 on Day 7 with methadone (last of the multiple doses of sofosbuvir with a washout period) versus the historical control in Study P2938-0212 (last of multiple dose without a washout period) (96 vs. 24 h). This difference contributed to the shorter estimate of t_{1/2} (9.42 h) in the terminal phase estimate on Day 7 in Study P2938-0212 (confounded by the distribution phase) compared with the t_{1/2} estimate (33.35 h) in this study with the coadministration of sofosbuvir and methadone.

Conclusion: Coadministration of sofosbuvir with methadone does not affect the PK of either R- or S-methadone. Coadministration of sofosbuvir with methadone in subjects on a stable methadone therapy does not affect the PK of sofosbuvir, GS-566500, or GS-331007 in a clinically relevant manner. Sofosbuvir and methadone can be coadministered without dose adjustment.

4.2.5 In vitro Studies (reviewed by Dr. Su-Young Choi)

1. Absorption

- AD-334-2003: Determination of the effect of concentration on the bidirectional permeability of GS-7977 through monolayers of Caco-2 Cells
- 821526: Evaluation of PSI-7977 and PSI-6206 as P-gp substrates and inhibitors using Caco-2 cell monolayers
- PC-PSI-7977-0006: In vitro interaction studies of PSI-7977 and PSI-6206 with BCRP (ABCG2) in the vesicular transport assay and in bidirectional transport studies on transfected MDCKII monolayers

2. Distribution and stability

- PC-PSI-7851-08-0016: Stability study of PSI-7851 in the whole blood of human, cynomolgus monkey, beagle dog and CD-1 mouse
- PC-PSI-785108-0013: PSI-7851 stability in human liver S9 fraction and plasma stability

3. Metabolism and elimination

- PC-PSI-7977-09-0001: Hydrolysis of PSI-7976 (R-diastereomer) and PSI-7977 (S-diastereomer), diastereoisomers of PSI-7851 (isomeric mixture of PSI-7976 and PSI-7977), by cathepsin A and carboxylesterase 1
- PC-PSI-7851-08-0026: Human Cathepsin A hydrolyzes PSI-7851
- PC-PSI-7851-09-0014: Hydrolysis of PSI-7851 by human carboxylesterase 1 and 2
- PC-PSI-7977-09-0004: Investigation of the human cytochrome P450, FMO, and UGT involvement in the metabolism of PSI-7977, PSI-352707, PSI-7411, and PSI-6206 using human liver microsomes
- PC-PSI-7977-11-0004: Comparison of PSI-7977 metabolism in human hepatocytes and peripheral blood mononuclear cells
- AD-334-2014: Test to monitor conversion from PSI-7977 to PSI-7976 in rat, dog and human plasma and human urine by LC-MS/MS

4. Drug interaction

- PC-PSI-7977-10-0005: In vitro evaluation of PSI-7977 as an inducer of cytochrome P450 expression in cultured human hepatocytes
- PC-PSI-7851-09-0009: Absence of effect of PSI-7851 and its metabolites on human cytochrome P450 isozymes
- AD-334-2013: In vitro assessment of human UGT1A1 inhibition potential of GS-7977, GS606965 and GS-331007
- PC-PSI-7977-11-0007: In vitro interaction studies of PSI-7977 and PSI-6206 with the human OATP1B1 and OATP1B3 uptake transporters
- AD-334-2004: In Vitro Interaction Studies of GS-7977 with Human OCT1 and BSEP Transporter
- AD-334-2005: In Vitro Interaction Studies of GS-331007 with Human Hepatic Transporters OCT1 and BSEP and Renal Transporters OAT1, OAT3 OCT2 and MATE1

- PC-PSI-07977-11-0002: The NSSA inhibitor, BMS790052, has no effect on the uptake and metabolism of PSI-7977 in primary human hepatocytes
- AD-334-2010: Effect of either cytochrome P450 or HCV inhibitors on Triphosphate Formation in Primary Human Hepatocytes Following Incubation with GS-7977
- AD-334-2002: Effect of HCV inhibitors from different classes GS-5885, GS-9451, GS-9010, GS-5816 and GS-9669 on the bidirectional permeability of GS-7977 through monolayers of Caco-2 Cells

Title: Determination of the effect of Concentration on the Bidirectional Permeability of GS-7977 through Monolayers of Caco-2 Cells (AD-334-2003)

Objective: To determine the effect of concentration on the bi-directional permeability of GS-7977 *in vitro* using a human colon carcinoma cell line (caco-2).

Methods

Caco-2 human intestinal epithelial cell monolayers cultured for 21 to 28 days plated on 12 well Transwell® dual chamber plates. Bi-directional permeability of GS-7977 at concentrations ranging from 10 μM to 2,800 μM was assessed. Assays were run in duplicate (n=2) in GS-7977 concentration dependency experiment, and in triplicate (n=3) when co-dosing GS-7977 with reference compounds atenolol and minoxidil. The membrane integrity and transport activity of the assay plate were certified with TEER value, atenolol permeability, lucifer yellow permeability, and propranolol permeability, and digoxin transport.

Results

The bidirectional permeability of GS-7977 is summarized in Table 1. GS-7977 showed partially saturable efflux with an efflux ratio decreasing from 49.7 at 10 μM to 7.3 at 2,800 μM . Low forward permeability was observed at all tested concentrations and the permeability was increased with concentration.

In a separate experiment, the permeability of GS-7977 was assessed in the presence of high and low permeability reference compounds minoxidil and atenolol, respectively. At 3,020 μM , the permeability was higher than that of atenolol (low permeability reference), but lower than that observed for minoxidil (high permeability reference) as shown in Table 2. 3,020 μM is the theoretical intestinal concentration achieved after a 400 mg dose of GS-7977.

Table 1. Concentration dependence of bidirectional permeability of GS-7977 through Caco-2 monolayers

Direction	Target Conc. (μM)	Initial Conc. (μM)	Recovery (%)	P _{app} (10^{-6} cm/s)			Efflux Ratio
				R1	R2	Avg.	
Forward	10	9.65	93	0.23	0.21	0.22	49.7
Reverse		10.5	105	9.61	12.3	10.9	
Forward	350	333	97	0.31	0.28	0.29	35.9
Reverse		355	106	8.62	12.4	10.5	
Forward	700	803	97	0.25	0.31	0.28	35.4
Reverse		770	110	9.6	10.2	9.92	
Forward	1,400	1550	111	0.47	0.31	0.39	13.9
Reverse		1645	116	4.78	6.11	5.45	
Forward	2,800	2560	111	0.42	0.35	0.38	7.3
Reverse		2930	115	2.52	3.05	2.79	

Table 2. Bidirectional caco-2 permeability results for GS-7977 co-dosed atenolol and minoxidil

Compound	Direction	Target Conc. (μM)	Recovery (%)	P _{app} (10^{-6} cm/s)	Efflux Ratio
GS-7977	Forward	3,020	96.3 \pm 1.55	0.708 \pm 0.333	5.81
	Reverse		102 \pm 5.84	4.11 \pm 0.0851	
Atenolol	Forward	100	96.1 \pm 12.3	0.515 \pm 0.365	NA
Minoxidil	Forward	10	98.7 \pm 2.21	6.64 \pm 1.22	

Conclusion

GS-7977 have partially saturable efflux with an efflux ratio decreasing from 49.7 at 10 μM to 7.3 at 2,800 μM . Low forward permeability was observed at all tested concentrations and the permeability was increased with concentration. These results suggest potential involvement of efflux transporters in GS-7977 absorption.

Title: Evaluation of PSI-7977 and PSI-6206 as P-gp Substrates and Inhibitors Using Caco-2 cell monolayers (8215026)

Objective: To evaluate ^{14}C -PSI-7977 and ^{14}C -PSI-6206 as substrates and/or inhibitors of P-glycoprotein (P-gp) utilizing a human colon carcinoma cell line (Caco-2 cells).

Methods:

a. Monolayer Culture

Caco-2 Monolayer cultures were prepared on Costar Transwell® polycarbonate membrane inserts. Cells suspended in supplemented DMEM were seeded onto wet and equilibrated membranes at an initial density of 4×10^4 cells/cm² and for 21 to 30 days.

b. Colon Carcinoma Cell Line (Caco-2) Permeability Assay

The apparent permeability of ¹⁴C-PSI-7977 and ¹⁴C-PSI-6206 was determined in both the apical to basolateral and the basolateral to apical directions, in triplicate at 1, 10, and 100 μM. The transport was initiated by the addition of the test article dosing solution to the donor compartment. Cell monolayers were incubated with test articles at 37°C for 1, 2, 3, and 4 hours. At each time point, the total volume of the donor and receiver compartments was removed to determine concentration. The TEER values of the monolayers prior to and following experiment were measured to confirm integrity of the Caco-2 cell monolayers. The apparent permeability of ¹⁴C-PSI-7977 or ¹⁴C-PSI-6206 was also determined in the presence of P-gp inhibitors cyclosporine A (10 μM) or verapamil (100 μM).

c. P-glycoprotein (P-gp) Substrate Assay

The apparent permeability of ¹⁴C-PSI-7977 or ¹⁴C-PSI-6206 was determined in both the apical to basolateral and basolateral to apical directions under the same conditions as described above in the presence of P-gp inhibitors cyclosporine A (10 μM) or verapamil (100 μM).

d. P-glycoprotein (P-gp) Inhibition Assay

The effects of PSI-7977 and PSI-6206 (0.2, 1, 5, 10 and 100 μM), cyclosporine A (10 μM), and verapamil (100 μM) on the P-gp mediated transport of ³H-digoxin (1 μM) were determined in triplicate. The incubation time was 1 hour.

e. Stability Test

The stability of ¹⁴C-PSI-7977 and ¹⁴C-PSI-6206 was tested in Caco-2 cells. Drugs were dosed at a final concentration of 10 μM at the apical and basolateral compartments (in duplicate) separately and then incubated at 37°C for 2 and 4 hours.

Analysis of Samples

The concentrations of each radiolabeled compound in samples were determined using liquid scintillation counting (LSC). The stability test samples collected from the apical and basolateral compartments were then analyzed by HPLC for profiling.

Characterization of Metabolites

Selected sample were analyzed for characterization by LC/MS/MS for structural identification. The Structures of metabolites were confirmed by co-chromatography, comparison of mass spectral characteristics with authentic reference standards, and accurate mass analysis.

Results

Apparent permeability and efflux ratio of ¹⁴C-PSI-7977 and ¹⁴C-PSI-6206

The apparent permeability of ¹⁴C-PSI-7977 and ¹⁴C-PSI-6206 (1, 10, and 100 μM) through Caco-2 monolayers was determined in the absence and presence of a P-gp inhibitor [cyclosporine A (10 μM) or verapamil (100 μM)]. In the absence of a P-gp inhibitor, the apparent permeability of ¹⁴C-PSI-7977 was in the range of 0.153 to 0.594 $\times 10^{-6}$ cm/s in the apical (A) to basolateral (B) direction and in the range of 7.35 to 9.60 $\times 10^{-6}$ cm/s in the B to A direction.

The efflux ratio of ^{14}C -PSI-7977 in the absence of a P-gp inhibitor ranged from 12.4 to 56.2. In the presence of cyclosporine A or verapamil, the efflux ratio was significantly reduced to a range of 2.82 to 8.38. These results indicate that ^{14}C -PSI-7977 is a substrate of P-gp.

In the absence of a P-gp inhibitor, the apparent permeability of ^{14}C -PSI-6206 was in the range of 0.184 to 1.75×10^{-6} cm/s in the A to B direction, and in the range of 0.273 to 4.60×10^{-6} cm/s in the B to A direction. The efflux ratio of PSI-6206 in the absence of a P-gp inhibitor was no higher than 2.63. In the presence of cyclosporine A or verapamil, the permeability and efflux ratio of ^{14}C -PSI-6206 were not significantly altered compared to the vehicle control groups. These results indicate that ^{14}C -PSI-6206 is not a substrate of P-gp.

Stability of ^{14}C -PSI-7977 and ^{14}C -PSI-6206 in Caco-2 Cells

^{14}C -PSI-7977 was metabolized significantly when sampled from the apical compartments, but not from the basolateral compartments, regardless which compartment was a donor. The major metabolite has been characterized by LC-MS/MS along with an authentic standard of PSI-352707, an immediate metabolite of ^{14}C -PSI-7977. ^{14}C -PSI-7977 is predominantly metabolized to PSI-352707 in Caco-2 cells. Further metabolism of PSI-352707 was not observed in Caco-2 cells in the current study likely due to poor expression of certain specific enzymes in Caco-2 cells. Because of significant metabolism in the apical compartment, the total radioactivity should represent both ^{14}C -PSI-7977 and its major metabolite (PSI-352707). On the other hand, ^{14}C -PSI-6206 was relatively stable in Caco-2 cells.

Reviewer comments: Results from in vitro studies (PC-PSI-7977-09-0001, PC-PSI-7851-08-0026, PC-PSI-7851-09-0014) indicated that the enzymes responsible for the conversion of PSI-7977 to PSI-352707 are CES1 (carboxylesterase-1) and CatA (cathepsin A), and these enzymes are known to be expressed in Caco-2 cells. Therefore, rapid metabolism of PSI-7977 in Caco-2 cells is likely due to the presence of CES1 and CatA.

Lack of inhibitory effects of ^{14}C -PSI-7977 and ^{14}C -PSI-6206 on P-gp mediated transport in Caco-2 cells

^{14}C -PSI-7977 or ^{14}C -PSI-6206 did not show any notable inhibition effect on P-gp mediated transport of ^3H -digoxin at concentrations up to $100 \mu\text{M}$ compared to the solvent control. The positive controls (P-gp inhibitors), verapamil and cyclosporine-A, inhibited P-gp mediated transport of ^3H -digoxin and thus decreased the efflux ratio of ^3H -digoxin.

Conclusion

^{14}C -PSI-7977 is extensively metabolized to PSI-352707 in Caco-2 cells. ^{14}C -PSI-7977 (and/or its major metabolite PSI-352707) in Caco-2 cells are likely substrates of P-gp in Caco-2 cells, but these compounds are not P-gp inhibitors.

Study title: *In vitro* interaction studies of PSI-7977 and PSI-6206 with BCRP (ABCG2) in the vesicular transport assay and in bi-directional transport studies on transfected MDCKII monolayers (PC-PSI-7977-0006)

Objective

The purpose of the study was to provide data on the interaction of PSI-7977 and PSI-6206 with BCRP.

- **Part 1a:** To investigate whether PSI-7977 and PSI-6206 is a substrate of BCRP (preliminary experiment).
- **Part 1b:** To investigate whether BCRP is the drug transporter responsible for the transport of PSI-7977 across MDCKII-BCRP monolayers.
- **Part 2a:** To investigate whether PSI-7977 and PSI-6206 are BCRP inhibitors.
- **Part 2b** To determine an IC₅₀ value of BCRP-mediated prazosin transport inhibition by PSI-7977

Methods

a. Vesicular transport inhibition assay

PSI-7977 and PSI-6206 were incubated with membrane vesicle preparations (total protein: 25 µg/well) and the probe substrate. Incubations were carried out in the absence or presence of 4 mM ATP to distinguish between transporter-mediated uptake and passive diffusion into the vesicles. PSI-7977 and PSI-6206 were added to the reaction. Reactions were started by the addition of 25 µL of 12 mM MgATP (or assay buffer for background controls and stopped by the addition of 200 µL of ice-cold washing buffer and immediate filtration via glass fiber filters. Filters were washed, dried and the amount of substrate inside the filtered vesicles determined by liquid scintillation.

b. MDCKII monolayer assays

Bidirectional transports of PSI-7977 and PSI-6206 were determined through parental and BCRP transfected MDCKII cell monolayers. Assay buffers with ¹⁴C-PSI-7977 or ¹⁴C-PSI-6206 at 3, 10 and 100 µM final concentrations were added to the appropriate apical (400 µL) or basolateral chambers (800 µL). Bidirectional transports of ¹⁴C-PSI-7977 and ¹⁴C-PSI-6206 on parental and BCRP transfected MDCKII cells were determined with liquid scintillation. Apical to basolateral permeability of lucifer yellow was assessed for low permeability control, and antipyrine was used as a control for high permeability. Prazosin efflux ratio was assayed as a positive control for BCRP function.

After incubation at 37 °C, aliquots (100 µL) were taken from the receptor chambers to determine the translocated amount of PSI-7977, PSI-6206 and controls, respectively. Bidirectional transport of PSI-7977 on parental and BCRP transfected MDCKII cells was determined in the presence and absence of the BCRP inhibitor Ko134 at 1 µM to confirm the specificity of the transport in MDCKII-BCRP cells.

c. Determination of the inhibitory potentials of PSI-7977 and PSI-6206 on the BCRP-mediated prazosin transport on MDCKII-BCRP and MDCKII parental monolayers

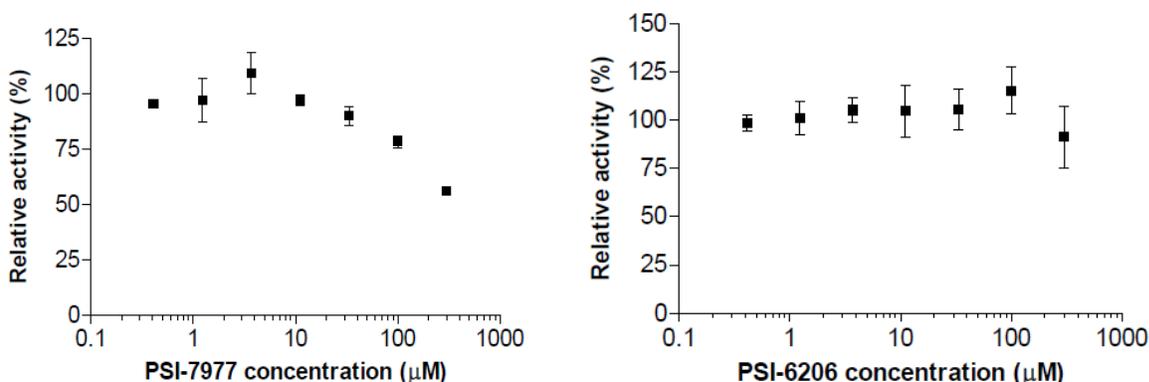
Bidirectional transport of prazosin (1 µM) on parental and BCRP transfected MDCKII cells was determined in the presence and absence of PSI-7977 (10 µM for Part 2a, 3.7 – 300 µM for Part 2b), PSI-6206 (10 µM), or Ko134 (the reference inhibitor, 1 µM). Apical to basolateral permeability of Lucifer Yellow was assessed for low permeability control and antipyrine was used as a control for high permeability. After incubation (60 min, at 37 °C), aliquots (100 µL) were taken from the receptor chambers to determine the translocated amount of prazosin (in the presence and absence of test articles). Samples containing prazosin were analyzed by scintillation counting.

Results

a. Vesicular transport inhibition assay

As a preliminary experiment, the *in vitro* inhibitions of E3S (estrone-3-sulfate) transport into BCRP expressing vesicles by PSI-7977 and PSI-6206 at seven concentrations (0.41, 1.2, 3.7, 11, 33, 100 and 300 μM) were measured. Little or no inhibition of the BCRP mediated E3S transport was observed in the applied concentration range for PSI-7977 and PSI-6206, respectively (Fig 1).

Fig 1. Modulation of BCRP-mediated probe substrate (E3S) transport by PSI-7977 (left) and PSI-6216 (right)



b. MDCKII-BCRP monolayer assay

MDCKII-BCRP monolayer efflux assay was conducted to evaluate whether PSI-7977 and PSI-6206 are substrates of the human BCRP.

<PSI-7977>

PSI-7977 showed equal permeability on MDCKII parental and transfected cells at 3 and 10 μM . At 100 μM , the basolateral to apical permeability was higher than the apical to basolateral, resulting in an efflux ratio (ER) of 3.33 on the transfected cells (Table 1). The observed ER was slightly higher than the cut-off ER value for being a substrate (ER > 2). This suggests that PSI-7977 is potentially a low affinity substrate of BCRP.

Bidirectional transport of PSI-7977 on parental and BCRP transfected MDCKII cells was determined in the presence and absence of the BCRP inhibitor Ko134 to confirm the specificity of the transport in MDCKII-BCRP cells (Table 1). In the presence of the BCRP inhibitor Ko134, the efflux ratio was decreased on transfected cells (2.17 to 0.72). This indicates that BCRP is responsible for the efflux transport of PSI-7977. Taken together, these results suggest PSI-7977 is a low affinity substrate of BCRP.

Table 1. Calculated permeability and efflux ratio values for PSI-7977 and controls

MDCKII parental cells				
Compound	P _{app} A-B	P _{app} B-A	ER	
Controls	antipyrene	57.12 ± 8.81	NA	ND
	Lucifer yellow	0.28 ± 0.04	NA	ND
prazosin	1 µM	32.9 ± 4.31	37.09 ± 3.27	1.13 ± 0.09
	+ 1 µM Ko134	38.82 ± 0.77	45.13 ± 4.21	1.10 ± 0.05
PSI-7977	100 µM	0.63 ± 0.12	3.18 ± 0.11	5.15 ± 0.93
	+ 1 µM Ko134	0.73 ± 0.12	2.2 ± 0.03	3.26 ± 0.39

MDCKII-BCRP cells				net ER	
Compound	P _{app} A-B	P _{app} B-A	ER	ER _T /ER _W	
Controls	antipyrene	50.72 ± 5.44	NA	ND	ND
	Lucifer yellow	0.87 ± 0.07	NA	ND	ND
prazosin	1 µM	5.19 ± 1.27	89.08 ± 3.97	17.84 ± 4.21	15.75 ± 0.25
	+ 1 µM Ko134	35.75 ± 10.06	36.89 ± 4.14	1.11 ± 0.46	1.01 ± 0.42
PSI-7977	100 µM	2.41 ± 0.51	5.04 ± 0.37	2.17 ± 0.54	0.42 ± 0.31
	+ 1 µM Ko134	2.71 ± 0.41	1.94 ± 0.01	0.72 ± 0.1	0.22 ± 0.19

< PSI-6206 >

No active transport of PSI-6206 across the monolayers has been observed at 3, 10, and 100 µM, which suggests that PSI-6206 is not a substrate of the human BCRP transporter.

c. Determination of inhibitory effects of PSI-7977 and PSI-6206 on BCRP-mediated transport (prazosin as a substrate for BCRP)

The inhibitory effect of PSI-7977, PSI-6206, and Ko134 (a known BCRP inhibitor) on prazosin transport across MDCKII-BCRP and MDCKII cells was determined. In the presence of 10 µM PSI-7977, the prazosin efflux ratio was reduced from 23 to 8.0, suggesting potential inhibitory effects of PSI-7977 on the BCRP-mediated transport (Table 2). No substantial changes in prazosin efflux ratio were observed in the presence of 10 µM PSI-6206.

Table 2. Bidirectional permeability and efflux ratio of prazosin in the presence or absence of PSI-7977

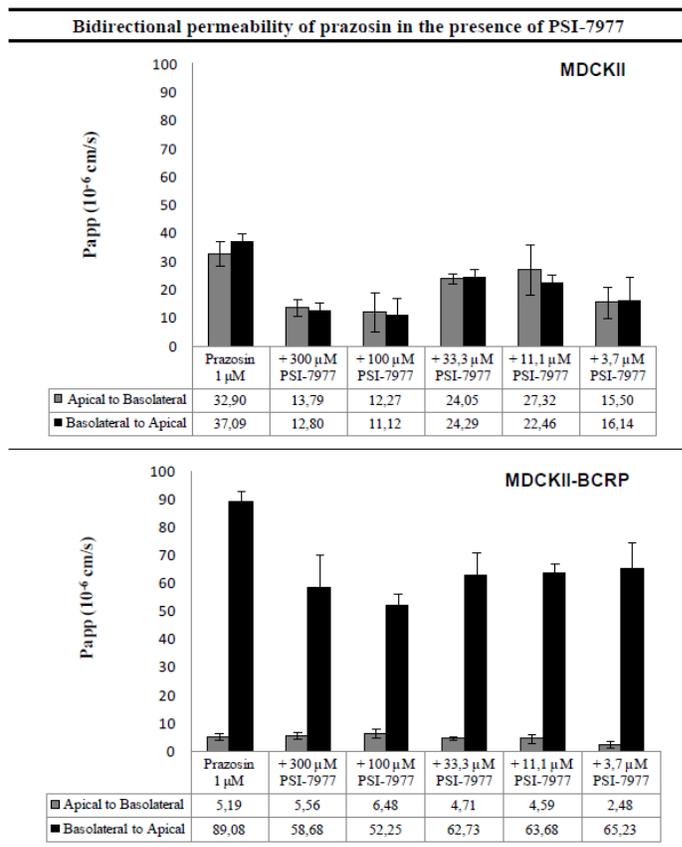
(a) Effects of PSI-7977

MDCKII parental cells				
Compound	P _{app} A-B	P _{app} B-A	ER	
Controls	antipyrene	71.62 ± 6.58	NA	ND
	Lucifer Yellow	0.16 ± 0.01	NA	ND
prazosin	1 µM	29.3 ± 5.78	28.9 ± 0.48	1.02 ± 0.23
	+ 1 µM Ko134	39.35 ± 2.47	38.88 ± 0.78	0.99 ± 0.07
	+ 10 µM PSI-7977	22.26 ± 0.1	37.57 ± 1.46	1.69 ± 0.06

MDCKII-BCRP cells				net ER	
Compound	P _{app} A-B	P _{app} B-A	ER	ER _T /ER _W	
Controls	antipyrene	74.09 ± 3.59	NA	ND	ND
	Lucifer Yellow	0.16 ± 0.04	NA	ND	ND
prazosin	1 µM	2.7 ± 0.03	62.76 ± 5.56	23.26 ± 2.31	22.88 ± 0.25
	+ 1 µM Ko134	68.14 ± 1.11	62.72 ± 4.1	0.92 ± 0.07	0.93 ± 0.1
	+ 10 µM PSI-7977	4.87 ± 1.13	63.96 ± 9.78	13.45 ± 2.42	7.97 ± 0.18

Further inhibition experiments were conducted to determine the IC_{50} of PSI-7977 on BCRP-mediated prazosin transport. PSI-7977 was applied at five concentrations (3.7, 11.1, 33.3, 100 and 300 μ M), but no concentration dependent effect was observed thus IC_{50} could not be determined (Fig 2). Partial inhibition was observed at each concentration, except for the lowest concentration tested.

Fig 2. Inhibitory effect of PSI-7977 at various concentrations on the BCRP-mediated transport of prazosin.



Conclusion

PSI-7977 is a low-affinity substrate for BCRP-mediated transport at 100 μ M, but not at 3, or 10 μ M. This is unlikely clinically relevant as this is a supratherapeutic concentration and the efflux ratio was marginally higher than the cut-off. PSI-7977 also slightly inhibited BCRP-mediated prazosin transport in a concentration-independent manner. PSI-6206 was neither a substrate nor an inhibitor of BCRP.

Title: Stability study of PSI-7851 in the whole blood of human, cynomolgus monkey, beagle dog and CD-1 mouse (PC-PSI-7851-08-0016)

Objective: To determine the stability of PSI-7851 in the whole blood of human, cynomolgus monkey, beagle dog, and CD-1 mouse

Method

PSI-7851 was prepared in methanol/water 50:50 to a final concentration of 4 µg/mL (stock solution). Then 30 µL of the stock solution were then spiked into 570 µL of human, cynomolgus monkey, beagle dog and CD-1 mouse fresh whole blood (final concentration: 200 ng/mL). The samples were gently mixed by tapping the tubes 3 to 4 times and incubated. Samples (50 µL) were removed at 0, 15, 30 and 60 minutes in duplicates and immediately mixed with 250 µL acetonitrile containing 0.5% formic acid and two internal standards (b) (4). The samples were extracted by vortexing and centrifugation (13,000 rpm for 10 min). Samples were analyzed by LC/MS/MS.

Results and conclusion

PSI-7851 was stable in whole blood of human, cynomolgus monkey and beagle dog when incubated for up to 60 min at 37 °C. PSI-7581 disappeared rapidly in mouse whole blood after incubation at 37 °C for 15 min.

Reviewer comments

The rapid degradation of PSI-7851 is due to a higher activity of esterase (CES) in plasma of rodent species (Bahar et al, J pharm sci 2012)

Title: PSI-7851 stability in human liver S9 fraction and plasma (PC-PSI-785108-0013)

Objective: To determine the stability of PSI-7851 in human liver S9 fraction and plasma

Methods

S9 Assay

The reaction mixture was prepared in a total volume of 1 mL containing 5 mM of MgCl₂, 50 mM of K₂HPO₄ (pH=7.4), 100 µM of PSI-7851, and 4 mg/mL of S9 fraction. The reaction was started by adding S9 fraction and 100 µL aliquots were taken at 0, 0.5, 1, 2, 4, 8, 24 hr. The reaction was stopped by mixing 300 µL of acetonitrile and the samples were centrifuged at 14,000 rpm for 30 min at 4 °C. The samples were analyzed by LC/MS/MS.

Plasma Assay

500 µL of human plasma was mixed with 500 µL of PBS containing 5 mM of MgCl₂. The reaction was started by adding 2 µL of a 50 mM stock solution of PSI-7851 to give a final concentration of 100 µM. 100 µL aliquots were taken at 0, 0.5, 1, 2, 4, 8, 24 hr. The reaction was stopped by mixing 300 µL of acetonitrile and the samples were centrifuged at 14,000 rpm for 30 min at 4 °C. The samples were analyzed by LC/MS/MS.

Results and conclusion

PSI-7851 was rapidly disappeared in human liver S9 fraction, and the half-life was 0.39 hours. PSI-7851 was relatively stable in human plasma (half-life > 24 hours)

Title: Hydrolysis of PSI-7976 and PSI-7977 (diastereoisomers of PSI-7851), by cathepsin A and carboxylesterase 1 (PC-PSI-7977-09-0001)

Objective: To identify the enzyme in human liver that hydrolyzes the terminal carboxylester of the two diastereoisomers of PSI-7851 (PSI-7976 and PSI-7977).

Methods

Human recombinant cathepsin A activation

Recombinant human cathepsin A (CatA) was purchased from (b) (4). CatA was activated by incubating with 1 µg/ml Cat L in 25 mM MES buffer pH 6.0 and 5 mM DTT for 30 minutes at 37 °C. Cat L was then inactivated by adding 10 µM of the protease inhibitor E64.

Purification of Carboxylesterase 1

The human liver cytosol (50 mL) was centrifuged at 10,000 x g at 4°C for 30 minutes to remove the cell debris. Ammonium sulfate was added to the supernatant to 20 % saturation. After 60 minutes the mixture was centrifuged at 10,000 x g for 20 minutes and ammonium sulfate was added to the supernatant to bring it to 50% saturation. After 30 minutes, the mixture was centrifuged for 30 minutes at 10,000 x g, and the pellet was resuspended in 14 mL of Buffer A (50 mM Tris HCl pH 7.5, 1 mM DTT, 1 mM EDTA, and 5% glycerol) followed by dialysis against Buffer A. The dialyzed solution was clarified by centrifugation and applied to an 8 mL Mono Q column (GE Healthcare, Piscataway, NJ) and proteins were eluted using a linear gradient of 50 mM to 1M NaCl. Fractions containing PSI-7851 hydrolase activity were pooled, concentrated and applied to a Superdex 200 10/300 GL column (GE Healthcare, Piscataway, NJ) equilibrated with Buffer A. It was further purified using Glycoprotein Isolation Kit (Thermo Scientific, Rockford, IL). Eluted protein was examined by SDS-PAGE and identified as human carboxylesterase 1 by MS peptide mapping and sequencing analysis (b) (4).

Hydrolysis of PSI-7976 and PSI-7977

Hydrolase activity assay was performed in a 100 µL reaction volume containing activated Cat A (0.1 µg) or purified human CES1 (0.4 µg), 100 µM of compound, 25 mM MES pH 6.0, 0.1 M NaCl, 1 mM DTT, and 0.1% NP40. After incubation for 10, 30, and 60 minutes at 37 °C, the reaction mixture was applied to an YM-10 Microcon filter (Millipore, Billerica, MA) to remove the protein. The flow-through from the filter was collected and analyzed on an. Rates of hydrolysis were calculated based on the ratio of the peak areas of substrate and product, PSI-352707.

Gene expression measured by RT-PCR

Total RNA was extracted from human primary hepatocytes from two donors and 1 µg of total RNA was used for cDNA synthesis using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Quantitative RT-PCR was performed using TaqMan® Universal PCR MasterMix and an ABI 7500 Real Time PCR System. Relative mRNA levels were calculated according to $\Delta\Delta CT$ method and expressed as fold change relative to HHPC-1 sample.

Western Blot Analysis

Protein extracts were prepared by incubating cells with pre-heated 1x NuPAGE® LDS Sample Buffer at 65 °C for 10 minutes. Approximately the same amounts of protein extract were resolved by gel electrophoresis using 10% Bis-Tris NuPAGE gels in MOPS running buffer and transferred to a nitrocellulose (NC) membrane. Blotting and antibody incubation were performed using Snap ID protein Detection System (Millipore, Billerica, MA) according to the manufacturer's protocol. Mouse monoclonal CatA and Goat polyclonal CES1 antibodies were diluted in the blocking buffer and corresponding HRP-conjugated secondary antibodies were used.

Results

1. Hydrolysis of PSI-7976 and PSI-7977 by human carboxylesterase 1 and cathepsin A

PSI-7976 or PSI-7977 at 100 μM was incubated for 10, 30, and 60 min in the presence of purified human CES1 or recombinant human CatA. As shown in Table 1, both stereoisomers were hydrolyzed by CES1 at the same rate. However, PSI-7977 was hydrolyzed by CatA approximately 20-fold faster than PSI-7976.

Table 1. Hydrolysis rates of PSI-7976 and PSI-7977 by CES1 and CatA

	Cat A	CESI
	Rate ($\mu\text{M}/\text{min}$)	Rate ($\mu\text{M}/\text{min}$)
PSI 7976	0.064 ± 0.004	0.526 ± 0.060
PSI 7977	1.121 ± 0.232	0.521 ± 0.109

2. RT-PCR Analysis of CatA and CES1 Gene Expression in primary human hepatocytes and Clone A cells.

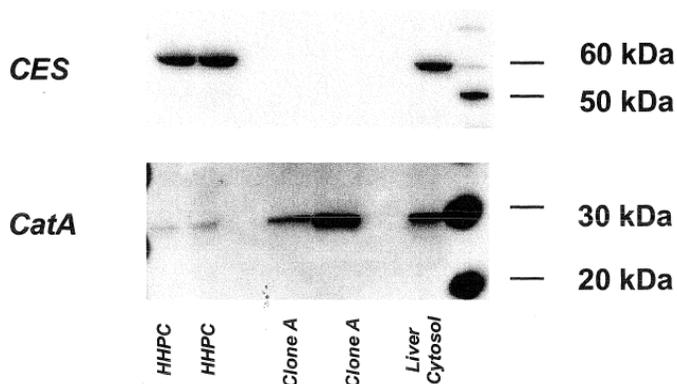
The level of CES1 and CatA mRNA gene expression in both Clone A cells and primary human hepatocytes (HHPC) was examined by real time PCR. Analysis of CES1 and CatA mRNA levels showed that CES1, which was expressed in HHPC, was not expressed in Clone A while CatA was expressed in both Clone A and HHPC from two donors (Table 2). The level of CatA expression in Clone A cells was approximately 5 times higher than in primary human hepatocytes.

Table 2. Relative mRNA expression levels as fold changes relative to HHPC-1 cells

Target gene	HHPC-1	HHPC-2	Clone A
CES1	1	1.49 (1.39-1.61)	<0.0001
CatA	1	0.87 (0.79-0.96)	4.91 (4.64-5.19)

3. Western Blot Analysis of Protein Expression in Cells

Western blot analysis was performed to examine the level CatA and CES1 protein expression in both Clone A cells and HHPC. Human liver cytosol was used as a positive control. The results showed that Clone A cells only expressed CatA, whereas HHPC expressed both enzymes (Figure 1).



Reviewer comments: The findings are consistent with RT-PCR results.

Conclusion

Both CESI and CatA are involved in hydrolysis of PSI-7851 and its two diastereoisomers. CatA preferentially hydrolyzed PSI-7977 whereas CES1 was not stereoselective. In Clone A cells, the major enzyme catalyzing the hydrolysis of PSI-7851 appears to be CatA as CESI expression was not detected in these cells. These differences in substrate specificity and in expression of the two enzymes in Clone A replicon cells and HHPC may explain the differences in intracellular metabolism and levels of the active triphosphate observed when the two cell types were incubated with each isomer in other in vitro studies using Clone A replicon cells. Furthermore, these differences may also explain the difference in activity between PSI-7976 and PSI-7977 observed in the replicon assay using Clone A cells.

Title: Human Cathepsin A Hydrolyzes PSI-7851 (PC-PSI-7851-08-0026)

Objective: To determine whether human cathepsin A (CatA) is involved in the metabolism of PSI-7851

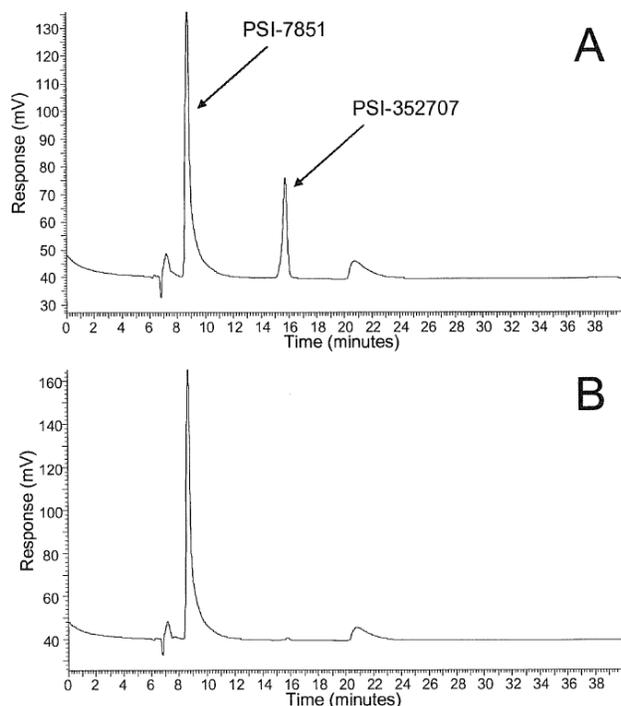
Methods:

Hydrolysis of PSI-7851 was performed in a 100 μ L reaction volume containing 1 μ g/mL activated Cat A, 100 μ M PSI-7851, 25 mM MES pH 6.0, 0.1 M NaCl, 1 mM DTT, and 0.1 % NP-40 for an hour. Then the reaction mixture was applied to anYM-10 Microcon filter (Millipore) and centrifuged at 12,000 rpm for 20 minutes to remove the protein. The flow-through from the filter was collected and analyzed by HPLC.

Results and Conclusion

When PSI-7851 was treated with human recombinant Cat A, PSI-352707 (retention time 15.8 min) was detected on HPLC chromatogram (Fig 1). In the presence of a Cat A inhibitor, VX950, conversion of PSI-7851 to the PSI-352707 was not observed. No PSI-352707 formation was observed when PSI-7851 was incubated in the reaction buffer for 1 hour in the absence of Cat A (data not shown). These results indicate that Cat A is most likely responsible for the hydrolysis of PSI-7851.

Fig 1. HPLC chromatograms after incubating PSI-7851 with human recombinant cathepsin A in the absence (A) and the presence (B) of VX950



Reviewer comments: The results suggest a potential drug interaction between VX-950 (telaprevir) and PSI-7851.

Title: Hydrolysis of PSI-7851 by human carboxylesterase (CES) 1 and 2 (PC-PSI-7851-09-0014)

Objective: To determine the mechanism of initial activation of PSI-7851

Methods: The hydrolysis reaction was performed in a 100 μ L reaction volume containing 1 μ g/mL enzyme, 50 μ M PSI-7851, 25 mM Tris-HCL pH 7.5. After incubation at 37 $^{\circ}$ C for 1 hour, the reaction mixture was applied to YM-10 Microcon filter to remove the protein. The flow-through from the filter was collected and analyzed on an HPLC using PARTISIL 10 SAX column. Activity of CES was confirmed by following the absorbance at 420 nm using 4-nitrophenyl acetate as a substrate.

Results and Conclusion

Incubation of PSI-7851 with recombinant human CES1 resulted in formation of its hydrolyzed metabolite PSI-352707 whereas CES2 was not able to hydrolyze PSI-7851. The activity of CES2 was confirmed using its prototypical substrate, 4-nitrophenylacetate. Therefore, PSI-7851 is a substrate of CES1, but not CES2.

Fig 1. HPLC chromatogram after incubating PSI-7851 with human recombinant CES1 (A) or CES2 (B)

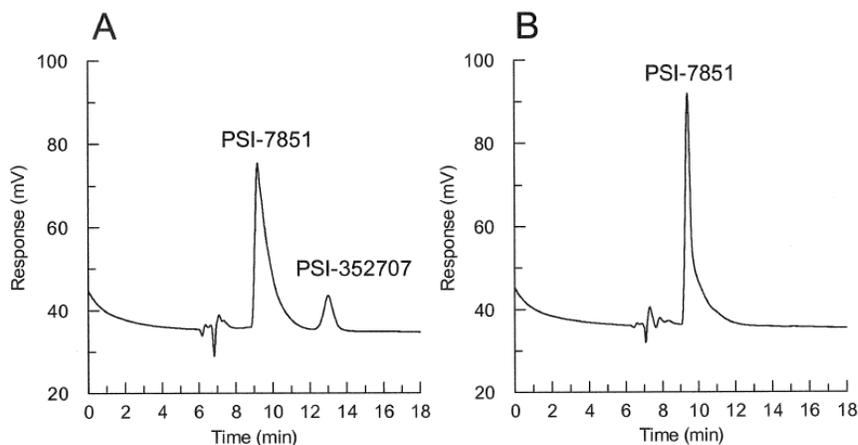


Figure 1: HPLC chromatograms after incubating PSI-7851 with human recombinant CES1 (A) or CES2 (B).

Title: Investigation of the human cytochrome P450, FMO, and UGT involvement in the metabolism of PSI-7977, PSI-352707, PSI-7411, and PSI-6206 using human liver microsomes (PC-PSI-7977-09-0004)

Objective: To investigate the involvement of human liver microsomal cytochrome P450 enzymes (CYPs), flavin-containing monooxygenases (FMOs), and uridine diphosphate glucuronosyltransferases (UGTs) in the metabolism of PSI-7977, PSI-352707, PSI-7411, and PSI-6206.

Methods

1. Determination of in vitro metabolism

2 μM of test article was incubated in a 1.0 mL incubation mixture containing MgCl_2 (3 mM), potassium phosphate buffer (100 mM, pH 7.4), NADPH (2 mM), and 0.5 mg/mL of human microsomal proteins. Pre-incubations were performed without NADPH for 5 minutes, and the reaction was initiated by adding NADPH. Aliquots (100 μL) were removed after 0, 5, 10, 20, 30, and 60 minutes of incubation, and were transferred to vials containing 100 μL acetonitrile and 1 μM of (b) (4) (internal standard). The disappearance of parent compound and the formation of its appropriate metabolites were monitored.

2. Heat inactivation of Flavin-containing monooxygenase (FMOs)

Microsomal heat liability experiments were performed to investigate if metabolism of test articles were mediated by FMOs. Incubations were performed as described in section 1, except the incubation mixtures were pre-incubated without NADPH at 45°C for 3 minutes, chilled on ice for 1 minute to lower the temperature to below 37°C.

3. Incubations with CYP inhibitor 1-aminobenzotriazole (ABT)

To investigate if metabolism of the test articles was mediated by CYPs, the test articles were incubated in human liver microsomes in the presence of CYP inhibitor, 1-aminobenzotriazole (ABT) 100 µM. The incubation conditions were similar to what was described in Section 1.

4. Incubations with UDPGA as co-factor

To investigate if the test articles were subject to phase II metabolism, each test article was incubated with human liver microsomes in the presence of uridine 5'-diphosphate glucuronic acid (UDPGA) 4 mM. Alamethicin was added to the incubations at the final concentration of 50 µg/mg to activate the UGT enzymes. The incubation conditions were similar to those described in Section 1.

5. Bioanalysis

The LC/MS/MS analytical method for PSI-7851, PSI-352707, PSI-7411, and PSI-6206 analysis in human plasma was based on the validated method from (b) (4) study 86-0901.

Results

1. Metabolism of PSI-7977 in human liver microsomes

The disappearance of PSI-7977 (Table 1) and appearance of PSI-352707 (Table 2) were observed in human liver microsomal incubations. This is thought to be primarily due to the presence of esterases and/or hydrolases in human liver microsomes.

The rate of metabolism of PSI-7977 and formation of PSI-352707 was decreased by approximately 50% in the presence of the CYP inhibitor, ABT. This suggests potential involvement of CYP isoforms in the metabolism of SOF.

Heat treatment of human liver microsomes or addition of UDPGA as a co-factor had no effect on the rate of PSI-7977 disappearance (Table 1), indicating no significant role of FMO or UGT in the metabolism of PSI-7977.

Table 1. Summary of PSI-7977 remaining after incubation with human liver microsomes

Incubation Time (min)	HLM	Heat	ABT	UDPGA
0	100	100	100	100
5	72	82	92	80
10	60	75	87	74
20	53	65	80	60
30	40	61	70	49
60	27	42	46	24

CYP: Human liver microsomes

Heat: Heat inactivation of FMO enzymes at 45°C

ABT: CYP Inhibition by 1-aminobenzotriazole (ABT)

UDPGA: Incubations with UDPGA as co-factor

Table 2. Summary of PSI-352707 formation after incubation of PSI-7977 with human liver microsomes

Incubation Time (min)	Peak Area Ratios (Analyte/IS)			
	HLM	Heat	ABT	UDPGA
0	0.143	0.043	0.000	0.006
5	0.792	0.271	0.194	0.374
10	0.965	0.497	0.303	0.542
20	1.140	0.584	0.517	0.838
30	1.171	0.695	0.760	1.142
60	1.476	0.948	1.260	1.711

CYP: Human liver microsomes

Heat: Heat inactivation of FMO enzymes at 45 °C

ABT: CYP Inhibition by 1-aminobenzotriazole (ABT)

UDPGA: Incubations with UDPGA as co-factor

Reviewer comments:

The results indicated the potential involvement of CYP isoforms in the metabolism of PSI-7977. However, the following observations suggest that CYP isoforms do not play a clinically relevant role in PSI-7977 metabolism: in vitro and clinical studies have shown that PSI-7977 is rapidly metabolized to GS566500 by high capacity esterases (Cat A and CES1); no other metabolite directly derived from PSI-7977 was detected in vitro or in vivo, and in vitro drug interaction studies with ritonavir and ketoconazole and in vivo drug interaction studies with efavirenz and darunavir/ritonavir indicated no clinically relevant changes to the metabolism of PSI-7977. Based on these observations, the sponsor did not further characterize the roles of individual CYP isoforms on the metabolism of PSI-7977 using purified CYP isozymes.

2. Metabolism of PSI-352707 in human liver microsomes

There was no significant decrease in concentration of PSI-352707 in the human microsome incubation mixture over the 1 h incubation period. There was no significant decrease in concentration of PSI-352707 observed in human liver microsomes in the presence of ABT or UDPGA.

3. Metabolism of PSI-7411(GS606965) in human liver microsomes

There was no significant change in PSI-7411 concentrations in human microsomes incubation mixture over the 1 hour incubation period. There was no significant decrease in concentration of PSI-7411 observed in human liver microsomes in the presence of ABT or after heat inactivation of FMOs. Incubation with UDPGA showed that approximately 70% of the PSI-7411 remained after the 1 h incubation, suggesting the disappearance of PSI-7411 involved glucuronosyltransferase activity.

Comments: The major pathway of PSI-7411 metabolism is phosphorylation by UMP-CMP and NDP kinases. The contribution and clinical significance of PSI-7411 glucuronidation is thought to be none to minimal as PSI-7411 glucuronide is not a major metabolite detected in vivo studies.

4. Metabolism of PSI-6206 in human liver microsomes

There was no significant decrease of PSI-6206 observed in the human liver microsomal incubations including human liver microsomes alone, with ABT or UDGPA, as well as heat inactivation.

Reviewer comments: PSI-6206 is the major circulating metabolite in vivo and results from a mass balance study indicated that the majority of PSI-6206 is eliminated unchanged in urine.

Conclusion

PSI-7977 was unstable in human liver microsomes most likely due to esterase activities in human microsomes. The metabolism of PSI-7977 was slower in the presence of CYP inhibitor (1-aminobenzotriazole), suggesting potential roles of CYP isoforms in PSI-7977 metabolism. FMO and UGT had no significant effect on PSI-7977 metabolism. PSI-352797 and PSI-6206 were stable after incubations with human liver microsomes under various conditions. The concentrations of PSI-7411 were decreased with the additions of UDPGA as a co-factor, suggesting potential involvement of UGT in the metabolism of PSI-7411.

Title: Comparison of PSI-7977 metabolism in human hepatocytes and peripheral blood mononuclear cells (PC-PSI-7977-11-0004)

Objective: To compare the metabolism of PSI-7977 in human hepatocytes and peripheral blood mononuclear cells (PBMC)

Methods

Cells were incubated with 5 μM [^{14}C]-PSI-7977 (133 dpm/pmol) at 37°C. At selected times, for human hepatocytes the extracellular medium was removed and the cell layer was washed with cold PBS. After trypsinization, cells were counted and centrifuged at 1,200 rpm for 5 min. For human PBM cells 1 mL of cells was transferred to a centrifuge tube and diluted with 9 ml cold PBS and pelleted by centrifugation at 1,200 rpm for 5 min. The cell pellets were suspended in 1 mL of cold 60% methanol and incubated overnight at -20°C. The samples were centrifuged at 14,000 rpm for 5 min and the supernatants were dried using a SpeedVac Concentrator. Dried samples were resuspended in water, and 50 μL aliquots were analyzed by ion exchange HPLC.

Results

In primary human hepatocytes, the following intracellular metabolites were detected after 48 hours of incubation with 5 μM of PSI-7977; PSI-6206, PSI-352707, PSI-7411, PSI-7410 and PSI-7409 (the major metabolite)

In non-stimulated and stimulated human PBMC for 48 hours with 5 μM of PSI-7977, the major peaks corresponded to PSI-352707 and PSI-7409 and the smaller peaks to PSI-6206 and PSI-7410. The total amount of radioactive metabolites and PSI-7409 was higher in primary human hepatocytes compared to PBM cells. However, the level of PSI-352707 was significantly higher in PBM cells than in hepatocytes suggesting that the conversion of PSI-352707 to PSI-7411 was the rate limiting step in the metabolism of PSI-7977 in PBM cells.

A time course experiment was performed to assess the effect of time on the metabolism of PSI-7977 in primary human hepatocytes (Fig 1). PSI-7851 was rapidly hydrolyzed to PSI-352707. The level of PSI-352707 reached its maximum at 2 to 4 hours incubation and then quickly declined. PSI-7411, PSI-7410, and PSI-7409 appear to have reached their maximum level after about 8 hours of exposure. At 4 hours of incubation, PSI-352707 was the metabolite in highest concentration. However, after 8 hours of incubation PSI-7409 became the predominant metabolite. Intracellular level of PSI-6206 was low throughout the time course.

In both non-stimulated and stimulated human PBM cells (Fig 2), PSI-7977 was hydrolyzed to PSI-352707. The level of PSI-352707 plateaued after 8 hours incubation. PSI-7409 reached its maximum level after 24 hours of exposure with the compound. Intracellular levels of PSI-7411, PSI-7410 and PSI-6202 were low throughout the time course.

Fig 1. Time dependency of PSI-7977 intracellular metabolism in human hepatocytes

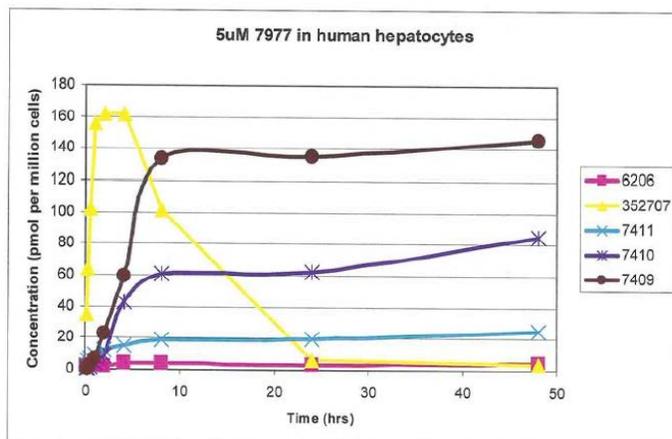
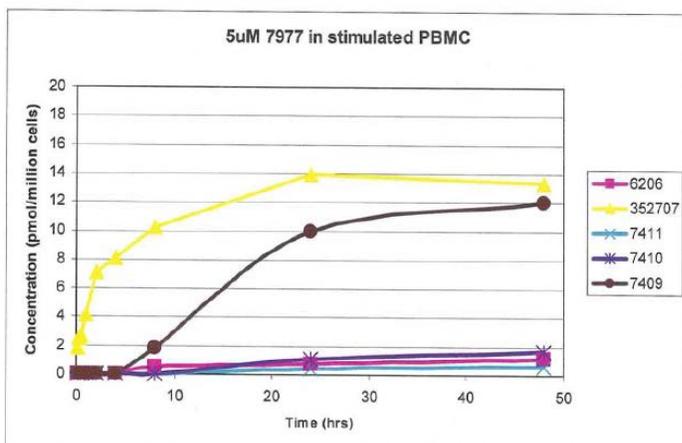
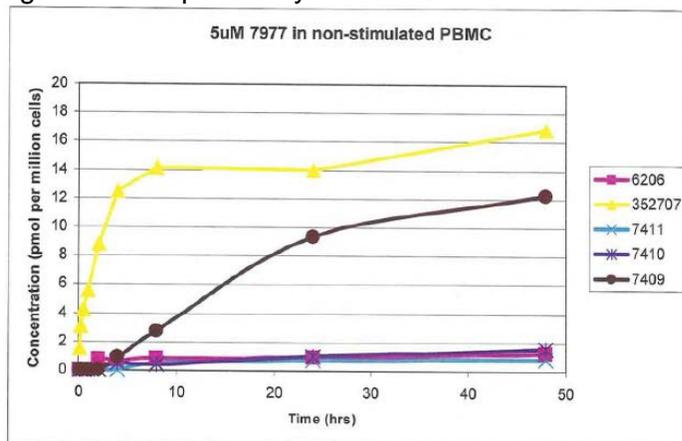


Fig 2. Time dependency of PSI-7977 intracellular metabolism in human PBMC



Conclusion

In human hepatocytes and PBMC, all known intracellular metabolites (PSI-6206, PSI-352707, PSI-7411, PSI-7410 and PSI-7409) were detected after 48 hours of incubation with 5 μM of PSI-7977. The major metabolites were PSI-7409 in human hepatocytes and PSI-352707 in PBMC, respectively.

Title: Test to Monitor Conversion from PSI-7977 to PSI-7976 in Rat, Dog and Human Plasma and Human Urine by LC-MS/MS (AD-334-2014)

Objective: To examine whether PSI-7977 can be converted to PSI-7976 *in vivo* and *in vitro* during sample handling and storage

Methods

In vitro samples of PSI-7977 were prepared at 2500, 5000 and 10,000 ng/mL in rat plasma (treated with 1 mM DDVP as an carboxylesterase inhibitor), dog plasma, human plasma and human urine, respectively.

After undergoing the following conditions, the *in vitro* samples were analyzed with LC-MS/MS to determine the relative level of PSI-7977 and PSI-7976:

- Before and after storing the samples in a -70°C freezer for 35 days
- Re-injection of test samples after storage at 4°C for 14 days
- Before and after bench top exposure at the temperature and duration of each validated method (16 hours on wet-ice bath for rat plasma, 6 hours at RT for dog plasma, 7 hours on wet-ice bath for human plasma and 6 hours on wet-ice bath for human urine)
- Before and after freeze/thaw cycles per each validated method (4 cycles for rat plasma, 8 cycles for dog plasma and 5 cycles for both human plasma and human urine)

Results and conclusion

There is no distinguishable difference in the percentage of PSI-7976 in solution, plasma, and urine under various storage and process conditions compared to freshly prepared samples. There is no evidence of *in vitro* conversion from PSI-7977 to PSI-7976 in plasma and urine during sample storage and processing.

Title: In vitro evaluation of PSI-7977 as an inducer of cytochrome P450 expression in cultured human hepatocytes (PSI-7977-10-0005)

Objective: To investigate the induction effects of PSI-7977 on the expression of cytochrome P450 (CYP) enzymes in human hepatocytes.

Methods

Three preparations of cultured human hepatocytes from three separate livers were treated once daily with dimethyl sulfoxide (DMSO, 0.1% v/v, vehicle control), one of three concentrations of PSI-7977 (1, 10 or 100 μM) or one of three known human CYP inducers, omeprazole (100 μM for CYP1A2), phenobarbital (750 μM for CYP2B6) and rifampin (10 μM for CYP3A4) for 3 days.

After treatment, the cells were harvested to isolate microsomes for the analysis of phenacetin O-dealkylation (a marker for CYP1A2 activity), bupropion hydroxylation (a marker for CYP2B6 activity) and testosterone 6 β -hydroxylation (marker for CYP3A4/5 activity). Additional hepatocytes from the same treatment groups were harvested with TRIzol® to isolate RNA, which was analyzed by qRT-PCR to assess the effect of PSI-7977 on CYP1A2, CYP2B6 and CYP3A4 mRNA expression levels.

Results

i) The effects of PSI-7977 on the mRNA expression level and activity of CYP1A2

PSI-7977 had little to no effect on CYP1A2 mRNA expression levels (less than 1.5-fold) and CYP1A2 activity at concentrations up to 100 μ M.

ii) The effects of PSI-7977 on the mRNA expression level and activity of CYP2B6

PSI-7977 increased CYP2B6 mRNA expression levels and activities in a concentration-dependent manner. mRNA expression levels of CYP2B6 were increased by 1.5-, 1.7-, and 2.0-fold in the presence of 1, 10, and 100 μ M of PSI-7977, respectively. The activities of CYP2B6 were also increased by 1.2- and 2.7-fold in the presence of 10 and 100 μ M of PSI-7977, respectively. Phenobarbital, a positive control for CYP2B6 induction, increased the mRNA expression level and activity of CYP2B6 by 15- and 18-fold, respectively.

ii) The effects of PSI-7977 on the mRNA expression level and activity of CYP3A4.

PSI-7977 increased CYP3A4 mRNA expression levels and activities. The mRNA expression levels of CYP3A4 were increased by 1.2-, 1.4-, and 2.7-fold in the presence of 1, 10, and 100 μ M of PSI-7977, respectively. However, the activities of CYP3A4 were minimally increased (1.2-fold increase by PSI-7977 100 μ M). Rifampin, a positive control for CYP3A4 induction, increased the mRNA expression level and activity of CYP3A4 by 14- and 6.9-fold, respectively.

Table 1. The effects of PSI-7977 or prototypical inducers on the mRNA expression levels of CYP1A2, CYP2B6 and CYP3A4 in cultured human hepatocytes

Treatment	Concentration	Fold increase ^a		
		CYP1A2	CYP2B6	CYP3A4
Dimethyl sulfoxide	0.1% (v/v)	1.00 \pm 0.00	1.00 \pm 0.00	1.00 \pm 0.00
PSI-7977	1 μ M	1.29 (n=2)	1.47 (n=2)	1.20 (n=2)
PSI-7977	10 μ M	1.41 \pm 0.31	1.67 \pm 0.81	1.35 \pm 0.31
PSI-7977	100 μ M	1.44 \pm 0.91	2.04 \pm 0.76	2.73 \pm 1.01
Omeprazole	100 μ M	203 \pm 115	NA	NA
Phenobarbital	750 μ M	NA	14.7 \pm 5.2	NA
Rifampin	10 μ M	NA	NA	14.0 \pm 7.1

a Values are relative to GAPDH and are the mean \pm standard deviation of three determinations (human hepatocyte preparations H974, H975 and H976) unless indicated otherwise (e.g., n = 2).

Table 2. The effects of PSI-7977 or prototypical inducers on the activities of CYP1A2, CYP2B6, and CYP3A4 (fold-increase compared to vehicle control)

Treatment	Concentration	Fold increase ^a		
		Phenacetin O-dealkylation (CYP1A2)	Bupropion hydroxylation (CYP2B6)	Testosterone 6β-hydroxylation (CYP3A4/5)
Dimethyl sulfoxide	0.1% (v/v)	1.00 ± 0.15 ^b	1.00 ± 0.62 ^b	1.00 ± 0.47 ^b
PSI-7977	1 μM	1.05 (n=2)	1.05 (n=2)	1.02 (n=2)
PSI-7977	10 μM	1.02 ± 0.02	1.24 ± 0.16	1.14 ± 0.30
PSI-7977	100 μM	1.05 ± 0.07	2.70 ± 0.20	1.21 ± 0.53
Omeprazole	100 μM	30.4 ± 5.5	8.30 ± 2.82	2.71 ± 0.82
Phenobarbital	750 μM	2.32 ± 0.25	18.1 ± 9.7	7.64 ± 2.84
Rifampin	10 μM	2.02 ± 0.47	7.07 ± 3.71	6.87 ± 2.65

a Values are the mean ± standard deviation of three determinations (human hepatocyte preparations H974, H975 and H976) unless indicated otherwise (e.g., n = 2).

b For vehicle controls, CV (Coefficient of Variance) (Rate standard deviation (pmol/mg microsomal protein/min) + Mean Rate (pmol/mg microsomal protein/min)) is calculated instead of standard deviation to give a more realistic representation of variance among control samples.

Reviewer comments: According to the current draft drug interaction study guidance (Feb 2012), an investigational product is considered an enzyme inducer when in vitro results are positive according to the predefined thresholds using basic models. The applicant did not propose predefined thresholds. Instead, the applicant stated that the induction effects of PSI-7977 is minimal as the increases in the mRNA levels and activities were less than 15% of those caused by the positive controls.

The in vivo drug interaction studies indicated that PSI-7977 is unlikely to induce CYP3A4 or CYP2B6 at clinical doses. The exposure of efavirenz (a sensitive CYP2B6 substrate) and darunavir (a sensitive CYP3A4 substrate) were not changed after 400 mg PSI-7977 administration.

Conclusion

PSI-7977 increased the mRNA expression levels of CYP2B6 and CYP3A4 (2.0- and 2.7-fold respectively at 100 μM). The CYP2B6 activity was also increased by 2.7-fold at 100 μM PSI-7977 while the CYP3A4 activity was minimally increased (1.2-fold at 100 μM PSI-7977). Based on the magnitudes of change, the clinical relevance of the concentrations tested, and *in vivo* drug interactions study results with efavirenz and darunavir, the induction effects of PSI-7977 on CYP3A4 and CYP2B6 are not considered clinically relevant. PSI-7977 at 100 μM caused little to no induction in CYP1A2.

Title: Absence of effect of PSI-7851 (GS-9851) and its metabolites on human cytochrome P450 isozymes (PC-PSI-7851-09-0009)

Objective: To determine the effects of PSI-7851 and its metabolites on human recombinant cytochrome P450 activity (CYP3A4, CYP1A2, CYP2C19, CYP2C9, CYP2C8, and CYP2D6)

Methods

BD bioscience CYP isoform inhibition screening kits were used in this study to measure the inhibitory activities of PSI-7851 and its metabolites. Concentrations of GS-9851 (PSI-7851), GS-331007 (PSI-6206), GS-461203 (PSI-7409), GS-607596 (PSI-7410), GS-606965 (PSI-7411), and GS-566500 (PSI-352707) tested were 100, 33.3, 11.1, 3.7, 1.23, 0.41, 0.14, and 0.05 μM.

The reaction mixture contained 16 μM NDAP+, 0.8 mM MgCl_2 , 0.8 mM glucose-6-phosphate, 0.4 unit/mL glucose-6-phosphate dehydrogenase, and desired concentrations of test compound or appropriate control inhibitor.

The reaction times differed depending upon CYP reaction; 15 min for CYP1A2 and CYP3A4, 30 min for CYP2C19 and CYP2D6, 40 min for CYP2C8, and 45 min for CYP2C9. Reactions were quenched by adding 75 μL of the quench solution. CYP activity was measured by recording fluorescence signal from the metabolite of the substrate. Blank values were subtracted from the sample values to obtain net fluorescence signal.

The following positive controls (known CYP isoform-specific inhibitors) were used in this study; ketoconazole (0.002-5 μM) for CYP3A4, furafylline (0.05-100 μM) for CYP1A2, tranlycypromine (0.05-100 μM) for CYP2C19, sulfaphenazole (0.005-10 μM) for CYP2C9, quercetin (0.02-40 μM) for CYP2C8, and quinidine (0.5-500nM) for CYP2D6.

Results

IC_{50} values obtained for PSI-7851 and its metabolites were $> 100 \mu\text{M}$ for CYP3A4, CYP1A2, CYP2C19, CYP2C9, CYP2C8, and CYP2D6 (Table 1). These results indicate that PSI-7851 and its metabolites are not inhibitors of human cytochrome P450. The IC_{50} values for each of the control compounds were consistent with values provided by the manufacturer (BD bioscience), confirming satisfactory experimental conditions for the assays (Table 2).

Table 1. IC_{50} values for PSI-7851 and its metabolites

Compound #	CYP3A4	CYP1A2	CYP2C19	CYP2C9	CYP2C8	CYP2D6
PSI 7851	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$
PSI 6206	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$
PSI 7409	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$
PSI 7410	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$
PSI 7411	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$
PSI 352707	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$	$>100\mu\text{M}$

Table 2. IC_{50} values for positive inhibitor controls

CYP450	Positive Inhibitor Control	Pharmasset IC_{50} (μM)	Manufacturer IC_{50} (μM)
CYP450, 3A4	Ketoconazole	0.007	0.002
CYP450, 1A2	Furafylline	3.2	2.5
CYP450, 2C19	Tranlycypromine	1.8	2.0
CYP450, 2C9	Sulfaphenazole	0.2	0.37
CYP450, 2C8	Quercetin	5.8	5.2
CYP450, 2D6	Quinidine	0.006	0.006

Reviewer Comments;

The sponsor did not provide detailed information on CYP isoform activities at each concentration of SOF, its metabolites, and positive controls as graphs and/or tables. The current data in these two tables are not sufficient to conclude whether the IC_{50} was appropriately

calculated and there is no effect of the test articles on the activities of CYP isoforms. In response to our information request, the sponsor will submit the detailed information of the study results by early September 2013.

Conclusion

PSI-7851 and its metabolites do not inhibit catalytic activities of human CYP isoforms (CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4).

Title: In vitro assessment of human UGT1A1 inhibition potential of GS-7977, GS-606965 and GS-331007 (AD-334-2013)

Objective: To evaluate the potential for GS-7977, GS-606965 and GS331007 to inhibit the catalytic activity of human UGT1A1

Methods

Test articles (0.4 – 100 μ M) were incubated with insect cell microsomal fraction containing baculovirus-expressed human UGT1A1 (Supersomes™) 0.25 mg/mL, alamethicin (25 μ g/mL) and UDP-glucuronic acid (5 mM), and the substrate, estradiol (10 μ M) for 30 min. 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 IC₅₀ value (the test compound concentration which produces 50% inhibition).

Results

A summary of the inhibitory potencies is presented in Table 1. Three test compounds (GS-7977, GS-606965, and GS-331007) showed no significant effect (IC₅₀ > 50 μ M) and so are unlikely to be clinically relevant inhibitors of UGT1A1 in vivo. Silybin and atazanavir, the positive control inhibitors reduced UGT1A1 activity with an IC₅₀ of 1.95 μ M and 0.22 μ M, respectively, confirming satisfactory experimental conditions for the assays.

Table 1. IC₅₀ values for inhibition of human UGT1A1 activity by GS-7977, GS-606965, and GS-331007 and positive control inhibitors

Compound	Role	IC ₅₀ (μ M)
GS-7977	Nucleotide prodrug	> 50
GS-606965	Nucleotide metabolite	> 50
GS-331007	Nucleoside metabolite	> 50
Atazanavir	Clinically relevant positive control	0.22
Silybin	Assay positive control	1.95

Estradiol 3-glucuronidation activity

Conclusion: None of the three compounds had a significant inhibitory effect on the activity of human UGT1A1

Reviewer comments

The sponsor did not provide detailed information on UGT1A1 activities at each concentration of SOF, its metabolites, and positive controls as graphs and/or tables. The current data in these

two tables are not sufficient to conclude whether the IC50 was appropriately calculated and there is no effect of the test articles on UGT1A1 activities. In response to our information request, the sponsor will submit the detailed information of the study results by early September 2013.

Title: In vitro interaction studies of PSI-7977 and PSI-6206 with the human OATP1B1 and OATP1B3 uptake transporters (PC-PSI-7977-11-0007)

Objective: To evaluate PSI-7977 and PSI-6206 as substrates and inhibitors of OATP1B1 and OATP1B3

Materials and methods

a. Inhibition experiments

Experiments were performed on Chinese hamster ovary (CHO) cells stably expressing OATP1B1 or OATP1B3. Uptake experiments were carried out at 37°C in 50 µL of Krebs-Henseleit buffer containing the respective probe substrate and the test drug (or solvent) (Table 1). After the experiment, cells were rinsed and lysed with 50 µL of 0.1 M NaOH. Fluo-3 transport (OATP1B3 activity) was determined by measuring fluorescence. ³H-estrone-3-sulfate transport (OATP1B1 activity) was determined by passing an aliquot (35 µL) from each well to liquid scintillation.

Table 1. Probe substrate and reference inhibitor for OATP1B1 and OATP1B3 inhibition assays

Transporter	Applying SOP	Incubation time	Probe substrate (concentration)	Reference inhibitor (concentration)
human OATP1B1	UPT-CHO-OATP1B1-E3S	10	[³ H]E3S (0.1 µM)	cerivastatin (100 µM)
human OATP1B3	UPT-CHO-OATP1B3-Fluo-3	10	Fluo-3 (10 µM)	fluvastatin (30 µM)

Table 2. Treatment groups in uptake transporter inhibition assays

Treatment groups in the 96-well plate format	No. of wells
TA in DMSO (0.41, 1.2, 3.7, 11, 33, 100 and 300 µM) on transfected cells	2 per TA concentration
TA in DMSO (0.41, 1.2, 3.7, 11, 33, 100 and 300 µM) on parental cells	2 per TA concentration
DMSO control on transfected cells	2
DMSO control on parental cells	2
Reference inhibitor in DMSO on transfected cells	2

b. Substrate experiments

The uptake of the test article by OATP1B1 or OATP1B3 was determined using cells overexpressing these transporters at two incubation time points (2 and 20 min) and at two concentrations (20 and 200 μM).

Results

Inhibition effects of PSI-7977 and PSI-6206 on uptake transporters

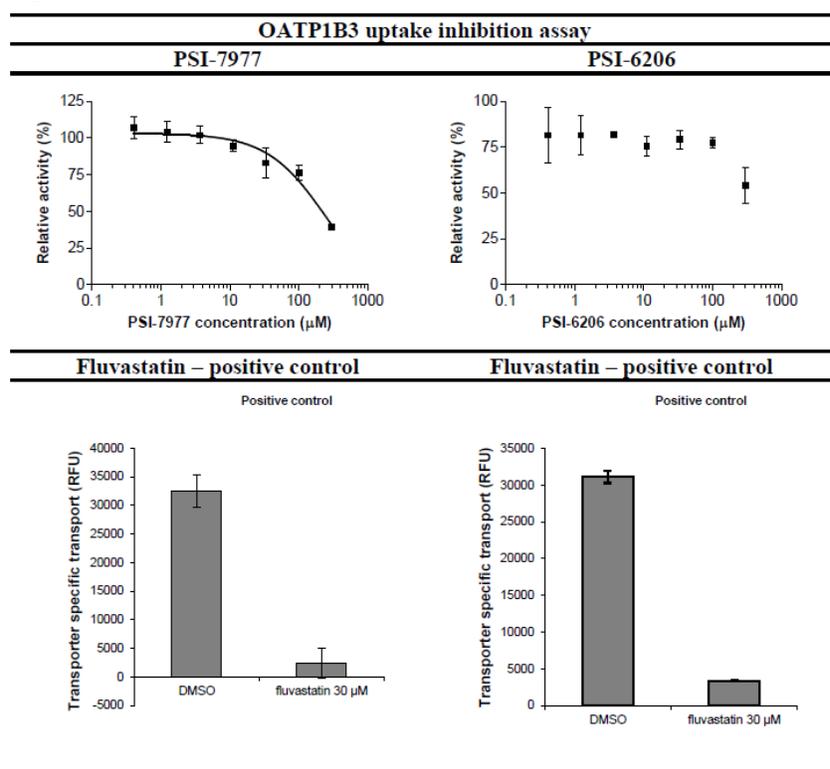
i) Effects of PSI-7977 and PSI-6206 on OATP1B1 transport activity

No inhibition of OATP1B1-mediated transport (using estrone sulfate as a probe substrate) was observed in the presence of PSI-7977 or PSI-6206.

ii) Effects of PSI-7977 and PSI-6206 on OATP1B3 transport activity

PSI-7977 inhibited OATP1B3-mediated uptake of Fluo-3 (a probe substrate) in a dose dependent manner. The IC_{50} value was 203.5 μM (Fig 1). The maximum inhibition (efficacy, %) was approximately 60%. PSI-6206 did not inhibit the OATP1B3-mediated uptake. Fluvastatin, a known inhibitor of OATP1B3, significantly inhibited the OATP1B3-mediated uptake.

Fig 1. Inhibition of OATP1B3-mediated Fluo-3 transport by PSI-7977, PSI-6206, or fluvastatin



<OATP1B1 or OATP1B3 mediated hepatic uptake of PSI-7977>

To determine if PSI-7977 was a substrate of OATP1B1 or OATP1B3, PSI-7977 (20 and 200 μM) was incubated with OATP1B1 or OATP1B3 expressing cells and control cells for 2 and 20 min. The accumulation of PSI-7977 in OATP1B1 and OATP1B3 expressing cells was not notable, indicating PSI-7977 was not a substrate of either transporter.

Fig 2. Accumulation of PSI-7977 in transporter-expressing and control cells in the OATP1B1 uptake transporter substrate feasibility assay

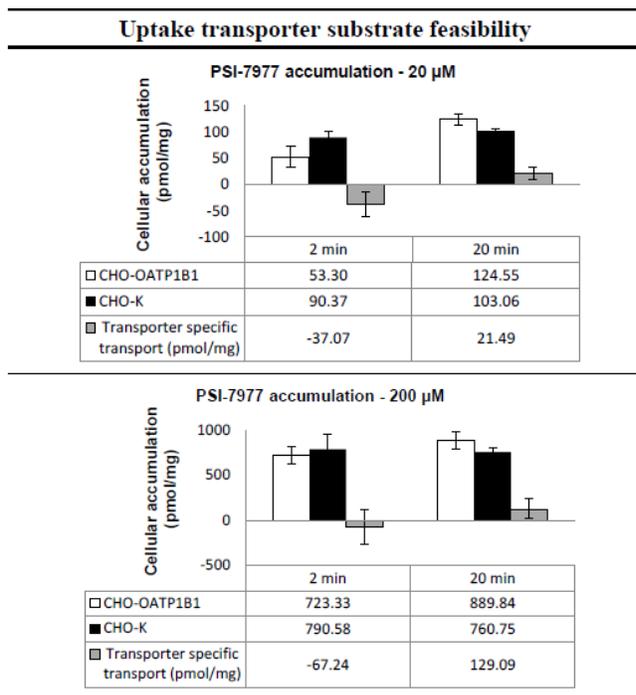
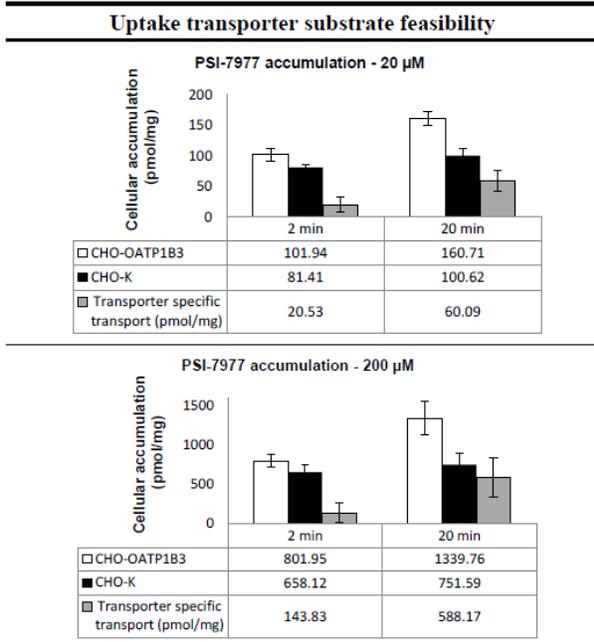


Fig 3. Accumulation of PSI-7977 in transporter-expressing and control cells in the OATP1B3 (uptake transporter substrate feasibility assay)



Reviewer comments: In Fig 3, it appears that there is a small but statistically significant difference in cellular accumulation of PSI-7977 in CHO-OATP1B3 cells compared to CHO cells

(control) after 20 min incubation. This suggests that PSI-7977 may be a weak substrate of OATP1B3 at a higher concentration. However, as the magnitude of difference appears to be minimal (< 2-fold), this is unlikely to be clinically relevant.

Conclusion

The accumulation of PSI-7977 in OATP1B1 and OATP1B3 expressing cells was less than 2-fold indicating PSI-7977 was not a substrate of either transporter. No inhibition of OATP1B1 activity was observed in the presence of PSI-7977. At concentrations above 10 μ M, PSI-7977 inhibited the OATP1B3 activities in a dose-dependent manner. The estimated IC₅₀ value for PSI-7977 in the OATP1B3 inhibition assay was ~200 μ M. PSI-6206 did not inhibit OATP1B1 or OATP1B3.

Title: In Vitro Interaction Studies of GS-7977 with Human OCT1 and BSEP Transporters (AD-334-2004)

Objective: To assess the potential for GS-7977 to interact with the human hepatic organic cation uptake transporter (OCT1) and bile salt export pump (BSEP) using transfected cell lines and membrane vesicles

Methods

1. OCT1 uptake transporter inhibition and substrate assay

Chinese hamster ovary (CHO) cell lines stably expressing human OCT1 protein as well as the control (parental) cells were used in these studies.

In the inhibition assay, cells were incubated for 20 minutes in buffer containing [¹⁴C]-metformin (4 μ M, an OCT1 substrate) and GS-7977. The amount of intracellular [¹⁴C]-metformin was determined by liquid scintillation counting. Results were compared to parent CHO cells (without OCT1) treated in the same manner. Verapamil (100 μ M) was used as a positive control and tested in parallel. In the substrate assay, transport was measured at two concentrations (10 μ M and 100 μ M) and at two time points (2 and 20 minutes). The amount of substrate inside the cells was determined by LC/MS/MS method. Results were compared to parent CHO cells (negative control). [¹⁴C]-tetraethylammonium chloride (TEA) and verapamil were used as a model substrate.

2. BSEP vesicular transport inhibition assay

GS-7977 was incubated with membrane vesicle preparations (total protein: 50 μ g/well) and the probe substrate taurocholate (2 μ M) in the absence or presence of ATP. Reactions were started by the addition of 25 μ L of 12 mM MgATP (or assay buffer for background controls), preincubated separately. Reactions were stopped after 5 min by the addition of 200 μ L of ice-cold washing buffer and immediate filtration via glass fiber filters. The filters were washed, dried and the amount of substrate inside the filtered vesicles determined by liquid scintillation. Cyclosporine A (20 μ M) was used as a positive control and tested in parallel.

Results

1. Inhibition of OCT1 and BSEP by GS-7977

At the highest tested concentration (100 μ M), GS-7977 showed weak inhibition of OCT1 with IC₅₀ approximately 100 μ M and maximum inhibition of 48% (Table 1). GS-7977 is not an inhibitor of BSEP. Positive controls (known inhibitors of OCT and BSEP, verapamil and

cyclosporine A, respectively) inhibited the transport activities > 90%, confirmed the function of the transporters in the applied cells and vesicles.

Table 1. Inhibition of human OCT1 and BSEP by GS-7977

Compound	Transporter	IC ₅₀ (μM)	Maximal inhibition (%) at the highest tested concentration (100 μM)
GS-7977	OCT1	~100	48
	BSEP	>100	18

2. OCT1 substrate assay

The accumulation of GS-7977 by OCT1 was not significantly higher than 2, indicating that GS-7977 is not a substrate for OCT1 transporter (Table 2). The accumulation ratio of TEA, an OCT1 sensitive substrate (a positive control) was 12.5 and TEA transport was inhibited by verapamil. This confirmed the function of the transporter in this experiment.

Table 2. Accumulation of GS-7977 in OCT1 transporter expressing and control cells in the OCT1 substrate assay

Compound	Concentration (μM) / Incubation Time (min)	Accumulation in transporter expressing cells (pmol/mg)	Accumulation in control cells (pmol/mg)	Transporter specific accumulation (fold)
GS-7977	10 / 2	27.07 ± 6.23	14.89 ± 4.99	1.82
	10 / 20	108.87 ± 57.84	46.14 ± 1	2.36*
	100 / 2	196.42 ± 38.41	199.57 ± 57.79	0.98
	100 / 20	720.99 ± 72.6	636.35 ± 42.32	1.13

* Due to the high standard deviation this value was not interpreted to indicate that GS-7977 is an OCT1 substrate

Conclusion

GS-7977, tested up to 100 μM, showed weak inhibition of the human hepatic transporters OCT1 and no inhibition of BSEP. GS-7977 is not a substrate for OCT1 transporter.

Title: In Vitro Interaction Studies of GS-331007 with Human Hepatic Transporters OCT1 and BSEP and Renal Transporters OAT1, OAT3 OCT2 and MATE1 (AD-334-2005)

Objective: To investigate GS-331007 interactions with the hepatic transporters BSEP and OCT1 and the renal transporters OCT2, OAT1, OAT3 and MATE1.

Materials and methods

1. BSEP vesicular transport inhibition assay

GS-331007 was incubated with membrane vesicle preparations and the probe substrate taurocholate (2 μM) in the absence or presence of ATP. Reactions were started by the addition of 25 μL of 12 mM MgATP (or assay buffer for background controls). Reactions were stopped after 5 min by the addition of 200 μL of ice-cold washing buffer and immediate filtration via glass fiber filters. The amount of substrate inside the filtered vesicles determined by liquid scintillation. Cyclosporine A (20 μM) was used as a positive control and tested in parallel.

2. OCT1, OCT2, OAT1, OAT3, and MATE1 transporter inhibition assays

The uptake transporter inhibition assay was conducted with cold GS-331007 and a labeled probe substrate and transporter specific accumulation of the probe substrate in the cells was measured. Cells and experimental conditions for uptake transporter inhibition assays are summarized in Table 1.

Table 1. Cells and experimental conditions for uptake transporter inhibition assays

Transporter	Cells	Probe substrate	Reference inhibitor
human OAT1 (CHO)	CHO cells	Para-aminohippuric acid	benzbromarone
human OAT3 (FlpIn)	FlpIn293 cells	Estrone-3-sulfate (E3S)	probenecid
human OCT1 (CHO)	CHO cells	metformin	verapamil
human OCT2 (CHO)	CHO cells	metformin	verapamil
human MATE1 (CHO)	CHO cells	metformin	quinidine

3. OCT1, OCT2, OAT1, OAT3 and MATE1 transporter substrate assays

The transporter specific uptake of GS-331007 was determined using cells overexpressing the OCT1, OCT2, OAT1, OAT3 or MATE1 transporters as well as the control (parental) cells. GS-331007 was incubated at 37±1°C at final concentrations of 1 and 10 µM. Incubations (2 min and 20 min) were carried out as described in the uptake transporter inhibition assays. GS-331007 intracellular concentrations were determined by LC/MS/MS. In case of OAT1, an additional experiment was performed in the presence of a known inhibitor of the transporter (200 µM benzbromarone).

Results

Transporter inhibition assays

Among the investigated transporters GS-331007 inhibited only the OAT1-mediated PAH transport in the tested concentration range (0.14, 0.41, 1.2, 3.7, 11, 33 and 100 µM) (Table 2). The maximum inhibition was 33% at 100 µM. No transporter specific interaction was detected in any other experiments (BSEP, OCT1, OCT2, OAT3 and MATE1). The reference inhibitors confirmed the function of the transporters in the applied cells and vesicles (> 90% inhibition).

Table 2. Inhibition of human BSEP, OCT1, OCT2, OAT1, OAT3, and MATE1 transporters by GS-331007

Vesicular transport inhibition		
Transporter	Maximum inhibition (% of control)	IC ₅₀ (μM)
BSEP	no inhibition	NA
Uptake transporter inhibition		
Transporter	Maximum inhibition (% of control)	IC ₅₀ (μM)
OCT1	no inhibition	NA
OCT2	no inhibition	NA
OAT1	33%	> 100
OAT3	no inhibition	NA
MATE1	no inhibition	NA

2. Transporter substrate assays

Transporter specific accumulation of GS- 331007 into OCT1, OCT2, OAT1, OAT3 and MATE1 transporter-expressing cells was investigated at two concentrations (1 μM and 10 μM) and two time points (2 and 20 minutes). No OCT1, OCT2, OAT3 or MATE1 transporter specific accumulation was detected in any of the investigated conditions. In case of OAT1, the fold accumulation was 2.74 at 10 μM after 20 minutes incubation time. The experiment was repeated in the presence and absence of an inhibitor of OAT1 (200 μM benzbromarone) (Fig 1). The substrate nature of GS-331007 could not be confirmed in this experiment, as the fold accumulation was 1.39 and did not change in the presence of the inhibitor (1.44). The positive control experiments using known substrates confirmed the function of the transporters in the applied cells and vesicles.

Table 3. Fold-accumulation of GS-331007 in OCT1, OCT2, OAT3, MATE1 and OAT1 expressing cells

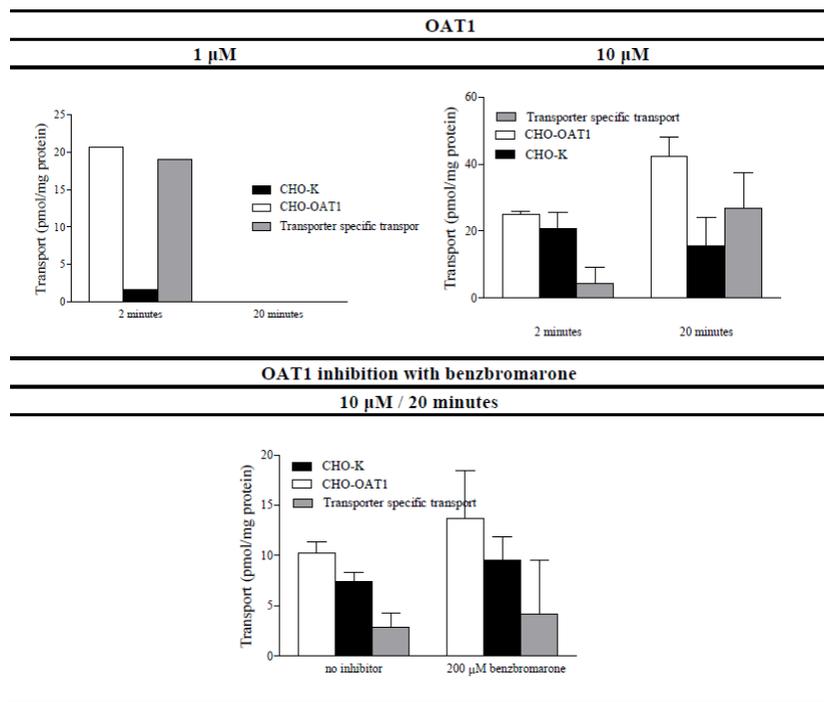
Condition (μM, min)	Fold-accumulation of GS-331007				
	OCT1	OCT2	OAT3	MATE1	OAT1
1 μM, 2 min	NA	NA	NA	NA	13.02 ^b
1 μM, 20 min	NA	0.58	NA	NA	NA
10 μM, 2 min	0.68	0.92	1.57	0.93	1.21
10 μM, 20 min	1.29	1.14	1.09	0.89	2.74 ^a
Fold-accumulation of positive controls ^c					
	22.7	9.9	11.7	13.2	3.4

a. The study was repeated with and without an inhibitor. The fold-accumulation obtained in the second experiment was 1.39 without an inhibitor and 1.44 with an inhibitor.

b. Only one of the triplicates could be measured. Therefore, this result is not reliable.

c. Metformin for OCT1, OCT2, and MATE-1, para-aminohippuric acid for OAT1, estrone-3-sulfate for OAT3

Fig 1. Accumulation of GS-331007 in OAT1 transporter expressing and control cell in the uptake transporter substrate assay



Conclusion

GS-331007 inhibited the OAT1-mediated PAH transport with a maximum inhibition of 33% at 100 μM (the highest tested concentration). No inhibition was observed for the other transporters tested (BSEP, OCT1, OCT2, OAT3 and MATE1). No OCT1, OCT2, OAT1, OAT3 or MATE1 transporter specific accumulation was detected in any of the investigated conditions.

Title: The NS5A inhibitor, BMS790052, has no effect on the uptake and metabolism of PSI-7977 in primary human hepatocytes (PC-PSI-7977-11-0002)

Objective: To determine if the NS5A inhibitor BMS790052 would inhibit the uptake and metabolism of PSI-7977 in primary human hepatocytes.

Methods

Primary human hepatocytes were incubated with 5 μM [¹⁴C]-PSI-7977 and increasing concentrations of BMS790052 (up to 50 μM). After 4 hour incubation, the cell layer was washed and trypsinized. Then intracellular fluid was collected by two-step centrifugation followed by concentration using SpeedVac

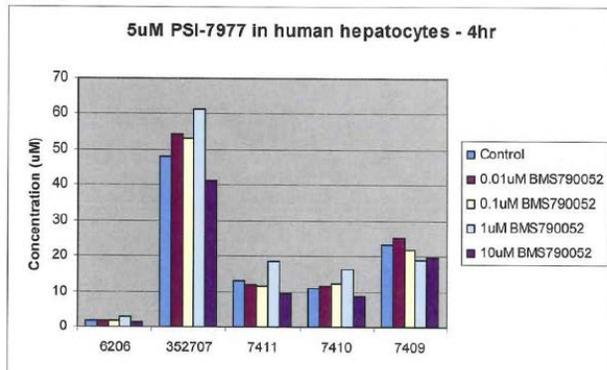
Results and Conclusion

The concentrations of intracellular metabolites of PSI-7977 (PSI-352707, PSI-7411, PSI-7410, and PSI-7419) were not changed in the presence of various concentrations of BMS-790052.

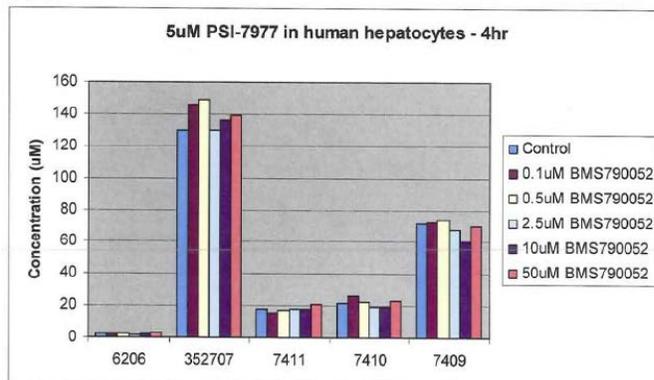
Therefore, BMS790052 did not appear to influence the uptake or intracellular metabolism of PSI-7977 in human hepatocytes.

Fig 1. Effect of BMS790052 on metabolism of PSI-7977 in human hepatocytes

Donor 1



Donor2



Title: Effect of either cytochrome P450 or HCV inhibitors on Triphosphate Formation in Primary Human Hepatocytes Following Incubation with GS-7977 (AD-334-2010)

Objective: To determine the effects of cytochrome P450 inhibitors (ketoconazole and ritonavir) or HCV inhibitors from different classes (GS-5885, GS-9451, GS-9190, GS-5861 and GS-9669) on the biotransformation of GS-7977

Methods

GS-7977 was incubated at 10 µM either alone or in combination with various cytochrome P450 inhibitors or HCV inhibitors for 2 hours in primary human hepatocytes. Following removal of extracellular media at select time points, cells were lysed, centrifuged, and supernatant was dried by MiniVac Duo concentrator. Dried samples were then reconstituted in mobile phase and analyzed by LC-MS/MS.

Results

Following 2 hour incubation similar levels of intracellular phosphorylated metabolites were observed when GS-7977 was incubated either alone or in combination with tested drugs (Table 1).

Table 1. Individual intracellular concentrations during a 2 hr continuous incubation with GS-7977 and inhibitors in primary human hepatocytes from a single donor.

Concentration (pmol/million) ^{a,b}						
GS compounds		GS-331007 (Nucleoside)	GS-606965 (Monophosphate)	GS-607596 (Diphosphate)	GS-461203 (Triphosphate)	GS-566500 (Intermediate Metabolite)
GS-7977	N=1	1.51	3.91	3.50	69.0	350
	N=2	2.13	3.9	3.47	70.5	327
GS-7977+GS-5885	N=1	2.04	5.54	3.01	47.7	305
	N=2	1.74	5.15	3.32	51.6	293
GS-7977+GS-9451	N=1	3.75	6.81	3.94	60.1	424
	N=2	2.38	5.62	3.47	54.4	351
GS-7977+GS-9190	N=1	2.93	5.7	3.10	53.4	315
	N=2	2.67	4.59	2.82	53.3	298
GS-7977+GS-5816	N=1	2.33	5.64	3.13	49.6	265
	N=2	1.92	6.35	3.10	49.3	294
GS-7977+GS-9669	N=1	0.897	3.69	2.97	52.1	305
	N=2	2.11	1.37	3.41	55.4	314
GS-7977+ritonavir	N=1	2.91	3.64	3.01	55.4	327
	N=2	3.99	3.44	3.41	61.7	330
GS-7977+ketoconazole	N=1	8.78	5.65	3.32	57.5	336
	N=2	8.78	5.9	3.60	62.8	322

a Intracellular concentration in μM can be estimated assuming an intracellular volume of 1 μL /million cells.

b Values represent the mean of duplicate wells.

Reviewer comments: Ketoconazole significantly increased the amount of GS-331007, the major circulating metabolite. However, the triphosphate concentration was the same compared to the control. This suggests that the concentration of GS-331007 may not reflect the concentration of the active metabolite.

Conclusion

Co-incubation of GS-7977 with either cytochrome P450 or HCV inhibitors did not cause a marked change in GS-7977 metabolism including the formation of the pharmacologically active triphosphate metabolite, GS-461203.

Title: Effect of HCV Inhibitors from Different Classes GS-5885, GS-9451, GS-9190, GS-5816 and GS-9669 on the Bidirectional Permeability of GS-7977 through Monolayers of Caco-2 Cells (AD-334-2002)

Objective: To assess the effect of HCV inhibitors from different classes on the bidirectional permeability of GS-7977 *in vitro* using a human colon carcinoma cell line (caco-2).

Methods

Caco-2 cell monolayers were cultured for twenty-one to twenty-eight days plated on 12 well Transwell® dual chamber plates. Apical side was dosed for apical-to-basolateral (A to B) assessment. Basolateral side was dosed for basolateral-to-apical (B to A) assessment. Donor side and receiver side were sampled at time points of 0 and 120 minutes and 60 and 120 minutes, respectively. GS-7977 concentrations were determined by LC/MS/MS.

Results

As summarized in Table 1, GS-7977 showed low apical to basolateral permeability (P_{app} of 0.25×10^{-6} cm/s) with efflux (efflux ratio = 43.6) in caco-2 cells when incubated alone at $10 \mu\text{M}$. Coincubation with CsA (cyclosporin A) or other HCV inhibitors increased the apical to basolateral permeability of GS-7977 and reduced the efflux ratio. Modest decreases (approximately 2-fold) in the efflux ratio were observed for GS-5885, GS-9190 and GS-9669. Moderate decreases (approximately 4-fold) in the efflux ratio were observed with GS-5816. CsA (a known p-gp inhibitor) and GS-9451 completely inhibited efflux transport.

Table 1. Effect of Cyclosporin A or HCV Inhibitors from Different Classes on the Bi-Directional Permeability of GS-7977 through Monolayers of Caco-2 Cells

Direction	Inhibitor	P_{app} (10^{-6} cm/s)					Efflux Ratio
		R1	R2	R3	R4	Avg.	
Forward	none	0.3	0.26	0.23	0.21	0.25	43.6
Reverse		10.8	10.8	9.61	12.3	10.9	
Forward	Cyclosporin A ($10 \mu\text{M}$)	0.3	0.27	0.65	0.62	0.46	1.4
Reverse		0.37	0.53	0.85	0.82	0.64	
Forward	GS-5816 ($1 \mu\text{M}$)	0.73	0.6	-	-	0.66	11.2
Reverse		7.22	7.56	-	-	7.39	
Forward	GS-5885 ($1 \mu\text{M}$)	0.21	0.49	-	-	0.35	22.9
Reverse		7.43	8.78	-	-	8.1	
Forward	GS-9190 ($10 \mu\text{M}$)	0.46	0.92	-	-	0.69	16.1
Reverse		10.5	11.7	-	-	11.1	
Forward	GS-9451 ($30 \mu\text{M}$)	0.78	1.41	-	-	1.09	1.1
Reverse		1.08	1.25	-	-	1.17	
Forward	GS-9669 ($100 \mu\text{M}$)	-	-	0.31	0.28	0.3	23.4
Reverse		-	-	7.05	6.82	6.93	

GS-7977 incubated at $10 \mu\text{M}$.

Conclusion

GS-7977 showed low permeability and efflux (efflux ratio = 43.6) through caco-2 cells when incubated alone at $10 \mu\text{M}$. Coincubation with HCV inhibitors decreased the efflux ratio of GS-7977, suggesting that the tested HCV inhibitors and cyclosporine A have the potential to increase the absorption of GS-7977 from the gastrointestinal tract due to their inhibition of GS-7977 efflux. In particular, efflux was nearly completely inhibited by cyclosporine A and GS-9451.

4.2.6 Pharmacogenomics Review (by Dr. Sarah Dorff)

OFFICE OF CLINICAL PHARMACOLOGY GENOMICS GROUP REVIEW

NDA/BLA Number	204671
Submission Date	04/08/2013
Applicant Name	Gilead Sciences Inc.
Generic Name	Sofosbuvir
Proposed Indication	Indicated in combination with other agents for the treatment of chronic hepatitis C (CHC) in adults
Primary Reviewer	Sarah Dorff, Ph.D.
Secondary Reviewer	Michael Pacanowski, Pharm.D., M.P.H.

1 Background

Sofosbuvir is an orally administered direct-acting antiviral agent (DAA) designed to inhibit the hepatitis C virus (HCV) non-structural 5B (NS5B) RNA dependent RNA polymerase via non-obligate chain termination during viral replication. The proposed indication is for use of sofosbuvir in combination with peginterferon and ribavirin (PEG+RBV) for individuals infected with HCV genotypes 1, 4, 5, and 6, or sofosbuvir in combination with ribavirin alone for HCV genotype 2 or 3 infections. Sofosbuvir is the first DAA proposed for HCV genotypes 2-6 and contains the first interferon-free regimen submitted for the treatment of HCV (in genotypes 2 and 3).

The single nucleotide polymorphism rs12979860 near the *IFNL3* (*IL28B*) gene encoding interferon-lambda 3 has been shown to be a strong predictor of sustained virologic response (SVR) in HCV genotype 1 patients receiving PEG+RBV-based therapies. However, the utility of *IFNL3* genotype in guiding treatment for genotypes 2-6 is less well established (Jimenez-Sousa et al., *BMC Med* 2013). The purpose of this review is to evaluate the influence of *IFNL3* genotype on response to sofosbuvir-containing regimens in the treatment of HCV and determine how information related to this impact should be addressed in labeling.

2 Submission Contents Related to Genomics

The development program for sofosbuvir consisted of 22 clinical trials including 4 Phase 2 trials that used *IFNL3* genotype as a stratification factor (P7977-0221, PROTON, ELECTRON, and ATOMIC). Subgroup analyses based on *IFNL3* genotype (rs12979860 CC vs. non-CC, as determined by PCR and allele specific probes) were performed in 4 pivotal Phase 3 studies (POSITRON, FISSION, FUSION, and NEUTRINO). The designs of the Phase 3 trials are summarized in table 1. Trial endpoint measurements were sustained virologic response at week 12 (SVR12) post-treatment.

Table 1. Phase 3 trials assessed for *IFNL3* genotype

Study	N	Genotype	Prior HCV Treatment	Treatment
P7977-1231 ^a (FISSION)	499	2, 3	Treatment-naïve	SOF+RBV for 12 weeks or PEG+RBV for 24 weeks
GS-US-334-0107 (POSITRON) ^b	280	2, 3	PEG intolerant, ineligible, or unwilling	SOF+RBV for 12 weeks or placebo for 12 weeks
GS-US-334-0108 (FUSION) ^a	202	2, 3	Treatment-experienced	SOF+RBV for 12 weeks or SOF+RBV for 16 weeks
GS-US-334-0110 (NEUTRINO) ^c	327	1, 4, 5, 6	Treatment-naïve	SOF+PEG+RBV for 12 weeks

SOF: sofosbuvir; RBV: ribavirin; PEG: peginterferon.

^a 3 subjects in FISSION and 6 subjects in FUSION were found to be genotype 1 and were excluded.

^b 9% of POSITRON study subjects were treatment-experienced (PEG intolerant), 43.5% were PEG ineligible, and 47.5% were PEG unwilling, respectively.

^c genotype 1a N = 255, genotype 1b N = 66, 1a/1b N = 1, genotype 4 N = 28, genotype 5 N = 1, genotype 6 N = 6

The sponsor has proposed descriptive labeling regarding the effect of *IFNL3* genotype on SVR12 rates in genotype 1 treatment-naïve subjects.

3 Key Questions and Summary of Findings

3.1 Does *IFNL3* genotype influence SVR rates to sofosbuvir-containing regimens similarly across HCV genotypes 1-6?

IFNL3 genotype is predictive of response to sofosbuvir treatment in subjects with genotype 1 infection, but not in those with HCV genotype 2, 3, or 4 infection. Insufficient data are available with regard to the effect of *IFNL3* genotype in HCV genotypes 5 and 6.

3.1.1 Distribution of *IFNL3* genotype (rs12979860) by trial

The frequency of *IFNL3* genotypes is summarized in Table 2. The study populations were predominantly white (87.2% for FISSION, 91.4% for POSITON, 86.6% for FUSION, 78.6% for NEUTRINO). The proportion of individuals with the *IFNL3* CC genotype was similar among treatment-naïve genotype 2 and 3 subjects at 54.6% and 57.0%, respectively (including PEG ineligible, intolerant or unwilling subjects). However, treatment-experienced subjects had a lower proportion of the more responsive *IFNL3* CC genotype (26.4% and 30.7% for genotypes 2 and 3, respectively) compared to treatment-naïve subjects, which is consistent with prior studies showing enrichment of non-CC genotype in treatment-experience HCV genotype 1 trials. NEUTRINO enrolled only treatment-naïve subjects, and the proportion of individuals with the *IFNL3* CC genotype was lower (29.1%) compared to treatment-naïve subjects with genotypes 2 and 3. The distribution of *IFNL3* genotype was not balanced across the randomized treatment arms.

Table 2. Distribution of *IFNL3* genotype by trial

<i>IFNL3</i> genotype, N (%)	FISSION		POSITRON		FUSION		NEUTRINO ^a
	Treatment-Naïve		PEG Ineligible, Intolerant, Unwilling		Treatment-Experienced		Treatment-Naïve
	SOF+RBV 12 weeks	PEG+RBV 24 weeks	SOF+RBV 12 weeks	Placebo 12 weeks	SOF+RBV 12 weeks	SOF+RBV 16 weeks	SOF+PEG+RBV 12 weeks
Genotype 1							
							N = 292
CC	-	-	-	-	-	-	86 (29.5%)
CT	-	-	-	-	-	-	158 (54.1%)
TT	-	-	-	-	-	-	48 (16.4%)
Genotype 2							
	N = 70	N = 67	N = 109	N = 34	N = 36	N = 32	
CC	31 (44.3%)	34 (50.7%)	45 (41.3%)	17 (50.0%)	7 (19.4%)	11 (34.4%)	-
CT	31 (44.3%)	22 (32.8%)	50 (45.9%)	14 (41.2%)	18 (50.0%)	19 (59.4%)	-
TT	8 (11.4%)	11 (16.4%)	14 (2.8%)	3 (8.8%)	11 (30.6%)	2 (6.3%)	-
Genotype 3							
	N = 183	N = 176	N = 98	N = 37	N = 64	N = 63	
CC	75 (41.4%)	72 (41.1%)	52 (53.1%)	12 (32.4%)	23 (35.9%)	16 (25.4%)	-
CT	89 (49.2%)	76 (43.4%)	34 (34.7%)	22 (59.5%)	33 (51.6%)	37 (58.7%)	-
TT	17 (9.4%)	27 (15.4%)	12 (12.2%)	3 (8.1%)	8 (12.5%)	10 (15.9%)	-
Genotype 4							
							N = 28
CC	-	-	-	-	-	-	4 (14.3%)
CT	-	-	-	-	-	-	21 (75.0%)
TT	-	-	-	-	-	-	3 (10.7%)

SOF: sofosbuvir; RBV: ribavirin; PEG: peginterferon.

^a HCV genotype 5 (N = 1, *IFNL3*: CT) and HCV genotype 6 (N = 6, *IFNL3*: 5 CC, 1 CT).

Data Source Tables: m5.3.5.1, P7977-1231 (FISSION), Section 15.1, Table 4.2; GS-US-334-0107 (POSITRON), Section 15.1, Ad Hoc Table 4.1; GS-US-334-0108 (FUSION), Section 15.1, Ad Hoc Table 4.1; m5.3.5.1, GS-US-334-0110 (NEUTRINO), Section 15.1, Table 3.

*Reviewer comment: Several imbalances in the distribution *IFNL3* genotype could complicate interpretation of the trial data if this factor influences responses.*

3.1.2 SVR12 by *IFNL3* genotype

3.1.2.1 Results in HCV Genotype 2 Infection

Subjects with HCV genotype 2 treated with sofosbuvir for 12 weeks had SVR12 rates of at least 85% across all *IFNL3* genotype subgroups (Table 3); this was consistent across all patient populations (e.g., treatment-naïve and treatment-experienced). Treatment-naïve subjects in exhibited SVR12 rates that approached 100%. Subjects who were treatment-experienced tended to have lower SVR12 rates compared to treatment-naïve subjects. In this trial, subjects with the non-CC genotype tended to have higher SVR12 rates with 16 weeks of treatment; a similar trend would have been expected in CC subjects, although the sample size is too small to draw meaningful conclusions. Overall, no consistent trends for differences in SVR12 were observed based on *IFNL3* CC versus non-CC genotype in subjects infected with HCV genotype 2.

3.1.2.2 Results in HCV Genotype 3 Infection

In general, subjects having HCV genotype 3 infection had lower SVR12 rates compared to other HCV genotypes (Table 3). SVR12 was achieved in only ~56% of subjects who were treatment naïve and received sofosbuvir for 12 weeks. In addition, *IFNL3* CC subjects had higher SVR12 after traditional treatment with PEG+RBV compared to treatment with sofosbuvir (75.0% vs. 57.3%). Genotype 3 CC subjects treated with the PEG-free regimen tended to have higher SVR12 rates than non-CC subjects. The rate of SVR12 was lower in treatment-experienced subjects, with SVR12s of 25-40% after 12 weeks of sofosbuvir treatment. Similarly, non-CC subjects tended to have lower response rates. However, treatment in this group with sofosbuvir for 16 weeks increased SVR12 to 62% regardless of *IFNL3* genotype, rates similar to those observed in treatment-naïve subjects. Overall, non-CC subjects tended to have lower SVR rates, although no consistent relationship between *IFNL3* genotype and SVR12 was observed in genotype 3 infected HCV subjects.

3.1.2.3 Results in HCV Genotype 1, 4, 5, 6 Infection

Response to sofosbuvir treatment in HCV genotype 1, 4, 5, or 6 infections exceeded 87.1% in the treatment-naïve trial. *IFNL3* non-CC subjects had significantly lower SVR12 rates ($p = 0.007$; Table 3), which was driven primarily by differences in subjects with genotype 1 (89% of the population). Nearly all subjects with HCV genotype 4 infection achieved SVR12 after treatment with sofosbuvir. All subjects with genotypes 5 and 6 did achieve SVR12, however, the small number of individuals preclude conclusions regarding *IFNL3* in these subgroups. These results are consistent with previous findings of the influence of *IFNL3* genotype on HCV genotype 1 treatment. The rate of SVR12 in non-CC subjects was similar to non-CC subjects treated with other recently approved DAAs (boceprevir or telaprevir).

Table 3. SVR12 rates by HCV genotype, *IFNL3* genotype, treatment arm, and trial.

<i>IFNL3</i> genotype, n/N (%)	FISSION			POSITRON			FUSION			NEUTRINO
	Treatment-Naïve			PEG Ineligible, Intolerant, Unwilling			Treatment-Experienced			Treatment-Naïve
	SOF+RBV 12 weeks	PEG+RBV 24 weeks	Prop Diff (95% CI)	SOF+RBV 12 weeks	Placebo 12 weeks	Prop Diff ^b (95% CI)	SOF+RBV 12 weeks	SOF+RBV 16 weeks	Prop Diff (95% CI)	SOF+PEG+RBV 12 weeks
Genotype 2										
	N = 70	N = 67		N = 109	N = 34		N = 36	N = 32		
CC	31/31 (100%)	28/34 (82.4%)	17.6% (4.7%- 34.5%)	40/45 (88.9%)	0/17 (0.0%)	88.9% (79.6%- 98.1%)	6/7 (85.7%)	9/11 (81.8%)	3.9% (-43.0%- 43%)	-
95% CI	88.8%- 100.0%	65.5%- 93.2%		75.9%- 96.3%	0.0%- 19.5%		42.1%- 99.6%	48.2%- 97.7%		-
Non-CC	37/39 (94.9%)	24/33 (72.7%)	21.2% (5.1%- 41.0%)	61/64 (95.3%)	0/17 ^a (0.0%)	95.3% (90.0%- 100.0%)	25/29 (86.2%)	21/21 (100.0%)	-13.8% (-31.7%- 2.9%)	-
95% CI	82.7%- 99.4%	54.5%- 86.7%		86.9%- 99.0%	0.0%- 19.5%		68.3%- 96.1%	83.9%- 100.0%		-
Genotype 3										
	N = 183	N = 176		N = 98	N = 37		N = 64	N = 63		
CC	43/75 (57.3%)	54/72 (75.0%)	-17.7% (-32.6%- 2.2%)	34/52 (65.4%)	0/12 ^a (0.0%)	65.4% (52.3%- 78.4%)	9/23 (39.1%)	10/16 (62.5%)	-23.4% (-53.0%- 10.4%)	-
95% CI	45.4%- 68.7%	63.4%- 84.5%		50.9%- 78.0%	0.0%- 26.5%		19.7%- 61.5%	35.4%- 84.8%		-
Non-CC	59/106 (55.7%)	55/103 (53.4%)	2.3% (-11.6%- 15.8%)	26/46 (56.5%)	0/25 (0.0%)	56.5% (42.0%- 71.0%)	10/41 (24.4%)	29/47 (61.7%)	-37.3% (-55.5%- 15.3%)	-
95% CI	45.7%- 65.3%	43.3%- 63.3%		41.1%- 71.1%	0.0%- 13.7%		12.4%- 40.3%	46.4%- 75.5%		-
Genotype 1,4,5,6										
										N = 327
CC	-	-	-	-	-	-	-	-	-	93/95 (97.9%)
95% CI	-	-	-	-	-	-	-	-	-	92.6%- 99.7%
Non-CC	-	-	-	-	-	-	-	-	-	202/232 (87.1%)
95% CI	-	-	-	-	-	-	-	-	-	82.1%- 91.1%

Prop Diff: difference in proportions; SOF: sofosbuvir; RBV: ribavirin; PEG: peginterferon.

^a one subject discontinued treatment and was imputed by reviewer as a treatment failure according to sponsor's treatment of subjects with missing SVR12 data.

^b difference in proportions computed by reviewer according to POSITRON statistical analysis plan formula: m5.3.1.5, Section 6.1.3.

Data Source Tables: m5.3.5.3, ISE, Table 3; m5.3.5.1, P7977-1231 (FISSION), Section 15.1, Table 8.5, GS-US-334-0107 (POSITRON), Section 15.1, Ad Hoc Table 8.2.1; GS-US-334-0108 (FUSION), Section 15.1, Ad Hoc Tables 38.1 and 38.2; m5.3.5.3, ISE, Table 2; m5.3.5.1, GS-US-334-0110 (NEUTRINO), Section 15.1, Table 7.2. POSITRON placebo and prop diff calculated by reviewer from 'adefeff' data file.

4 Summary and Conclusions

The applicant evaluated the role of *IFNL3* genotype on SVR12 in subjects infected with HCV. *IFNL3* genotype was associated with modestly lower SVR12 rates in treatment-naïve subjects with genotype 1, 4, 5, or 6 infection after 12 weeks of combination treatment with sofosbuvir, peginterferon, and ribavirin. However, even among non-CC's, SVR12 rates were similar to those previously observed with triple drug regimens containing telaprevir or boceprevir. These results should be depicted in labeling.

Based on recent meta-analyses of published literature, the effect of *IFNL3* genotype on treatment response in HCV genotypes 2 and 3 is less pronounced than in genotype 1. While some of the study populations showed trends toward lower SVR12 rates in non-CC subjects (i.e., treatment-naïve genotype 2, PEG-intolerant/ineligible/unwilling genotype 3, treatment-experienced genotype 3), no consistent correlation was found between *IFNL3* genotype and the rate of SVR12. Despite the lack of a consistent effect of *IFNL3* genotype in these settings, it is reasonable to include these results in labeling in a manner that is consistent with other baseline prognostic factors.

Treatment-experienced genotype 3 subjects treated with sofosbuvir for 16-weeks had higher SVR12 rates compared to the 12-week regimen. It is reasonable to suspect that longer treatment durations would benefit treatment-naïve genotype 3 patients as well, considering that the 12-week sofosbuvir regimen did not produce better response rates than PEG+RBV, at least among CC subjects. Among treatment-experienced genotype 2 subjects, 16 weeks of treatment also produced higher SVR12 rates, although the same trend was not observed among CC subjects because of the small sample size. Though not directly studied, patients who have poor baseline characteristics that are more likely to fail the first course of treatment (with PEG) could benefit from a 16-week sofosbuvir-containing regimen. However, it does not appear that *IFNL3* genotype alone would be sufficient to identify patients that would benefit from longer treatment durations.

Several imbalances between treatment arms were noted for the distribution of *IFNL3* genotypes. However, it does not seem that *IFNL3* has a major influence on SVR12 for the studied regimens, and as such, it is unlikely to significantly affect interpretation of the trial results.

5 Recommendations

The Genomics and Targeted Therapy Group recommends that SVR12 rates be depicted according to *IFNL3* genotype for HCV genotypes 1-4.

5.1 Post-marketing studies

None.

5.2 Labeling Recommendations

Pending.

4.2.7 Pharmacometrics Review (by Dr. Jeffry Florian)

OFFICE OF CLINICAL PHARMACOLOGY:
PHARMACOMETRIC REVIEW

Application Number	NDA 204671
Submission Number (Date)	April 9, 2013
Drug Name	Sofosbuvir
Proposed Indication	In combination with other agents for the treatment of chronic hepatitis C (CHC) in adults
Clinical Division	DAVP
Primary CP Reviewer	Jenny Zheng, Ph.D.
Primary PM Reviewer	Jeffry Florian, Ph.D.
Secondary CP Reviewer	Shirley Seo, Ph.D.
Secondary PM Reviewer	Yaning Wang, Ph.D.
Sponsor	Gilead Sciences

1 SUMMARY OF FINDINGS

1.1 Key Review Questions

The purpose of this review is to address the following key questions.

1.1.1 Is there evidence of an exposure-response efficacy relationship for sofosbuvir (SOF) or GS-331007 exposure and sustained virologic response at week 12 of follow-up in genotype 1, genotype 2, or genotype 3 subjects?

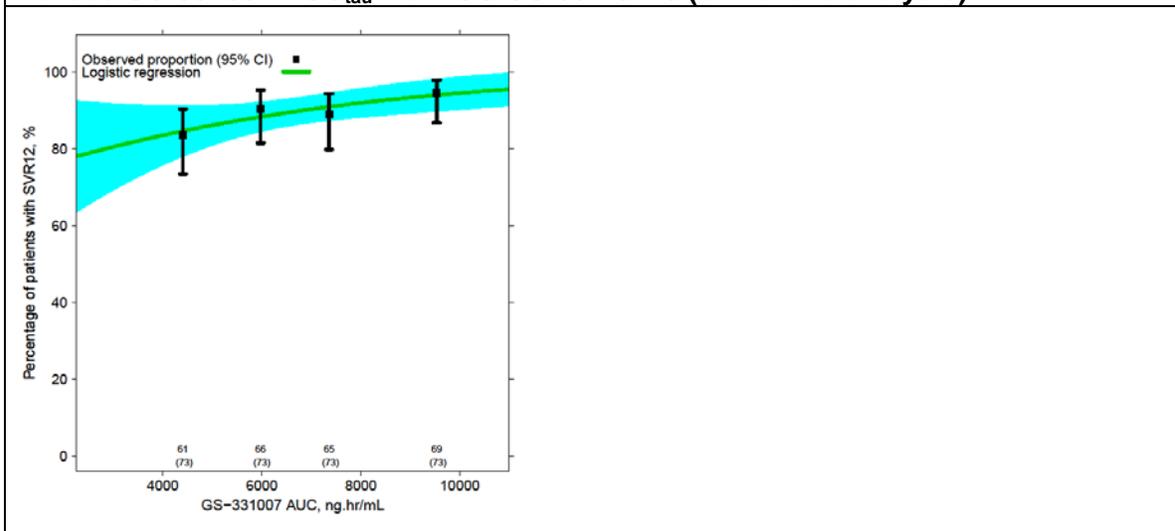
Exposure-response analyses were based on SOF and GS-331007 (primary circulating metabolite; inactive) AUC_{tau} for genotype 1, genotype 2, and genotype 3 subjects based on data from the following Phase III trials: GS-US-334-0108 (treatment-experienced genotype 2/3, SOF/RBV for 12 or 16 weeks), GS-US-334-0110 (treatment naïve genotype 1, SOF/RBV/PEG for 12 weeks), and P7977-1231 (treatment-naïve genotype 2/3, SOF/RBV for 12 weeks). The primary endpoint evaluated in these analyses was sustained virologic response at week 12 of follow-up (SVR12). Also evaluated were various on-treatment virologic assessments at week 1, 2, and 4 of treatment based on the percentage of subjects with virologic measurements not detected. These analyses are limited as neither SOF or GS-331007 are the active moiety, the sparse sampling in the Phase III trials, and only a single dose was evaluated in the Phase III trials. Additional data from multiple doses or on exposures for the active moiety would be required to better identify the role of SOF dose on the exposure-response relationships.

Genotype 1

Univariate analysis of the results from GS-US-334-0110 (n=292 genotype 1 subjects with pharmacokinetic data) identified an exposure-response relationship between GS-331007 AUC_{tau} and SVR12 (Figure 1), but no relationship between SOF AUC_{tau} and SVR12. Subjects with GS-331007 in the lowest exposure quartile had an SVR12 rate of 84% compared to 95% in the highest exposure quartile. Similar analyses performed based on on-treatment virologic response at week 1, 2, and 4, however, indicated that the percentage of subjects with virologic measurements not detected were more likely in S0000

those subjects in the lowest exposure quartile (8%, 52%, and 88%, respectively) compared to subjects in the highest exposure quartile (1%, 36%, and 84%, respectively). In addition, multivariate analysis including GS-331007 AUC_{tau} as well as other predictive factors such as genotype subtype, cirrhosis status, IL28B genotype (CC versus non-CC), RBV dose (mg/kg), resulted in rejection of GS-331007 AUC_{tau} as a significant predictor for SVR12. Finally, as displayed above in the metabolic pathway, GS-331007 is the end-step metabolite from SOF and it is uncertain how GS-331007 exposures may be related to concentrations of the active triphosphate compound. Altogether, while an exposure-response efficacy relationship was identified for GS-331007, it cannot be concluded that subjects on the lower range of exposures observed in GS-US-334-0110 would be less likely to have a response compared to subjects in the highest exposure quartile.

Figure 1: Percentage of Patients Achieving Sustained Virologic Response (SVR12) Versus GS-331007 AUC_{tau} from GS-US-334-0110 (univariate analysis)



Genotype 2

Exposure-response analyses using SOF and GS-331007 AUC_{tau} were conducted for genotype 2 subjects based on the data from P7977-1231 (treatment-naïve, n=70) and GS-US-334-0108 (treatment-experience; SOF/RBV 12 weeks: n=36; SOF/RBV 16 weeks: n=32). Due to the small number of subjects in each of these treatment arms numeric comparisons were performed between subjects above and below the median SOF and GS-331007 AUC_{tau}. In the treatment-naïve study there was no difference in response between subjects below (97% [34/35]) and above (97% [34/35]) the median GS-331007 exposures (7000 ng-hr/mL). Twenty-seven of the 70 genotype 2 subjects did not have SOF data available from sparse sampling. These subjects were excluded from the binned analysis for SOF AUC_{tau} with respect to SVR. Similar to the observations for GS-331007 AUC_{tau} there was no difference in response between those subjects above (95% [21/22]) and below (95% [20/21]) the median SOF AUC_{tau} (757.8 ng-hr/mL). In the treatment experienced study, numeric trends were observed based on GS-331007 AUC_{tau} for 12-weeks (below median: 83% [15/18]; above median: 88% [16/18]) and 16-weeks (below median: 88% [14/16]; above median: 100% [16/16]). There were only 25 genotype 2 subjects with SOF AUC_{tau} available in the 12-week and 16-week treatment arms, so no analyses were performed with respect to SOF AUC_{tau}. Similar to the conclusions for genotype 2 treatment-naïve subjects, the small sample size and low number of treatment failures hinders interpreting these numeric trends as a result of GS-S0000

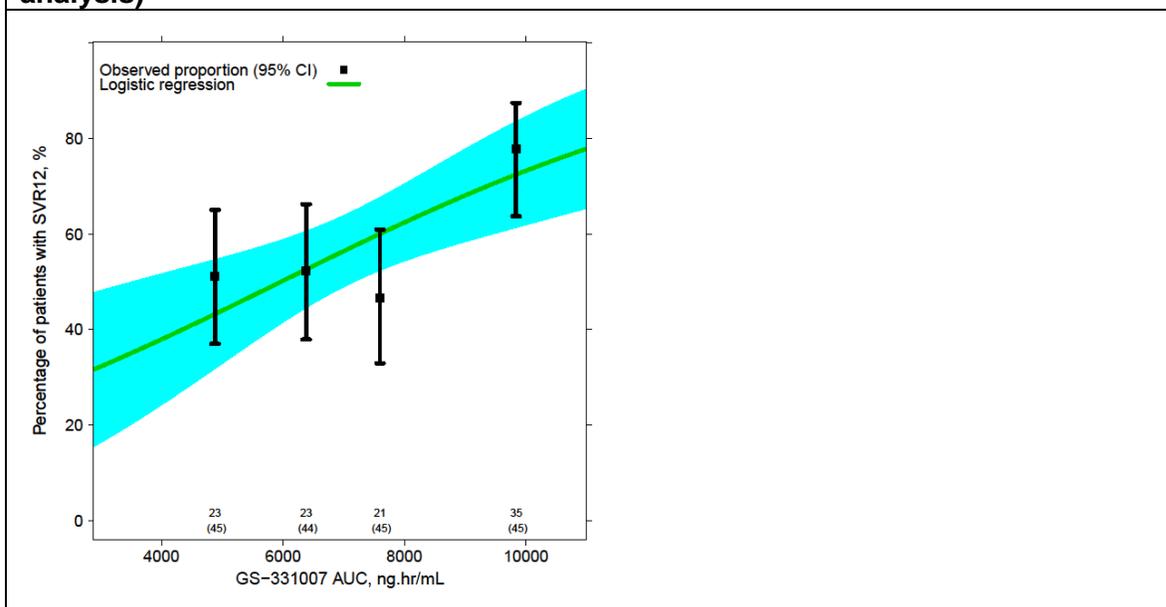
GS-331007 exposures. Finally, no clear relationship between GS-331007 AUC_{tau} and on-treatment virologic response at week 1, 2, or 4 could be determined from the available data for either treatment-naïve or treatment-experienced genotype 2 subjects.

Genotype 3

Exposure-response analyses using SOF and GS-331007 AUC_{tau} was conducted for genotype 3 subjects based on the data from P7977-1231 (treatment-naïve, n=179) and GS-US-334-0108 (treatment-experience; SOF/RBV 12 weeks: n=64; SOF/RBV 16 weeks: n=63).

Univariate analysis of the results from P7977-1231 identified exposure-response relationship between GS-331007 AUC_{tau} and SVR12 (Figure 2). The response in the lowest quartile for GS-331007 AUC_{tau} was 51% compared to 78% in the highest quartile. Similar to the results for genotype 1 subjects, multivariate analysis of factors impacting genotype 3 response (IL28B, cirrhosis, RBV mg/kg, baseline viral load) resulted in removal of GS-331007 as a significant predictor of response. No relationship was identified between SOF AUC_{tau} and SVR12. Furthermore, no relationship was identified between SOF or GS-331007 AUC_{tau} and on-treatment virologic response at week 1, 2 and 4.

Figure 2: Percentage of Genotype 3 Patients Achieving Sustained Virologic Response (SVR12) Versus GS-331007 AUC_{tau} (left) from P7977-1231 (univariate analysis)

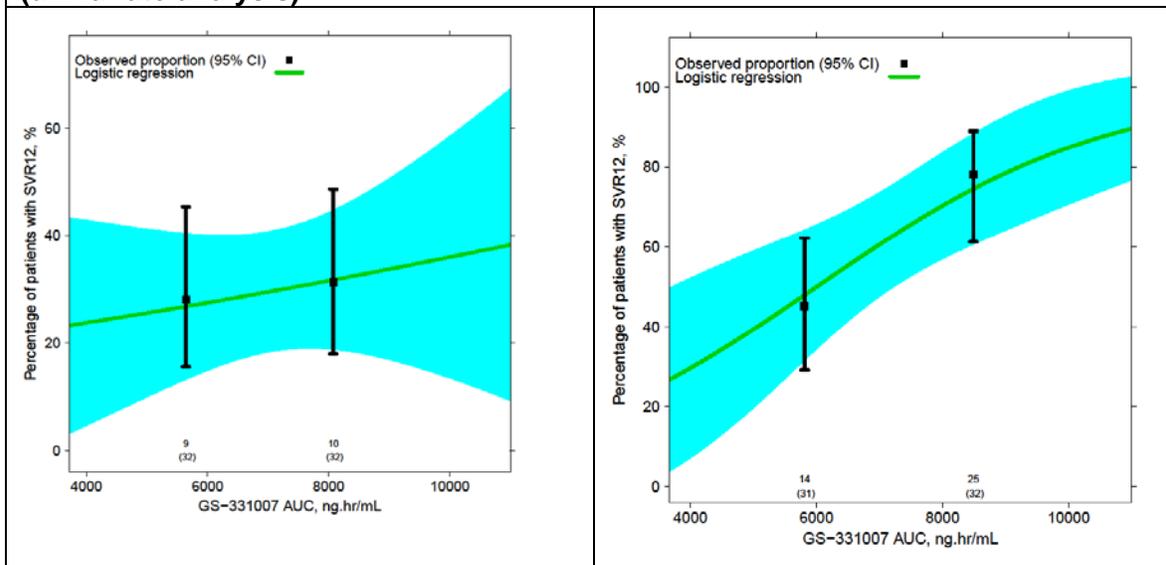


GS-331007 AUC_{tau} was also identified as a significant factor for response in treatment-experienced subjects administered SOF/RBV 16-weeks (Figure 3, right) but not for SOF/RBV 12-weeks (Figure 3, left). SVR12 in subjects with GS-331007 exposures less than the median (7062 ng·hr/mL) was 28% and 45% for 12- and 16-weeks compared to 31% and 78% in subjects with exposures above the median. Multivariate analysis retained GS-331007 AUC_{tau} as a predictor of response, but this was primarily driven by the higher response rate observed for 16-weeks in subjects with exposures above the median (response rate in subjects below the median had only modest improvement). These observations, as well as the lack of any on-treatment differences in virologic

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response at week 1, 2, or 4 with respect to GS-331007 AUC_{tau} suggest that treatment duration may be a confounding factor for this exposure response relationship analysis. In addition, the increase in SVR12 rate with 16-week treatment duration compared to 12-week treatment duration in those subjects with exposures above the median GS-331007 AUC_{tau} supports that longer treatment durations in this population may result in higher SVR response rates, particularly those subjects with GS-331007 exposure below the median.

Figure 3: Percentage of Genotype 3 Patients Achieving Sustained Virologic Response (SVR12) Versus GS-331007 AUC_{tau} for 12-weeks (left) and 16-weeks (univariate analysis)



1.1.2 Is there evidence of an exposure-response safety relationship for SOF or GS-331007 exposure and common adverse events or cardiovascular adverse events?

Two separate exposure-response safety analyses were conducted based on: i) a pooled analysis of Phase III subjects administered SOF 400 mg and RBV in GS-US-334-0108, GS-US-334-0107, and P7977-1231; and ii) subjects administered SOF 400 mg, RBV, and pegylated interferon in GS-US-334-0110.

In each of these pooled population, exposure-response relationships could not be identified for the most common adverse events observed during the Phase III SOF trials (e.g., headaches, diarrhea, nausea). Logistic regression models were evaluated for SOF and GS-331007 AUC_{tau} and no significant relationships were identified.

Exposure-response safety analyses were also evaluated for dyspnea and system organ class cardiac disorders to identify if the SOF or GS-331007 exposures from the Phase III trials were associated with any cardiac adverse events. This analysis was based on the pooled Phase III population and identified that any grade dyspnea and any grade cardiac events were more likely in subjects with higher GS-331007 exposures. However, the significance of these adverse events relationships should be interpreted with caution. First, the overall number of cardiac events in the Phase III population administered SOF was 19 out of 991 patients with PK data available (6 of 327 in SOF/PEG/RBV [1.8%] and 13 of 664 in SOF/R [1.9%]). This event rate was lower than the cardiac event rate observed the P/R control arm from P7977-1231 (11 of 243 [4.2%]). In addition, the

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adverse event listings under this system organ class were predominantly grade 1 and include palpitations, tachycardia, bradycardia, and ventricular extrasystoles (see review by the Medical Officer, Dr. Poonam Mishra for additional details). These adverse events could also be confounded by concomitant administration of RBV to all patients during the Phase III trials which are known to cause anemia. Additional analyses looking for associations between creatinine kinase elevations exceeding the upper limit of normal (>336 U/L for males and >176 U/L for females) and SOF or GS-331007 exposures also did not demonstrate any drug-exposure association with these elevations.

Exposure-response safety analyses could not be performed for either pegylated-interferon or RBV as pharmacokinetic data for these compounds were not collected during the Phase III trials. However, a weight-base exposure-response safety analysis was performed for the two pooled populations described above to assess whether increased mg/kg RBV dosing was associated with increased likelihood of anemia.

Anemia adverse events occurred in 6.1% (10 of 163) of subjects administered SOF/RBV with the lowest mg/kg RBV dosing (6.4-12.6 mg/kg) compared to 14.4% (24 of 166) in subjects with the highest mg/kg RBV dosing (15-20 mg/kg). RBV dose reductions were also more frequent in the quartile with the highest mg/kg RBV dosing (15 of 166 [9.0%]) compared to the lowest quartile (8 of 163 [4.9%]). These trends remain despite the use of the weight based RBV dosing approved for genotype 1 subjects (1000 mg for body weight <75 kg and 1200 mg for body weight >75 kg) in the Phase III trials rather than the approved 800 mg RBV dose for use with genotype 2 and 3 subjects (in combination with pegylated interferon).

A similar relationship between RBV mg/kg dosing and both anemia and RBV dose reduction was observed in GS-US-334-0110 where genotype 1, 4, 5, and 6 subjects (twenty-eight genotype 4, one genotype 5, and six genotype 6 subjects were included in GS-US-334-0110) were administered SOF/RBV/PEG. Anemia adverse events (9 of 82 [11.0%]) and RBV dose reductions (8 of 82 [9.8%]) were less frequent in the lowest mg/kg RBV dosing quartile (7-12 mg/kg) compared to anemia adverse events (22 of 82 [26.8%]) and RBV dose reductions (19 of 82 [23.2%]) in the highest mg/kg RBV dosing quartile (15-20 mg/kg). The increase in anemia event rate in GS-US-334-0110 compared to the genotype 2/3 Phase III trials is likely due to the addition of PEG with SOF/RBV in GS-US-334-0110.

1.1.3 Do the exposure-response efficacy relationships in genotype 2 and genotype 3 subjects support the use of RBV at 1000/1200 mg based on body weight?

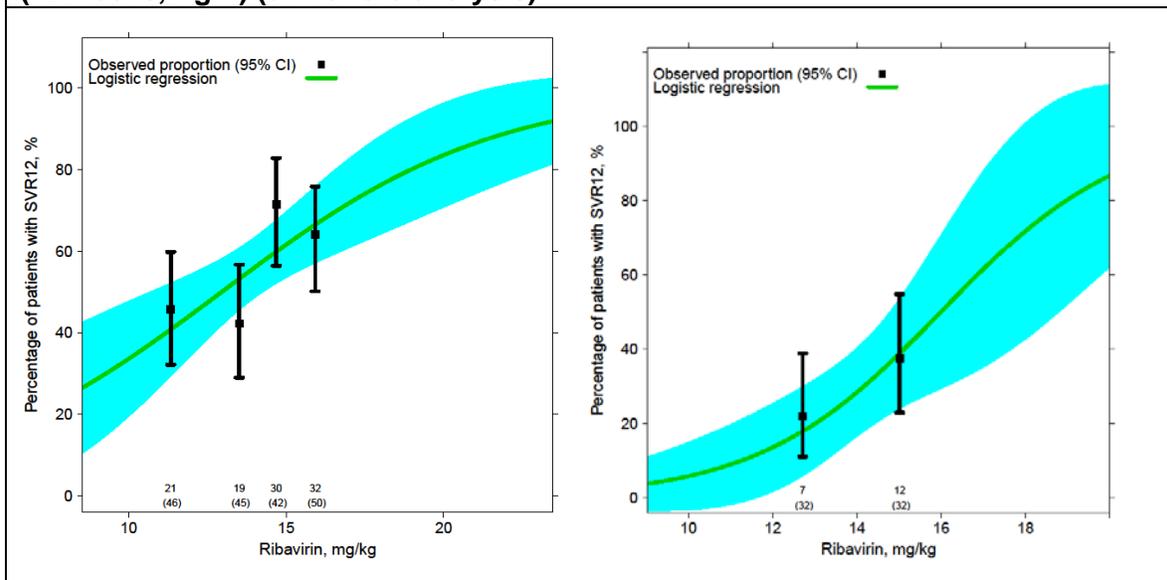
The exposure-response relationships for genotype 3 subjects from P7977-1231 and GS-US-334-108 support the use of RBV 1000/1200 mg based on body weight in this population. Due to the overall small number of genotype 2 subjects in these two studies, the high SVR rate (described above in question 1.1.3), and as the RBV efficacy relationship is limited to using mg/kg RBV dose rather than RBV concentration (data was not collected), it is inconclusive whether a lower dose of RBV may have resulted in a similar SVR rate in genotype 2 subjects. However, as the adverse events observed in the genotype 2/3 Phase III trials were manageable through RBV dose reduction, there is no evidence necessitating the use of a lower RBV dose in this population.

RBV concentration data was not collected in these studies, so instead RBV dose adjusted for body-weight was evaluated against SVR12 in genotype 3 subjects from P7977-1231. Univariate and multivariate analyses demonstrate that weight-based RBV was significant associated with increased likelihood of SVR12 (odds ratio of 1.20 for a 1

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mg/kg increase in RBV; p=0.03) (Figure 4). This relationship was also supported by the 12-week treatment results from GS-US-334-0108 (odds ratio 1.27 for a 1 mg/kg increase in RBV dose; p-value=0.002), but not the 16-week treatment results from GS-US-334-0108. The reason for these diverging results may be interplay between exposure and treatment duration that cannot be deconvoluted given the available treatment data.

Figure 4: Percentage of Genotype 3 Patients Achieving Sustained Virologic Response (SVR12) Versus RBV Mg/kg from P7977-1231 (left) and GS-US-334-0108 (12 weeks, right) (univariate analysis)



The appropriateness of the 1000/1200 mg RBV dose is further illustrated by comparing SVR12 and anemia rates in genotype 3 subjects from P7977-1231, grouped by RBV mg/kg dosing bands. RBV dose was adjusted based on body weight, with subjects weighing less than 75 kg receiving RBV 1000 mg and subjects weighing 75 kg or more receiving RBV 1200 mg. RBV mg/kg was calculated using the RBV dose each subject was administered dividing by the subject's body weight. Subjects receiving a higher RBV mg/kg dose performed better regardless of the subject's original RBV dose category. For example, the SVR12 rate was 52% in subjects administered RBV 1000 mg whose weight-based RBV dose was between 8.5 - <14.1 mg/kg. In contrast, the SVR12 rate was 70% in subjects administered the same RBV dose, but whose weight-based RBV dose was 14.1 - <23.5 mg/kg (Table 1, left). As expected, anemia was also more likely in those subjects receiving a higher weight-based RBV dose (Table 1, right)

Table 1: SVR12 (left) and Anemia Rate (right) in Genotype 3 Subjects from P7977-1231, Grouped by RBV Dose and Weight-based RBV Dose

SVR12 % (n/N)		RBV dose		Anemia %, (n/N)		RBV dose	
		1000 mg	1200 mg			1000 mg	1200 mg
RBV Dose	8.5 - <14.1 mg/kg	52% (11/21)	43% (29/68)	RBV Dose	8.5 - <14.1 mg/kg	5% (1/21)	1% (1/68)

	14.1 <23.5 mg/kg	-	70% (33/47)	64% (28/44)		14.1 <23.5 mg/kg	-	6% (3/47)	7% (3/44)
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It should be noted that for a fixed RBV dose of 800 mg, 95% of the genotype 3 subjects from P7977-1231 (173 of 183) would have had a weight-based RBV dose <14.1 mg/kg and 24% (44 of 183) would have had a weight-based RBV dose <8.5 mg/kg. Given the retention of weight-based RBV dose in the final efficacy model as well as the observed trend in SVR12 with respect to RBV mg/kg dose, the use of 1000/1200 weight-based dosing of RBV in genotype 3 subjects is supported.

A similar table for genotype 1 subject (GS-US-334-0110, SOF/PEG/RBV) shows similar trends of increased SVR12 in subjects administered receiving higher weight-based RBV (Table 2). However, it should be noted that RBV 1000/1200 mg is approved for this genotype and was used in this trial.

Table 2: SVR12 (left) and Anemia Rate (right) in Genotype 1 Subjects from GS-US-334-0110, Grouped by RBV Dose and Weight-based RBV Dose

SVR12 % (n/N)		RBV dose		Anemia %, (n/N)		RBV dose	
		1000 mg	1200 mg			1000 mg	1200 mg
RBV Dose	7.2 - <13.7 mg/kg	86% (12/14)	82% (107/130)	RBV Dose	7.2 - <13.7 mg/kg	14% (2/14)	15% (19/130)
	13.7 - <20.0 mg/kg	99% (68/69)	94% (73/78)		13.7 - <20.0 mg/kg	30% (21/69)	21% (16/78)

Given the above analyses and the overall positive results from the Phase III trials where RBV 1000/1200 mg was evaluated, the reviewer recommends continued use of RBV 1000/1200 mg in this population.

1.1.4 Does the data in genotype 3 treatment-experienced subjects from GS-US-334-0108 support the use of 16-weeks SOF/RBV in genotype 3 treatment-naïve subjects?

Yes, data in support of a 16-week treatment duration in genotype 3 treatment-naïve subjects comes from three sources: i) empirical results that extending the treatment duration from 12- to 16-weeks in genotype 3 treatment-experienced subjects increased SVR12; ii) logistic regression modeling provided by the sponsor; and iii) a bridging analysis involving odds ratio calculations performed by the statistics reviewer. For additional details on the third item, please see the review by Dr. Qi.

i) GS-US-334-0108 Genotype 3 Results

The sponsor evaluated two treatment durations (12-weeks versus 16-weeks) in genotype 3 subjects in GS-US-334-0108. The results indicated an improvement in SVR from 30% (19/64) to 62% (39/63) by extending the treatment duration from 12-weeks to 16-weeks. As previous analyses have demonstrated that PEG/RBV treatment-experienced subjects are represented within the treatment-naïve population, and the treatment-naïve SVR12 rate was 56% (102/183), which can be approved upon, it is appropriate to utilize a 16-week treatment duration in genotype 3 treatment-naïve subjects.

ii) Multivariate Model GS-US-334-0108 and P7977-1231

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Multivariate analysis of results from these two trials identified cirrhosis status, baseline HCV RNA, treatment duration, sex, weight-based RBV dose, and IL28B as factors predictive of response. A combined multivariate model was fit to data from both trials. Next, the model was used to predict SVR12 rate in the treatment-naïve subjects under the assumptions that the relationships identified for treatment-experienced subjects would be similar in treatment naïve populations and that the treatment-duration in treatment-naïve subjects was extended to 16-weeks. The predicted SVR12 rate in the genotype 3 subjects if administered 16-weeks of SOF/RBV was 81%. This was similar to the predicted SVR12 rate from the applicant's analyses based on Bayesian logistic regression (81% without cirrhosis interaction; 78% with cirrhosis interaction)

iii) Bridging analysis based on odds ratios

A bridging analysis was conducted by the statistics reviewer, Dr. Qi. Briefly, the reviewer assumed a constant odds ratio comparing 12-weeks and 16-weeks in genotype 3 treatment-experienced subjects was the same as the odds ratio for 12-weeks and 16-weeks in treatment-naïve subjects. The equation for this calculation is shown below:

$$\frac{P_{TN,16}/(1 - P_{TN,16})}{P_{TN,12}/(1 - P_{TN,12})} = \frac{P_{TE,16}/(1 - P_{TE,16})}{P_{TE,12}/(1 - P_{TE,12})}$$

For the observed SVR12 rates in treatment-experienced subjects (12-weeks: 30%; 16-weeks 62%) and the observed SVR12 rate in treatment-naïve subjects (12-weeks: 56%), this results in a calculated SVR12 rate for treatment-naïve subjects administered 16-weeks of 83% (95% CI of 69-92%).

1.1.5 Does the data in genotype 1 treatment-naive subjects from GS-US-334-0110 support the use of 12-weeks SOF/PEG/RBV in genotype 1 treatment-experienced subjects?

Yes, data supporting the efficacy of 12-weeks SOF/PEG/RBV in genotype 1 treatment-experienced subjects is available from a: i) previous analyses demonstrating that P/R non-responders are represented within the treatment-naïve population; ii) subset analysis of difficult to treat subjects in GS-US-334-0110 based on baseline characteristics; and iii) a comparison of response rates across in prior null responders, prior partial responders, and a treatment-naïve subjects with multiple poor baseline predictive factors from multiple HCV programs.. Attempt at predicting the response of SOF/PEG/RBV in prior null responders using a logistic regression model describing results of GS-US-334-0110 and a representative demographic of prior null responders from previous submissions (dataset of prior treatment status, IL28B genotype, baseline viral load, cirrhosis status, gender, and body weight) resulted in an over-estimate of the SVR12 rate (80% (95% CI: 73-86%). The applicant has not evaluated any genotype 1 prior P/R-nonresponders with SOF/PEG/RBV in their development program, and the basis for extending the indication to include prior P/R-nonresponders would be based on the following three items:

i) P/R nonresponders represented within the treatment-naïve population

Previous experience with the treatment-naïve population and the treatment-experienced population (Florian J et al. *Hepatology* 2012, Liu J et al. *Clinical Infectious Diseases* 2012, and Liu J et al. *Hepatology* 2012) has indicated that the P/R nonresponders were putatively included in the treatment-naïve population. In general, about 50% of subjects in treatment-naïve trials fail treatment with PEG/RBV and are subsequently classified as

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P/R-nonresponders. Therefore, treatment-naïve response rates in excess of 50% are evidence that a DAA+PEG/RBV regimen is effective in patients who would be classified as P/R-nonresponders. Given the overall high SVR rate in GS-US-334-0110 (89-90%), which is similar or higher than the SVR rate in other approved HCV regimens that also are effective in P/R-nonresponders, it is highly likely that SOF/PEG/RBV is also effective in P/R-nonresponders with similar or higher effect size. However, this rationale does not provide an estimate of the response rate in specific difficult to treat subgroups. To address this question, two additional analyses were performed based on difficult to treat subjects from GS-US-334-0110 and previously submitted HCV programs.

ii) Subset of more difficult to treat subjects from GS-US-334-0110

Less likely to response subjects, characterized as subjects with the following baseline factors that are predictive of reduced response (e.g., with baseline non-CC IL28B genotype, higher baseline HCV RNA, and cirrhosis) in the treatment-naïve population could be considered as putative P/R-nonresponder. The SVR12 rates in these subjects with multiple baseline factors associated with reduced likelihood of response was 71% (37/52; 95%CI: 57-83%) and exceeds the PEG/RBV response rate previously observed for null and partial responders (5-10%, and 15-20%, respectively).

A within study comparison of this result is not possible as GS-US-334-0110 did not include a PEG/RBV control arm. Similarly, a comparison between this calculated response rate in a putative treatment-naïve subset and response rates in P/R nonresponders could not be performed as P/R-nonresponders were not evaluated with SOF/PEG/RBV.

Table 3: Comparison of SVR12 Rate From GS-US-334-0110 Among Subjects with Various Baseline Predictors of Response

Baseline Factor		SOF/PEG/RBV	
		SVR12 (n/N)	%
IL28B	CC	98% (84/86)	
	non-CC	86% (177/206)	
Baseline HCV RNA	Baseline HCV RNA < 800K	96% (47/49)	
	Baseline HCV RNA > 800K	88% (214/243)	
Metavir Score	F0-F2	91% (128/141)	
	F3-F4	82% (68/83)	
	Missing	96% (65/68)	
Cirrhosis Status	Cirrhotic	81% (42/52)	
	Non-cirrhotic	91% (216/237)	

	Missing	100% (3/3)
non-CC/High baseline VL/F3-F4		71% (37/52)

68 subjects had neither fibrosis staging information nor were listed as cirrhotic, 65 were treatment responders and 3 were treatment failures.

Of the three treatment failures, all were non-CC and had baseline viral load >800K IU/mL. Two subjects stopped treatment at week 2 (adverse event, protocol violation) and one stopped treatment at week 6 (withdrew consent). Including these three subjects as failures results in an estimated SVR12 of 71% (37/52). Thirty of the subjects missing fibrosis staging who responded to treatment were non-CC and high viral load subjects.

- iii) Subset of more difficult to treat subjects from GS-US-334-0110 and previous submissions

Analogous analyses were performed for other recent hepatitis C submissions, and summary results are shown in **Table 4**. Using a similar analysis among treatment-naïve subjects with poor baseline predictive factors, SOF administered with PEG/RBV for 12-weeks may have a similar response rate to other approved hepatitis C drugs. While there is uncertainty regarding a precise SVR12 rate for SOF/PEG/RBV in prior P/R-nonresponders, it is likely that SOF would be effective in prior P/R-nonresponders.

Table 4: Comparison of SVR12 Rate From Various Direct-Active Antiviral Treatment-Naïve and Treatment-Experienced Studies. The SVR Rates Shown for the Treatment-Naïve Studies are Based on the Subset of Subjects with Multiple Poor Baseline Predictive Factors (non-CC, baseline viral load >800K, F3 or F4 fibrosis staging)

Drug	Treatment-naïve		Prior Null	Prior Partial	Prior Null	Prior Partial
	DAA/PEG/RBV	PEG/RBV	DAA/PEG/RBV	PEG/RBV		
Telaprevir	43% (31/72) [BID]	46% (30/66) [TID]		32% (47/147)	59% (57/97)	5% (2/37) 15% (4/27)
Boceprevir (P05101; P06806)	43% (10/23)	-	20% (1/5)	38% (20/52)	46% (53/115)	- 7% (2/29)
Simeprevir (C208/216 combined)	51% (36/73)	-	8% (3/38)	49% (49/101)	66% (91/137)	19% (3/16) 9% (2/23)
Sofosbuvir (0110)	71% (37/52)	-	-	-	-	-

It should be noted that while the above analyses provide supportive evidence that SOF/PEG/RBV is likely to be effective in P/R-nonresponders, it is not sufficient to determine the optimal regimen in such patients. In particular, subjects with HCV RNA >25 IU/mL at week 2 were less likely to achieve SVR (80%, 20/25), compared to subjects with HCV RNA <25 IU/mL but detectable (87%, 108/124) or HCV RNA not detected (94%, 131/140). Subjects in the putative analysis above (n=49; this analysis S0000

does not include the three subjects with missing fibrosis data), were more likely to have HCV RNA >25 IU/mL (14%; 7/49) or <25 IU/mL detected (59.2%, 29/49) compared to the other genotype 1 subjects in GS-US-334-0110 (7.5% [18/240] with HCV RNA >25 IU/mL and 39.6% [95/240] with HCV RNA <25 IU/mL detected). As the treatment failures were predominantly relapses, the above data suggests that longer treatment duration in subjects included in the putative analysis, and similarly, subjects who are prior null or prior partial responders may benefit from longer SOF/PEG/RBV treatment durations (i.e., 16 weeks).

1.1.6 Does the data from the SOF Phase III trials demonstrate concordance between SVR12 and SVR24 assessments in interferon-containing (PEG/RBV or SOF/PEG/RBV) and interferon-free (SOF/RBV) regimens?

Yes, the available data from two of the sponsor’s trials (P7977-1231 and GS-US-334-0107) supports that assessments at week 12 of follow-up (SVR12) and week 24 of follow-up (SVR24) were concordant for SOF/RBV and PEG/RBV treatment in genotype 2 and 3 subjects. SVR24 data was unavailable from GS-US-334-0108 or GS-US-334-0110, so no concordance assessments could be performed for those two trials.

Data from P7977-1231 (SOF/RBV and PEG/RBV) and GS-US-334-0107 (SOF/RBV and placebo) was assessed for concordance between SVR12 and SVR24 assessments. Of the 253 subjects from the SOF/RBV treatment arm in P7977-1231, SVR12 and SVR24 data was available from 238 subjects. Fifteen subjects were excluded from the analysis due to missing data (n=10) or imputation of the SVR12 response as successful (n=5). Of the 243 subjects from the PEG/RBV treatment arm in P7977-1231, SVR12 and SVR24 data were available from 91 subjects (144 subjects with missing data and 8 subjects with imputation of SVR12 as successful). Of the 207 subjects from the SOF/RBV treatment arm in GS-US-334-0107, SVR12 and SVR24 data was available from 195 subjects (12 subjects with missing data).

Summary tables for concordance between SVR12 and SVR24 for these treatment arms are shown below in Table 5. Overall, assessments at week 12 of follow-up were concordant with those at week 24 of follow-up. Two subjects on SOF/RBV from P7977-1231 were categorized as responders based on SVR12 but subsequently relapsed by SVR24 (one relapse at follow-up week 16 and one relapse at follow-up week 20). Likewise, there was one subject classified as failing to achieve SVR12 who had HCV RNA not detectable at a subsequent visit and was classified as achieving SVR24. Results from the P7977-1231 PEG/RBV treatment arm showed a high positive predictive value, but the available data was limited. The GS-US-334-0107 SOF/RBV treatment arm showed 100% concordance between SVR12 and SVR24 in those subjects with both SVR12 and SVR24 data available. The positive predictive value based on data from the SOF/RBV treatment arm in P7977-1231 was 98.8% (161/163). The overall positive predictive value between SVR12 and SVR24 in SOF/RBV treatment arms was 99.4% (314/316). This data shows that the use of SVR12 as the primary endpoint in interferon-free trials may be reasonable, but it will continue to be evaluated as additional data from other interferon-free regimens becomes available.

Table 5: Concordance Between SVR12 and SVR24 from P7977-1231 and GS-US-334-0107 Treatment Arms

P7977-1231 (SOF/RBV)		
P7977-1231, SOF/RBV	SVR24	
	Yes	No

SVR12	Yes	161	2
	No	1	74

P7977-1231 (PEG/RBV)

P7977-1231, PEG/RBV		SVR24	
		Yes	No
SVR12	Yes	21	1
	No	0	69

GS-US-334-0107 (SOF/RBV)

GS-US-334-0107, SOF/RBV		SVR24	
		Yes	No
SVR12	Yes	153	0
	No	0	42

1.2 Recommendations

- Based on the outcome of the pivotal trials in genotype 2 subjects and the above analyses, a regimen of 12-weeks SOF combined with RBV 1000/1200 mg, based on body weight, is recommended for approval in treatment-naïve and treatment-experienced subjects. Patients with poor baseline predictive factors, such as cirrhosis, may benefit from longer treatment duration (i.e., 16-weeks).
- Based on the outcome of the pivotal trials in genotype 3 subjects and the above analyses, a regimen of 16-weeks SOF combined with RBV 1000/1200 mg, based on body weight, is recommended for approval in treatment-naïve and treatment-experienced subjects. The available data does not rule out that even longer treatment durations (i.e., 24 weeks) may provide additional benefit.
- Based on the outcome of the pivotal trial in genotype 1 subjects and the above analyses, a regimen of 12-weeks SOF/PEG/RBV is recommended for approval in treatment-naïve subjects. The bridging analyses shown above suggest that SOF would have at least similar effectiveness in prior P/R-nonresponders as other approved regimens. However, the available data is not sufficient to determine the optimal treatment duration in difficult-to-treat treatment-naïve subjects or prior null/partial responders, nor is the information sufficient to obtain a precise estimate of the anticipated SVR rate in prior null and partial responders.

1.3 Label Statements

See the clinical pharmacology review above for labeling comments.

2 PERTINENT REGULATORY BACKGROUND

Sofosbuvir is a novel nucleotide prodrug that inhibits hepatitis C virus (HCV) ribonucleic acid (RNA) replication in vitro and rapidly suppresses HCV RNA, and when used in combination with ribavirin (RBV) with or without pegylated interferon (PEG), results in high sustained virologic response (SVR) rates in subjects with chronic HCV infection.

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The proposed indication for SOF is for use in combination with other agents for the treatment of chronic hepatitis C (CHC) in adults. Pending regulatory approval, SOF will be the first nucleotide prodrug for the treatment of CHC infection. The recommended oral dose of SOF is one 400-mg tablet once daily with or without food. The sponsor has submitted 5 studies to support the use of SOF in genotype 1, 2, 3, 4, 5, and 6 subjects, as well as the treatment of chronic hepatitis C adults awaiting transplant

3 RESULTS OF SPONSOR'S ANALYSIS

3.1 Introduction

The applicant developed a population pharmacokinetic model to explore the impact of intrinsic and extrinsic factors on SOF and GS-331007 exposure in healthy volunteers and HCV-infected subjects. In addition, SOF and GS-331007 pharmacokinetic parameters were used by the applicant to explore exposure-response efficacy and safety relationships in treatment-naïve and treatment-experienced HCV-infected subjects (genotype 1 [only treatment-naïve], genotype 2, and genotype 3).

3.2 Population PK Model (SOF)

Report 5.3.3.5 Population Pharmacokinetic of Sofosbuvir

The objectives of the analysis were: 1) to build a population PK model of SOF; and 2) to evaluate the effect of physiologic and demographic covariates on the PK of SOF.

3.2.1 Data

Data from a total of 14 studies, including 7 in healthy subjects (P7977-0111, GS-US-334-0131, P7977-0613, P7977-0814, P7977-0915, P7977-1318, and P7977-1819; n = 296) and 7 studies in hepatitis C virus (HCV)-infected subjects (GS-US-334-0107 (POSITRON), GS-US-334-0108 (FUSION), GS-US-334-0110 (NEUTRINO), P2938-0212 (NUCLEAR), P7977-0221, P2938-0515, and P7977-1231 (FISSION), n = 1078). Data from a total of 1374 subjects were used in the analysis (**Table 6**).

Table 6: Studies used in the population pharmacokinetic analysis of SOF
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Study	Population	Phase	Sampling (Intensive/Sparse)	Number of Subjects Included in Population PK Analyses (N = 1374) ^a
GS-US-334-0131	Healthy	1	Intensive	88
P7977-0111	Healthy	1	Intensive	24
P7977-0613	Healthy	1	Intensive	60
P7977-0814	Healthy	1	Intensive	15
P7977-0915	Healthy	1	Intensive	30
P7977-1318	Healthy	1	Intensive	40
P7977-1819	Healthy	1	Intensive	39
P2938-0212	HCV-Infected	1	Intensive	24
P2938-0515	HCV-Infected	1	Intensive	17
P7977-0221	HCV-Infected	2	Intensive + Sparse	49
P7977-1231	HCV-Infected	3	Sparse	254
GS-US-334-0107	HCV-Infected	3	Sparse	206
GS-US-334-0108	HCV-Infected	3	Sparse	201
GS-US-334-0110	HCV-Infected	3	Sparse	327

a Numbers include subjects with all values below the limit of quantitation (BLQ) for SOF.

Sponsors SOF population PK report, pg 10

Demographics and a summary of subject covariates are summarized below in **Table 7**

Table 7: Summary of Demographics and Covariates in the SOF Population PK Analysis

Covariate	Average (\pm SD)	Min–Max	n
Age	47.4(\pm 12.5)	18–78	1374
Gender	-	-	873 (Male), 501 (Female)
Race	-	-	1129 (White), 167 (Black), 44 (Asian), 34 (Other)
Ethnicity	-	-	264 (Hispanic or latino), 1109 (Non-hispanic or non- latino)
Patient status	-	-	296 (Healthy subjects), 1078 (HCV patients)
Body weight (kg)	82.7(\pm 17.8)	42.6–162	1374
BMI (kg/m ²)	28.1(\pm 5.2)	17.3–56.1	1374
BSA (m ²)	2.0(\pm 0.2)	1.3–2.9	1374
Creatinine Clearance ^a (mL/min)	116(\pm 32.9)	12.2–281	1373
eGFR ^b (mL/min/1.73m ²)	89.2(\pm 20.1)	6.6–180.4	1373
Cirrhosis	-	-	204
Concomitant Medications			
Proton Pump Inhibitors	-	-	195
H2 Receptor Antagonists	-	-	51
Calcium Channel Blockers	-	-	87
Statins	-	-	52
Diuretics	-	-	113
SSRIs	-	-	164
Anticoagulants	-	-	137

a Estimated by Cockcroft-Gault formula
b Estimated Glomerular Filtration Rate (MDRD formula)
Note: Subjects for whom demographic information was unavailable were not included in the covariate model selection.

Sponsors SOF population PK report, pg 19

3.2.2 Methods

A mixed effect modeling approach using NONMEM v.7.2 software was applied in analyzing pooled SOF concentration-time data. Various structural PK models as well as inclusion of interindividual variability (IIV) terms on structural model parameters were tested during all model-building processes. Different error models to best describe any residual error were tested as well. The best base model was chosen based on graphical examinations, the accuracy and meaningfulness of parameter estimates, and a drop of more than 10.8 (equal to a significance level of 0.001) in the objective function value (OFV) as provided by NONMEM v.7.2.

First order with conditional estimates (FOCE) method implemented in NONMEM v.7.2. Covariates considered for analysis included age, gender, race, body weight, body mass index, body surface area, formulation, combination therapy (PEG+RBV or RBV), treatment duration, creatinine clearance, eGFR, hepatic impairment, cirrhosis, healthy versus HCV-infected, and concomitant medication. Decision of the testing of a S0000

characteristic as a covariate was based on examination of the graphs as well as existence of a scientific rationale for a potential effect. Those subject characteristics deemed to be significantly correlated with a model parameter were then formally tested as covariates in the model. The performance of the model was checked with graphical inspection of the model fits, visual and numerical predictive checks, and bootstrapping for confidence intervals of population PK parameters.

Different datasets were used during the model building process. Log-transformed concentration data (Dataset p12v2.xpt) were used to develop the base model. Upon the availability of data from the Phase 3 studies, this data set was expanded to include all available data (Dataset p123v3.xpt) and used in the final model building steps.

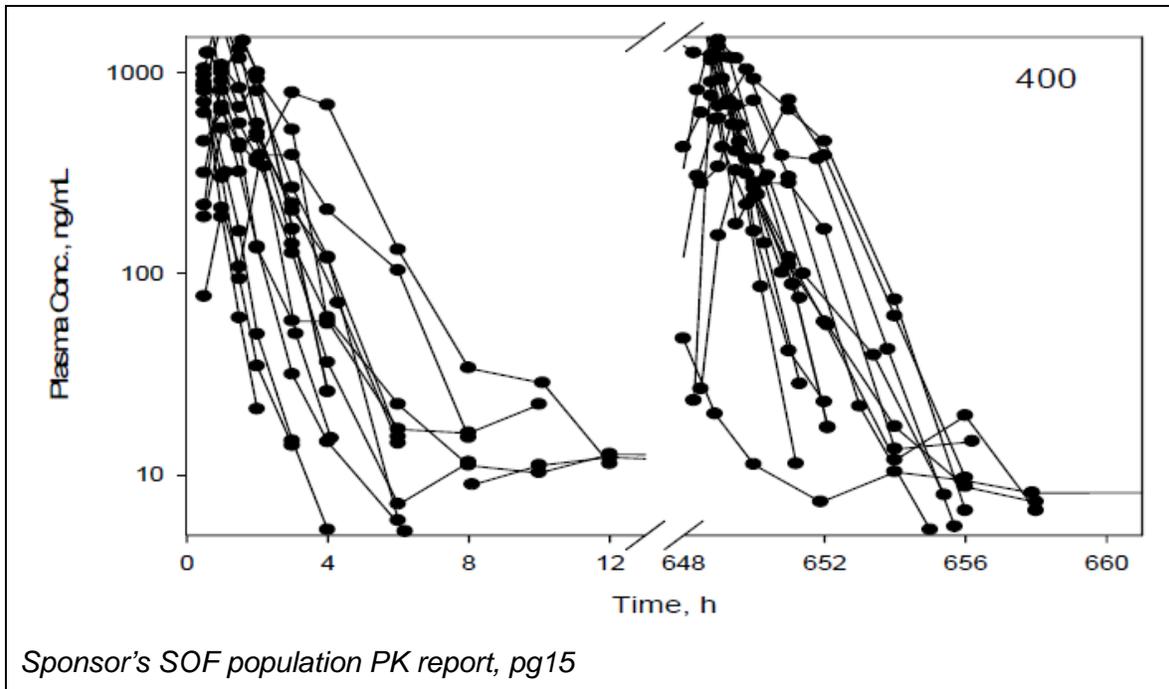
Following successful model validation, the final model was used to simulate SOF exposures in 1372 subjects from all 14 studies. Plasma SOF concentrations were simulated at prespecified time points (0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, 20, and 24 hours postdose) at steady state for each subject in each study assigning a dose of 400 mg. Noncompartment analysis (NCA) was performed on the simulated SOF concentrations using WinNonLin v.5 (Pharsight Corp. Sunnyvale, CA) and estimates of AUC_{tau} and C_{max} values were generated. In the Phase 3 studies, 197 subjects had no measurable SOF plasma concentration across all study visits; therefore, since the only covariate in the final model was patient status, these subjects were all assigned the typical value in the population for parameter estimates

3.2.3 Results

3.2.3.1 Individual SOF Plasma Concentration Time-Course

Sofosbuvir concentration versus time data for a representative study (P7977-0221) is shown in Figure 5.

Figure 5: SOF Plasma Concentrations Versus Time After Single and Multiple Doses of SOF 400 mg in P7977-0221 (n-15)



A 1-compartment PK model with first order absorption rate constant and absorption lag time provided a good description of the PK of SOF in both healthy and HCV-infected subjects. An effect of patient status (HCV-infected versus healthy subjects) on the oral clearance (CL/F) and absorption rate (KA) was observed and included in the final model. Final parameters estimates are listed in Table 8 and goodness of fit plots are displayed in Figure 6.

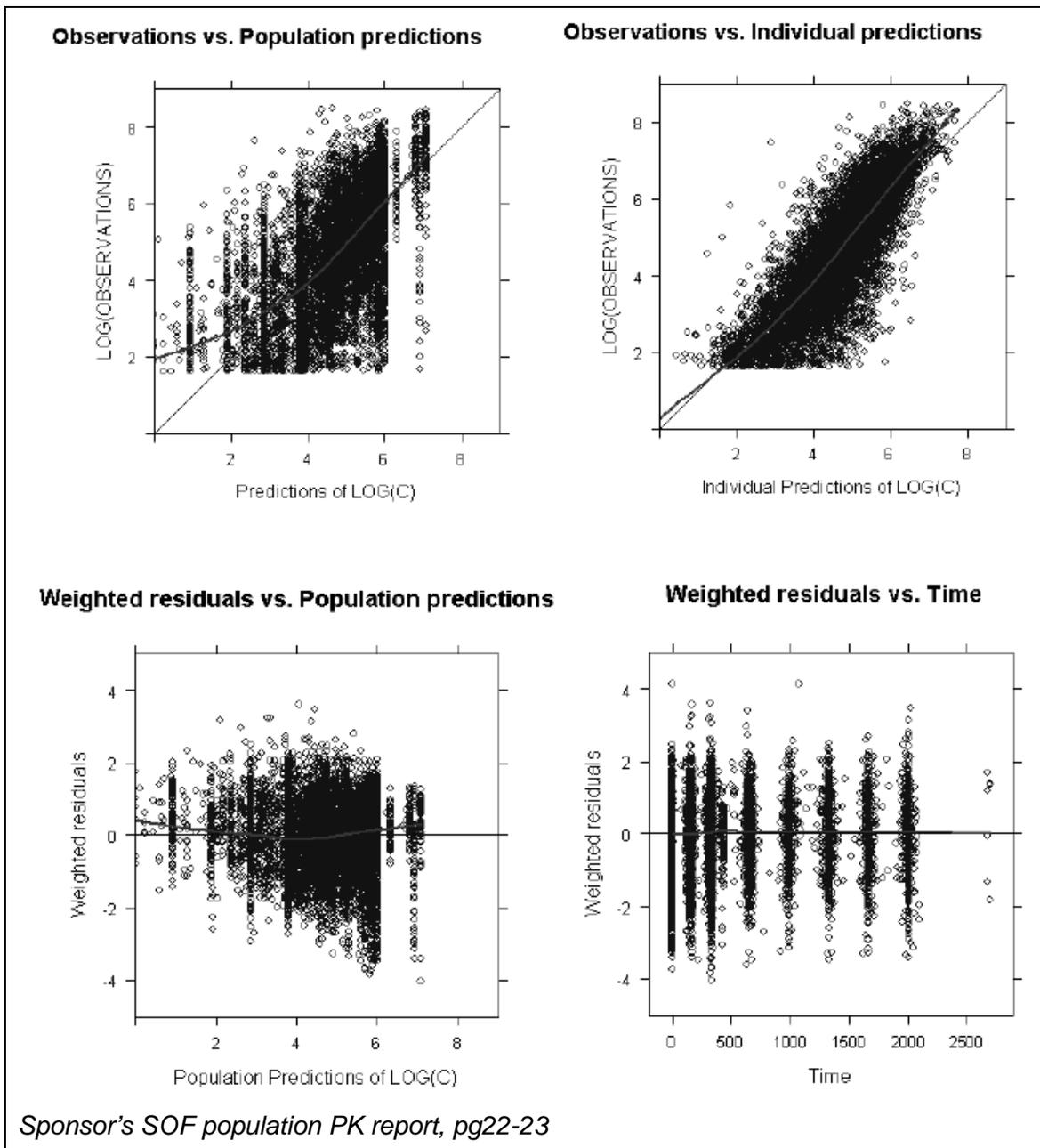
Table 8: SOF Population PK Parameters from the Final Model

Parameter	Estimate (RSE%)	IIV (RSE%)
CL/F (L/h)	652 (3.0)	0.507 (10.9)
V/F (L)	127 (26.5)	1.73 (43.8)
KA (h ⁻¹)	0.96 (4.0)	0.437 (16.1)
TLAG (h)	0.1 (27.9)	-
PAT on CL/F	-165 (16.4)	-
PAT on KA	-0.303 (13.9)	-
σ ² (proportional)	0.95 (3.0)	-

IIV: interindividual variability, CL/F: oral clearance, V/F: apparent volume of distribution KA: first-order absorption rate constant from the transit to the central compartment, TLAG: lag-time for first-order absorption process, σ²: variance of proportional error term. RSE values were obtained through bootstrapping (n=360).

Sponsors SOF population PK report, pg 21

Figure 6: Goodness of Fit Plots for the final SOF Population PK Model



The typical CL/F of SOF was estimated as 652 L/hr with an IIV of 0.51. The typical V/F of the central compartment was estimated as 127 L with an IIV of 1.73. SOF absorption was modeled as a first-order absorption rate (k_a) of 0.96 h⁻¹ with an IIV of 0.44. In addition, a lag-time of 0.1 hr was included in the final model.

A statistically significant, positive correlation was observed between patient status (healthy volunteer versus HCV-infected) and SOF CL/F and k_a . The effect was described with a linear function with a slope population estimate of -0.165. Inclusion of this covariate resulted in only a slight decrease in the IIV for SOF k_a and CL/F. No other covariates were identified as having clinically relevant effects on SOF PK.

3.2.3.2 Noncompartmental Analysis of Simulation Results for SOF

The final model was used to simulate steady-state SOF concentrations after 20 daily doses of 400 mg SOF in all subjects and a NCA analysis was performed on simulated concentrations. The predicted exposures from subjects in GS-US-334-0107, GS-US-334-0108, GS-US-334-0110, and P7977-1231 were used for a subsequent PK/PD analysis

Population PK estimates of SOF C_{max} were underestimated by approximately 40% compared to intensive sampling studies; of note, AUC was well predicted by population PK analyses. Data from Phase 1 and 2 studies have shown little evidence of a relationship between SOF C_{max} and safety parameters given the low and transient exposure of SOF. As such, the primary parameter for interpretation of data from population PK analyses for SOF was AUC_{tau}.

In the Phase 3 studies, 197 subjects had no measurable SOF plasma concentration across all study visits, therefore, since the only covariate in the final model was patient status, these subjects were all assigned the typical value in the population for parameter estimates (CL/F, V/F, and KA), which resulted in all 197 subjects having the same estimate of SOF AUC_{tau} and C_{max}. PK/PD (efficacy) analyses for SOF were conducted including and excluding these subjects.

Covariate analysis revealed that patient status (HCV-infected vs. healthy subjects) affects the CL/F and KA of SOF. Mean (%CV) SOF AUC_{tau} in Phase 3 study HCV infected subjects was 36% higher than healthy subjects (860 vs. 634 h*ng/mL, respectively). Sofosbuvir NCA parameter estimates are summarized by study in **Table 9**.

Table 9: Simulated SOF NCA Parameters Summarized by Study
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		Mean (CV%) Simulated PK Parameters Upon SOF 400 mg at Steady State ^a	
Study		SOF AUC _{tau} (h·ng/mL)	SOF C _{max} (ng/mL)
GS-US-334-0131	N	88	88
	Mean	664	386
	CV%	31.2	49.9
P7977-0111	N	24	24
	Mean	630	274
	CV%	22.8	34.2
P7977-0613	N	60	60
	Mean	523	364
	CV%	31.9	42.1
P7977-0814	N	15	15
	Mean	563	358
	CV%	21.9	37.7
P7977-0915	N	30	30
	Mean	677	440
	CV%	35.3	43.9
P7977-1318	N	40	40
	Mean	576	265
	CV%	27.4	43.3
P7977-1819	N	39	39
	Mean	831	444
	CV%	40.5	34.1

P2938-0212	N	24	24
	Mean	656	461
	CV%	28.7	28.3
P2938-0515	N	17	17
	Mean	887	529
	CV%	33.5	28.9
P7977-0221	N	49	49
	Mean	1190	492
	CV%	44.2	48.1
P7977-1231	N	252	252
	Mean	810	358
	CV%	28.0	19.2
GS-US-334-0107	N	206	206
	Mean	816	362
	CV%	28.3	23.4
GS-US-334-0108	N	201	201
	Mean	798	368
	CV%	32.8	27.1
GS-US-334-0110	N	327	327
	Mean	964	386
	CV%	41.8	32.0

Sponsors SOF population PK report, pg 207-8

Reviewer's Comments: The reviewer was able to recreate the analysis performed by the sponsor, and in general, the reviewer agrees with the results. It is recognized that due to the rapid half-life of SOF, it is difficult to characterize the PK, especially given the sparse sampling schedule used in the majority of the Phase II and III trials. However, the reviewer has three general concerns about the population PK approach utilized by the sponsor, though; the reviewer was also not able to address these issues with the available data:

- 1) The conclusion that HCV-infected subjects have higher SOF exposure, while a significant covariate in the model, cannot necessarily be concluded based on the available study data. While the lack of a PK effect from PEG is supported as there were no differences between SOF exposure as part of a*

PEG/RBV or RBV regimen, the difference in SOF exposure between healthy volunteers and HCV-infected subjects may instead be due to an interaction with RBV. All HCV-infected studies, with the exception of 8 subjects in P2938-0212 were administered SOF/RBV or SOF/PEG/RBV. While the number of subjects in P2938-0212 was small, the PK in these subjects (AUC 538 ng·hr/mL and C_{max} 603 ng/mL) was similar to that observed in healthy volunteers (AUC 634 ng·hr/mL and C_{max} 366 ng/mL). Assessment of both SOF and RBV concentrations in ongoing or future studies involving SOF/RBV will be necessary to determine if the difference in SOF PK is due to an interaction with RBV or disease status.

- 2) Given the limitations noted above in Section 1, a joint population PK model of both SOF and GS-331007 (and other metabolites) may have been more appropriate to inform whether GS-331007 exposure was a direct or indirect measure of efficacy. While no discussion of a combined SOF/GS-331007 modeling approach was discussed by the sponsor, the reviewer's attempts at a joint model were not successful, either.
- 3) While SOF is a precursor entity to the active metabolite, the short half-life makes it difficult to relate to either efficacy or safety with the sampling scheme utilized by the sponsor. This is further apparent as 197 subjects in the Phase III population did not contribute any information to the SOF population PK analysis (and should not have been used in the PK/PD analysis). The reviewer recommends exploratory collection of the intracellular metabolites, including the active triphosphate, from peripheral blood mononuclear cells in an ongoing or future study to assist in better understanding the relationship between SOF administration and accumulation of the active metabolite.

3.3 Population PK Model (GS-331007)

Report 5.3.3.5 Population Pharmacokinetic of GS-331007

The objectives of the analysis were: 1) to build a population PK model of GS-331007; and 2) to evaluate the effect of physiologic and demographic covariates on the PK of GS-331007

3.3.1 Data

Data from a total of 18 studies, including 7 in healthy subjects (P7977-0111, GS-US-334-0131, P7977-0613, P7977-0814, P7977-0915, P-7977-1318, and P7977-1819; n = 294) and 11 studies in hepatitis C virus (HCV)-infected subjects (GS-US-334-0107 (POSITRON), GS-US-334-0108 (FUSION), GS-US-334-0110 (NEUTRINO), P2938-0212 (NUCLEAR), P7977-0221, P7977-0422 (PROTON), P2938-0515, P7977-0523 (ELECTRON), P2938-0721 (QUANTUM), P7977-0724 (ATOMIC), and P7977-1231 (FISSION); n = 1795). Data from a total of 2089 subjects were used in the analysis (Table 10).

Table 10: Studies used in the population pharmacokinetic analysis of GS-331007

Study	Population	Phase	Sampling (Intensive/Sparse)	Number of Subjects Included in Population PK Analyses (N = 2089)
GS-US-334-0131	Healthy	1	Intensive	88
P7977-0111	Healthy	1	Intensive	24
P7977-0613	Healthy	1	Intensive	60
P7977-0814	Healthy	1	Intensive	15
P7977-0915	Healthy	1	Intensive	28 ^a
P7977-1318	Healthy	1	Intensive	40
P7977-1819	Healthy	1	Intensive	39
P2938-0212	HCV-Infected	1	Intensive	24
P2938-0515	HCV-Infected	1	Intensive	17
P2938-0721	HCV-Infected	2	Intensive + Sparse	152
P7977-0221	HCV-Infected	2	Intensive + Sparse	49
P7977-0422	HCV-Infected	2	Intensive + Sparse	120
P7977-0523	HCV-Infected	2	Intensive + Sparse	120
P7977-0724	HCV-Infected	2	Intensive + Sparse	327
P7977-1231	HCV-Infected	3	Sparse	252 ^b
GS-US-334-0107	HCV-Infected	3	Sparse	206
GS-US-334-0108	HCV-Infected	3	Sparse	201
GS-US-334-0110	HCV-Infected	3	Sparse	327

a 2 subjects (Subjects 306 and 308, end-stage renal disorder [ESRD] group) were inadvertently censored from the data set from P7977-0915.

b 2 subjects from Study P7977-1231 (Subjects 310149, and 310659) were not included in the population PK analysis due to missing dose records.

Sponsors GS-331007 population PK report, pg 10

Demographics and a summary of subject covariates are summarized below in **Table 11**.

Table 11: Summary of Demographics and Covariates in the GS-331007 Population PK Analysis

Covariate	Average (\pm SD)	Min–Max	n
Age	48.2 (\pm 11.9)	18 – 78	2089
Gender	-	-	1312 (Male), 777 (Female)
Race	-	-	1717 (White), 237 (Black), 54(Asian), 81 (Other)
Ethnicity	-	-	363 (Hispanic or Latino), 1725 (Nonhispanic or nonlatino)
Subject Status	-	-	294 (Healthy Subjects), 1795 (HCV Subjects)
Body Weight (kg)	82.0 (\pm 17.3)	42.6–162	2089
BMI (kg/m ²)	27.8 (\pm 5.1)	17.3–56.1	2089
BSA (m ²)	2.0 (\pm 0.2)	1.3–2.9	2089
Creatinine Clearance ^a (mL/min)	116 (\pm 32.4)	12.2–281	2088
eGFR ^b (mL/min/1.73m ²)	90.0 (\pm 19.4)	7.0–193	2088
Cirrhosis	-	-	218
Concomitant Medications			
Proton Pump Inhibitors	-	-	291
H2 Receptor Antagonists	-	-	91
Calcium Channel Blockers	-	-	124
Statins	-	-	93
Diuretics	-	-	182
SSRIs	-	-	322
Anticoagulants	-	-	200

a Estimated by Cockcroft-Gault formula

b Estimated Glomerular Filtration Rate (MDRD formula)

Note: Subjects for whom demographic information was unavailable were not included in the covariate model selection.

Sponsors GS0331007 population PK report, pg 20

3.3.2 Methods

Similar methods to those outlined for the SOF population PK modeling analysis were used for developing the GS-331007 population PK model. The GS-331007 model assumed a daily dose of 400 mg, the same as used in the SOF population PK model.

First order with conditional estimates (FOCE) method implemented in NONMEM v.7.2. Covariates considered for analysis included age, gender, race, body weight, body mass index, body surface area, formulation, combination therapy (PEG+RBV or RBV), treatment duration, creatinine clearance, eGFR, hepatic impairment, cirrhosis, healthy versus HCV-infected, and concomitant medication. Decision of the testing of a characteristic as a covariate was based on examination of the graphs as well as existence of a scientific rationale for a potential effect. Those subject characteristics

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deemed to be significantly correlated with a model parameter were then formally tested as covariates in the model. The performance of the model was checked with graphical inspection of the model fits, visual and numerical predictive checks, and bootstrapping for confidence intervals of population PK parameters.

Different datasets were used during the model building process. Log-transformed concentration data (Dataset gs4.xpt) were used to develop the base model. Upon the availability of data from the Phase 3 studies, this data set was expanded to include all available data (Dataset p123v1.xpt) and used in the final model building steps.

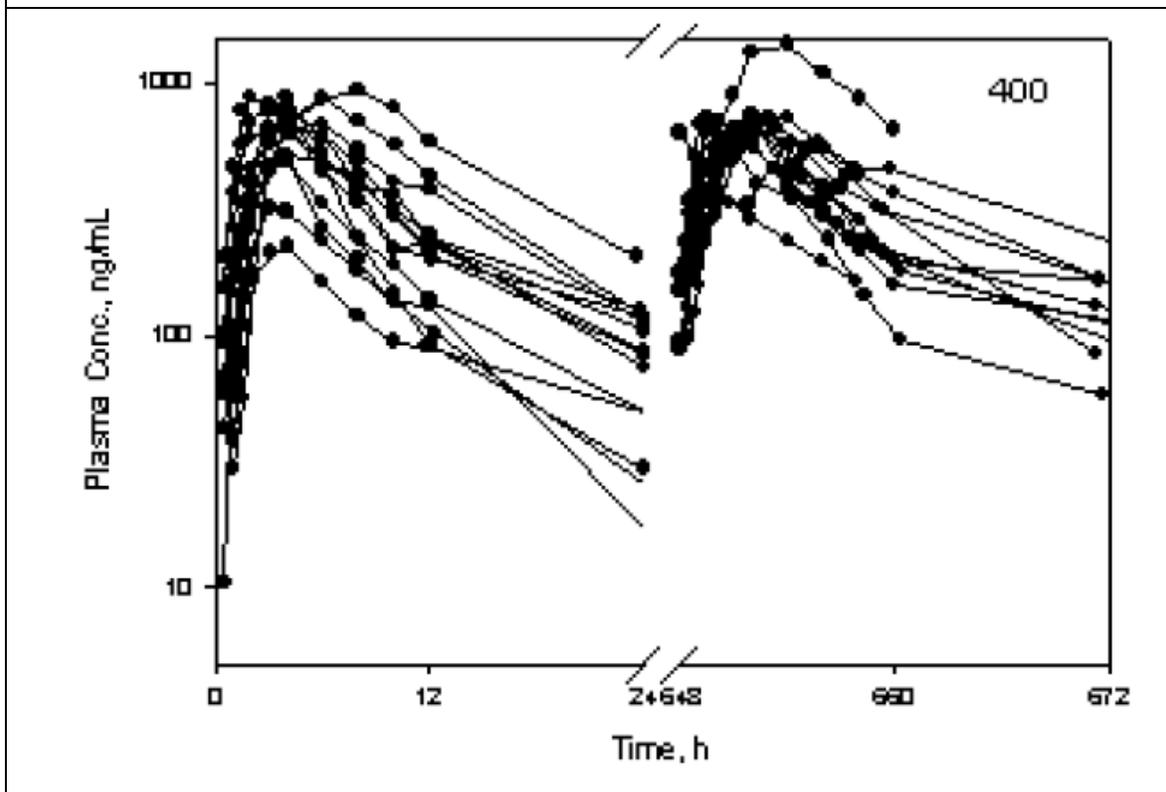
Following successful model validation, the final model was used to simulate GS-331007 exposures in all 2089 subjects from all 18 studies. Plasma GS-331007 concentrations were simulated at prespecified time points (0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, 20, and 24 hours postdose) at steady state for each subject in each study assigning a dose of 400 mg. Noncompartment analysis (NCA) was performed on the simulated GS-331007 concentrations using WinNonLin v.5 (Pharsight Corp. Sunnyvale, CA) and estimates of AUC_{τ} and C_{max} values were generated.

3.3.3 Results

3.3.3.1 Individual GS-331007 Plasma Concentration Time-Course

GS-331007 concentration versus time data for a representative study (P7977-0221) is shown in **Figure 7**.

Figure 7: GS-331007 Plasma Concentrations Versus Time After Single and Multiple Doses of SOF 400 mg in P7977-0221 (n-15)



A 2-compartment PK model with zero and first order absorption rate constant and absorption lag time provided a good description of the PK of GS-331007 in both healthy and HCV-infected subjects. An effect of creatinine clearance and HCV genotype (differences between healthy subjects, genotypes 1/4/6, genotype 2, and genotype 3) on CL/F was observed and included in the final model. Final parameters estimates are listed in **Table 12** and goodness of fit plots are displayed in **Figure 8**.

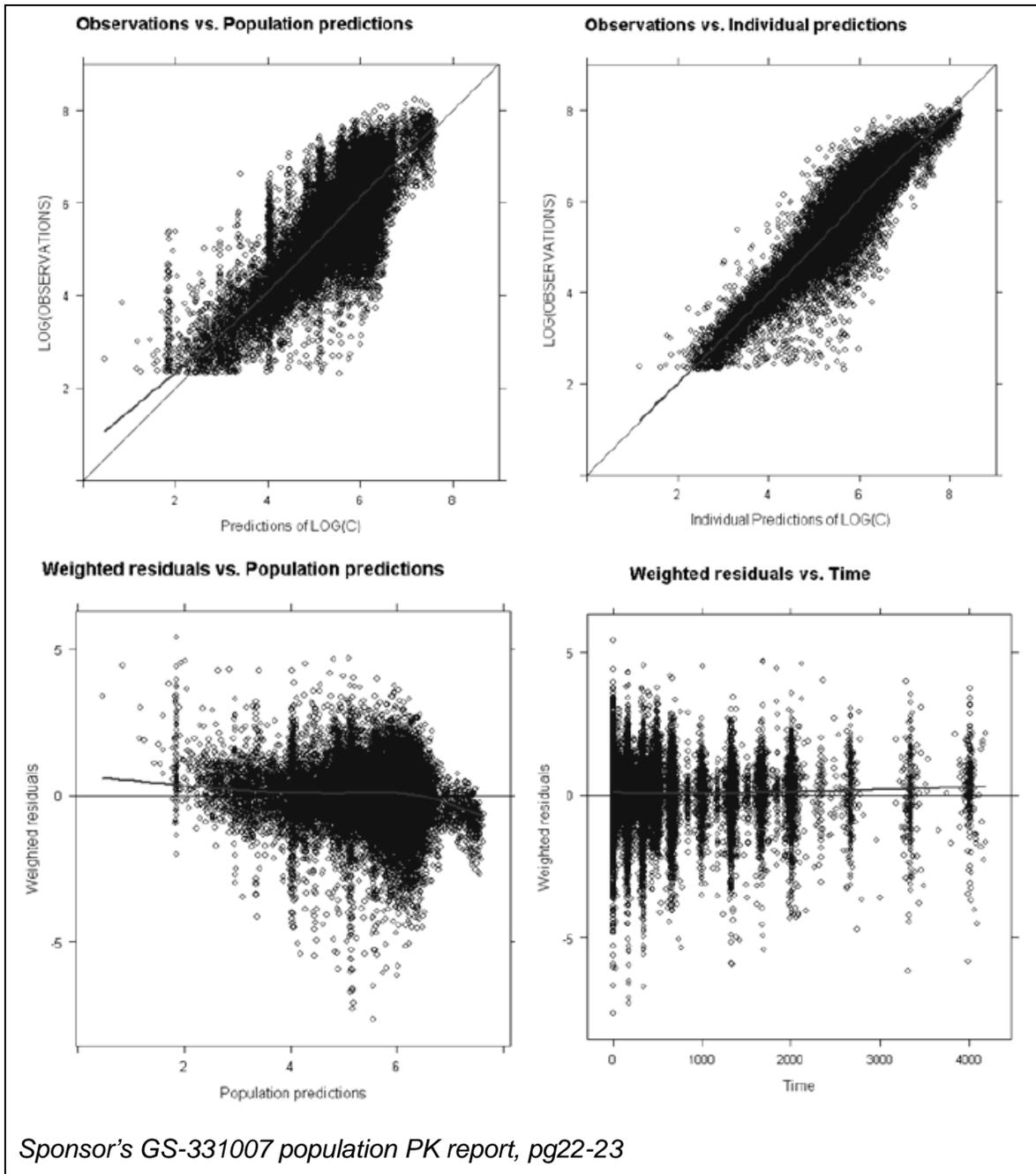
Table 12: Population PK Parameters for the Final GS-331007 Model

Parameter	Estimate (RSE%)	IIV (RSE%)
CL/F (L/h)	39.5 (2.7)	0.11 (4.7)
V/F (L)	218 (4.3)	0.8 (12.4)
CLD/F (L/h)	13.0 (6.3)	-
VP/F (L)	594 (4.7)	-
KA (h ⁻¹)	0.05 (6.4)	0.49 (5.6)
TLAG (h)	0.28 (0.6)	-
FR	0.61 (2.6)	-
D2	4.4 (1.1)	-
CLCR on CL/F	0.336 (8.1)	-
HCVGT=1,2 on CL/F	19.5 (7.1)	-
HCVGT=3 on CL/F	15.8 (10.2)	-
HCVGT=4 on CL/F	16.1 (10.8)	-
HCVGT=5,6,7 on CL/F	24.5 (11.4)	-
σ^2 (proportional)	0.192 (2.5)	-
COV(CL/F,V/F)	-	0.147 (9.7)
COV(CL/F,KA)	-	-0.144 (8.9)
COV(V/F,KA)	-	-0.472 (8.7)

IIV: interindividual variability, CL/F: oral clearance, V/F: apparent volume of distribution, CLD/F: apparent distributional clearance, VP/F: apparent volume of the peripheral compartment, KA: first-order absorption rate constant from the transit to the central compartment, FR: fraction of dose that absorbed via the first-order route, D2: duration of the zero-order absorption from the transit to the central compartment, TLAG: lag-time for first and zero-order absorption processes, σ^2 : variance of proportional error term. RSE values were obtained through bootstrapping (n = 639).

Sponsors GS-331007 population PK report, pg 21

Figure 8: Goodness of Fit Plots for the final GS-331007 Population PK Model



The typical CL/F of GS-331007 was estimated to be 39.5 L/h with an IIV of 0.11. The V/F of the central compartment was estimated as 218 L with an IIV of 0.80. GS-331007 absorption was modeled using zero-order absorption with duration 4.4 hr and a first-order absorption rate (k_a) of 0.046 h⁻¹ with an IIV of 0.49. In addition, a lag-time of 0.275 hr was included in the final model.

A statistically significant, positive correlation was observed between creatinine clearance and GS-331007 oral clearance. The effect was centered at the mean creatinine clearance value of 116.0 mL/min and described with a linear function with a slope population estimate of 0.336. The effect, however, was modest and the resulting

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decrease in the IIV term associated with GS-331007 oral clearance was 15.7%. The inclusion of the HCV genotype effect on the oral clearance as a discrete variable resulted in the reduction of the IIV by 67.1% (mostly due to differences between HCV-infected and healthy individuals). No other demographic or formulation characteristics were found to have any clinically relevant effects on GS-331007 PK.

3.3.3.2 Noncompartmental Analysis of Simulation Results for GS-331007

The final model was used to simulate steady-state GS-331007 concentrations (20 daily doses of SOF) in all subjects and a NCA analysis was performed on simulated concentrations. The predicted exposures from subjects in Studies GS-US-334-0107, GS-US-334-0108, GS-US-334-0110, and P7977-1231 were used for a subsequent PK/PD analysis

Based on the non-compartmental analyses from population PK modeling, comparable GS- 331007 exposure (AUCtau and Cmax) were observed across the 3 HCV genotype subgroups; mean (%CV) AUCtau: 6840 (32.1), 7520 (28.1), and 7300 (30.2) ng*h/mL, Cmax: 560 (39.5), 606 (35.1), 584 (36.2) ng/mL for HCV genotype 1/4/5/6, genotype 2, and genotype 3 respectively. Mean (%CV) GS-331007 AUCtau and Cmax in Phase 3 study HCV- infected subjects (pooled across all genotype subgroups) was 39% and 49% lower than healthy subjects (7200 vs. 11,900 h*ng/mL and 582 vs. 1140 ng/mL, respectively. Across quartile of creatinine clearance, GS-331007 AUCtau and Cmax exhibited a mean difference of 35% and 26%, respectively, between the midpoints of the lowest and highest quartiles. GS-331007 NCA parameter estimates are summarized by study in **Table 13**.

Table 13: Simulated GS-331007 NCA Parameters Summarized by Study

		Mean (CV%) Simulated PK Parameters Upon SOF 400 mg at Steady State ^a	
Study		GS-331007 AUC _{tau} (h·ng/mL)	GS-331007 C _{max} (ng/mL)
GS-US-334-0131	N	88	88
	Mean	11,100	1060
	CV%	17.9	24.2
P7977-0111	N	24	24
	Mean	12,800	1290
	CV%	21.7	21.6
P7977-0613	N	60	60
	Mean	10,100	1030
	CV%	20.0	21.0
P7977-0814	N	15	15
	Mean	11,300	1050
	CV%	19.1	16.0
P7977-0915	N	28	28
	Mean	42,000	2640
	CV%	91.5	61.4
P7977-1318	N	40	40
	Mean	13,300	1200
	CV%	22.2	22.7
P7977-1819	N	39	39
	Mean	11,200	1120
	CV%	21.6	21.4
P2938-0212	N	24	24
	Mean	9780	1090
	CV%	20.1	21.3

P2938-0515	N	17	17
	Mean	11,000	1180
	CV%	42.2	37.1
P2938-0721	N	152	152
	Mean	7980	659
	CV%	29.5	32.6
P7977-0221	N	49	49
	Mean	7820	710
	CV%	27.1	31.2
P7977-0422	N	120	120
	Mean	7020	631
	CV%	29.6	28.2
P7977-0523	N	120	120
	Mean	7180	582
	CV%	31.4	37.4
P7977-0724	N	327	327
	Mean	6710	538
	CV%	32.0	37.9
P7977-1231	N	252	252
	Mean	7370	600
	CV%	30.2	37.0
GS-US-334-0107	N	206	206
	Mean	7520	601
	CV%	28.0	34.1
GS-US-334-0108	N	201	201
	Mean	7200	569
	CV%	30.4	36.6
GS-US-334-0110	N	327	327
	Mean	6860	563
	CV%	32.0	39.2

Sponsors GS-331007 population PK report, pg 325-7

Reviewer's Comments: Similar to the SOF population PK analysis, the reviewer was able to recreate the analysis performed by the sponsor. While the inclusion of separate covariates for genotype 1/4/5/6, genotype 2, and genotype 3 subjects was numerically significant, the different CL/F estimates resulted in a less than 10% difference in AUC and C_{max} (also noted by the sponsor). As it is unanticipated that the different HCV genotypes are contributing to a difference in GS-331007 PK, it is likely this is a numeric artifact from sampling used in the trials or a minor interaction due to PEG. The reviewer has the same concern regarding development of a joint population PK model for SOF and GS-331007. In addition, the reviewer had one additional concern regarding the developed GS-331007 population PK model:

- 1) The conclusion that HCV-infected subjects have higher GS-331007 exposure, while a significant covariate in the model, cannot necessarily be concluded based on the available study data. While the lack of a PK effect from PEG is supported as there were no differences between GS-331007 exposure as part of a PEG/RBV or RBV regimen, the difference in GS-331007 exposure between healthy volunteers and HCV-infected subjects may instead be due to an interaction with RBV. All HCV-infected studies, with the exception of 8 subjects in P2938-0212 were administered SOF/RBV or SOF/PEG/RBV. While the number of subjects in P2938-0212 was small, the PK in these subjects (AUC 9638 ng·hr/mL and C_{max} 1378 ng/mL) was similar to that observed in healthy volunteers (AUC 11900 ng·hr/mL and C_{max} 1140 ng/mL). Assessment of SOF, GS-331007, and RBV concentrations in ongoing or future studies involving SOF/RBV will be necessary to determine if the difference in GS-331007 PK is due to an interaction with RBV or disease status.*

3.4 PK/PD Efficacy and Safety of SOF and GS-331007 in HCV-infected Subjects

Report 2.7 summary-clin-pharm.pdf: Clinical Pharmacology Summary

3.4.1 Dose Selection

In P7977-0221, SOF combined with a 48-week course of PEG/RBV, resulted in higher rates of SVR12 and SVR24 compared with PEG/RBV alone. SVR12 and SVR24 rates were greatest in the SOF 200 mg+PEG/RBV (72.2% and 83.3%, respectively) and SOF 400 mg+PEG/RBV (86.7% and 80.0%, respectively) groups versus the SOF 100 mg+P/R (56.3% and 56.3%, respectively) and placebo+P/R groups (50.0% and 42.9%, respectively). As SVR12 and SVR24 rates were lowest and breakthrough and relapse rates were highest in the SOF 100 mg+PEG/RBV group, SOF 200 mg+PEG/RBV and SOF 400 mg+PEG/RBV were the therapeutic doses selected to be evaluated in subsequent studies for longer treatment duration

In Study P7977-0422 (PROTON) subjects with genotype 1 HCV infection received SOF 200 mg or 400 mg+PEG/RBV for 12 weeks followed by 12 weeks of PEG/RBV. Virologic breakthroughs during treatment with PEG+RBV following 04treatment with SOF+PEG/RBV were more common in the SOF 200 mg+PEG/RBV group compared with the SOF 400 mg+PEG/RBV group, suggesting that the SOF 400-mg dose provided greater antiviral activity

3.4.2 PK/PD Efficacy Analyses

The PK/PD analyses of GS-331007 and SOF exposure-efficacy relationships from Phase 3 studies (graphical representation and univariate logistic regression) were performed in subjects with genotype 2 or 3 HCV infection administered SOF+RBV and subjects with genotype 1, 4, 5 or 6 HCV infection administered SOF+PEG/RBV in the Phase 3 Studies P7977-1231 (FISSION), GS-US-334-0107 (POSITRON), GS-US-334-0108 (FUSION), and GS-US-334-0110 (NEUTRINO). The primary endpoint for efficacy was SVR12. Population PK derived SOF and GS-331007 exposures were used in these analyses.

Based on SVR12 data from these Phase 3 studies, it was clear that treatment responses can vary substantially between these 2 genotypes. Graphical displays and statistically significant univariate logistic regression analysis assessing the relationship between GS-331007 AUCtau (continuous and categorical variable) and SVR12 across studies ($p < 0.05$) suggested potentially meaningful PK/PD relationships. Because PK was statistically significant in most univariate (PK only) analyses, exploratory multivariate logistic regression analyses exploring the impact of PK within the context of clinical predictors of efficacy was performed

Multivariate analysis was conducted using a multistage approach. Initially, a set of clinical, demographic, and baseline characteristics were assessed using univariate logistic regression. Univariate logistic regression modeling provided an estimate, in isolation, of the association of each predictor to SVR12. All univariate logistic regression results were tabulated including odds ratios, 95% CIs, and p-values. Multivariate logistic regression modeling jointly estimated the most important characteristics associated with SVR12. All characteristics assessed in the univariate analyses were included in a multivariate logistic-regression analysis using a stepwise selection procedure to determine which characteristics were independent predictors of SVR12. Predictors were selected for entry into the multivariate model if the p-value for that predictor was less than 0.10 and retained in the multivariate model if the p-value for the predictor was less than 0.05.

The results of the multivariate logistic regression analyses based on clinical, demographic, and baseline characteristics with and without exposure data (AUCtau of GS-331007) and SVR12 are presented for genotype 3 and genotype 1 subjects below

Table 14: Multivariate Logistic Regression Assessing the Association of Clinical, Demographic, and Baseline Characteristics With and Without Exposure Data on SVR12 in Genotype 3 Subjects

	P7977-1231 (FISSION) Treatment Naive		GS-US-334-0107 (POSITRON) IFN Ineligible, Intolerant, Unwilling		GS-US-334-0108 (FUSION) Treatment Experienced			
	SOF+RBV 12 Weeks		SOF+RBV 12 Weeks		SOF+RBV 12 Weeks		SOF+RBV 16 Weeks	
	Odds Ratio (95% Confidence Limit)	P-value	Odds Ratio (95% Confidence Limit)	P-value	Odds Ratio (95% Confidence Limit)	P-value	Odds Ratio (95% Confidence Limit)	P-value
Multivariate Regression With Clinical, Demographic, and Baseline Characteristics								
Sex (Female vs Male)	—	—	—	—	—	—	3.636 (1.040, 12.716)	0.043
Cirrhosis (No vs Yes)	2.772 (1.277, 6.020)	0.010	7.741 (1.994, 30.046)	0.003	—	—	—	—
Baseline HCV RNA (< 6 vs ≥ 6 log ₁₀ IU/mL)	2.259 (1.195, 4.271)	0.012	—	—	—	—	—	—
Weight-based RBV dose at baseline (mg/kg/day) (continuous)	1.258 (1.082, 1.462)	0.003	—	—	1.591 (1.110, 2.280)	0.011	—	—
Multivariate Regression With Clinical, Demographic, and Baseline Characteristics and SOF Exposure								
GS-331007 AUC ₀₋₂₄ population quartiles (PQ2 vs PQ1)	0.826 (0.355, 2.092)	0.74	1.037 (0.293, 3.676)	0.96	0.673 (0.138, 3.290)	0.63	9.135 (1.547, 53.942)	0.015
GS-331007 AUC ₀₋₂₄ population quartiles (PQ3 vs PQ1)	0.837 (0.339, 2.071)	0.70	1.523 (0.450, 5.158)	0.50	0.239 (0.038, 1.505)	0.13	6.600 (1.236, 35.246)	0.027
GS-331007 AUC ₀₋₂₄ population quartiles (PQ4 vs PQ1)	1.797 (0.636, 5.075)	0.27	7.947 (1.845, 34.224)	0.005	0.650 (0.113, 3.725)	0.63	11.009 (1.697, 71.417)	0.012
Sex (Female vs Male)	—	—	—	—	—	—	3.728 (0.891, 15.595)	0.071
Cirrhosis (No vs Yes)	2.590 (1.177, 5.699)	0.018	8.491 (1.882, 38.297)	0.005	—	—	—	—
Baseline HCV RNA (< 6 vs ≥ 6 log ₁₀ IU/mL)	2.424 (1.242, 4.730)	0.009	—	—	—	—	—	—
Weight-based RBV dose at baseline (mg/kg/day) (continuous)	1.226 (1.039, 1.448)	0.016	—	—	1.717 (1.139, 2.587)	0.010	—	—
PQ (1, 2, 3, 4) = (first, second, third, fourth) population quartile								
Note: Only predictors identified in multivariate logistic regression analysis using the full analysis set and the GS-331007 AUC ₀₋₂₄ were included in the model.								
Note: Missing cirrhosis status was imputed as 'No', and missing IL28B genotype was imputed as 'CC'.								
Note: One subject in Study GS-US-334-0108 with missing ethnicity data was removed from the multivariate logistic regression due to the stepwise algorithm.								
Note: The quartiles were calculated based on all of the data from all Phase 3 studies using the inverse of the empirical distribution function of the AUC ₀₋₂₄ for GS-331007 (SAS method 5). The quartiles were as follows: PQ1: AUC ₀₋₂₄ ≤ 5744.7 ng·h/mL; PQ2: 5744.7 ng·h/mL < AUC ₀₋₂₄ ≤ 6975.1 ng·h/mL; PQ3: 6975.1 ng·h/mL < AUC ₀₋₂₄ ≤ 8440.2 ng·h/mL; and PQ4: AUC ₀₋₂₄ > 8440.2 ng·h/mL.								
<i>Sponsors Clinical Efficacy Summary pg 224-5</i>								

Table 15: Multivariate Logistic Regression Assessing the Association of Clinical, Demographic, and Baseline Characteristics With and Without Exposure Data on SVR12 in Genotype 1 Subjects

	GS-US-334-0110 (NEUTRINO)	
	SOF+PEG+RBV 12 Weeks	
	Odds Ratio (95% Confidence Limit)	P-value
Multivariate Regression With Clinical, Demographic, and Baseline Characteristics		
Cirrhosis (No vs Yes)	3.924 (1.662, 9.265)	0.002
IL28B genotype (CC vs non-CC)	7.989 (1.815, 35.168)	0.006
Weight-based RBV dose at baseline (mg/kg/day) (continuous)	1.384 (1.153, 1.662)	< 0.001
Multivariate Regression With Clinical, Demographic, and Baseline Characteristics and Exposure Data		
GS-331007 AUC _{0-24h} population quartiles (PQ2 vs PQ1)	3.137 (0.955, 10.303)	0.059
GS-331007 AUC _{0-24h} population quartiles (PQ3 vs PQ1)	0.959 (0.351, 2.623)	0.94
GS-331007 AUC _{0-24h} population quartiles (PQ4 vs PQ1)	2.029 (0.596, 6.913)	0.26
Cirrhosis (No vs Yes)	4.106 (1.704, 9.893)	0.002
IL28B genotype (CC vs non-CC)	7.762 (1.772, 34.007)	0.007
Weight-based RBV dose at baseline (mg/kg/day) (continuous)	1.367 (1.127, 1.658)	0.002

Sponsors Clinical Efficacy Summary pg 224-5

Due to the lower SVR12 rates achieved in treatment-experienced genotype 3 HCV-infected subjects in the Phase 3 studies, HCV RNA reductions at the earliest measured time point (Week 1) were examined to rule out differences in early viral kinetics. Acute viral kinetics at the earliest measured time point (week 1) in all Phase 3 studies showed comparable HCV RNA reduction in genotype 2 (4.5 log₁₀ IU/mL) or genotype 3 (4.3 log₁₀ IU/mL) HCV-infected subjects. These data suggest that treatment duration is drivers of SVR12 in genotype 3 subjects.

Reviewer's comments: The reviewer identified similar predictive factors during the reviewer's analysis. In all cases except for 16-week duration in GS-US-334-0108, GS-331007 was not included in the final multivariate model. This relationship was primarily driven by an increased SVR12 rate in subjects with exposures above the median, but SVR12 rate was unchanged from the 12-week results in subjects below the median. This suggests that duration and not exposure may have been key factors in the observed response.

Weight-based RBV dose was also significant in all genotype 3 evaluations except for the 16-week duration assessment. This supports the RBV 1000/1200 mg dose evaluated in the Phase III trials may have provided additional benefit, but is not conclusive that the higher RBV doses are necessary for optimal response in this population. There could be other factors to consider, including RBV concentration, the active triphosphate concentration, and body weight independent of dose. However, as this was evaluated in Phase III, the safety events were manageable, and none of the efficacy analyses can rule out a benefit of the RBV doses evaluated, the reviewer does not recommend a change to the RBV dose. A similar weight-based RBV relationship was observed in

genotype 1 subjects from GS-US-334-0110, and the impact of weight-based RBV in SOF treatment will continue to be assessed in future submissions.

The reviewer conducted on treatment virologic assessments similar to the applicant and was also not able to identify any on treatment differences between genotype 2 and 3 subjects, nor was the reviewer able to identify a metric predictive of treatment failure. For additional details, please see the reviewer's analysis below.

3.4.3 PK/PD Safety Analyses

Pharmacokinetic-pharmacodynamic analyses of the GS-331007 and SOF exposure-safety relationships were performed using GS-331007 and SOF exposures derived from population [POSITRON], GS-US-334-0108 [FUSION], and GS-US-334-0110 [NEUTRINO]) versus safety parameters that included frequently observed AEs or laboratory abnormalities.

The applicant did not identify any significant relationships between GS-331007 AUC_{tau} or C_{max} and SOF AUC_{tau} and incidence of AEs for fatigue, headache, nausea, insomnia, or irritability, nor did the applicant identify any relationship between GS-331007 AUC_{tau} or C_{max} and SOF AUC_{tau} and change in hemoglobin.

4 REVIEWER'S ANALYSIS

4.1 Introduction

This is the original submission of sofosbuvir (SOF), a hepatitis C virus (HCV) NS5B-directed inhibitor and is first in class. The applicant is seeking approval of SOF in combination with ribavirin (RBV) 1000/1200 mg, based on body weight (1000 for <75 kg and 1200 for >75 kg), over 12-weeks in genotype 2 subjects and 16-weeks in genotype 3 subjects. The applicant is also seeking approval for SOF/PEG/RBV over 12-weeks in genotype 1 treatment-naïve subjects. The reviewer evaluated the dosing rationale, the relationship between the exposure of relevant entities (SOF and its primary circulating metabolite GS-331007) and the response (safety and efficacy) and predictors of response for each of these analyses. The results of these analyses are presented below.

4.2 Objectives

Analysis objectives are:

1. To assess the appropriateness of the selected SOF 400 mg q.d. dose based on Phase III efficacy (sustained virologic response at week 12 of follow-up; SVR12) and safety relationships in genotype 1, genotype 2, and genotype 3 subjects
2. Determine if the RBV dose evaluated in genotype 2 and 3 subjects (1000/1200 mg for <75/>75 kg, respectively) is supported by the available Phase III data
3. Determine if the evidence supports extension of the SOF/RBV to 16-weeks in genotype 3 treatment-naïve subjects
4. Determine if there is sufficient evidence to support effectiveness of SOF/PEG/RBV in genotype 1 prior P/R-nonresponders.

4.3 Methods

4.3.1 Data Sets

Data sets used are summarized in Table 16.

Table 16: Analysis Data Sets

Study Number	Name	Link to EDR
GS-US-334-0108	adeff.xpt, adeffout.xpt, adhcvout.xpt	\\Cdsesub1\evsprod\NDA204671\0000\m5\datasets\gs-us-334-0108\analysis\adam\datasets
GS-US-334-0107	adeff.xpt, adeffout.xpt, adhcvout.xpt	\\Cdsesub1\evsprod\NDA204671\0000\m5\datasets\gs-us-334-0107\analysis\adam\datasets
GS-US-334-0110	adeff.xpt, adeffout.xpt, adhcvout.xpt	\\Cdsesub1\evsprod\NDA204671\0000\m5\datasets\gs-us-334-0110\analysis\adam\datasets
P7977-1231	adeff.xpt, adeffout.xpt, adhcvout.xpt	\\Cdsesub1\evsprod\NDA204671\0000\m5\datasets\p7977-1231\analysis\adam\datasets
pk-pd	adpkpd.xpt	\\Cdsesub1\evsprod\NDA204671\0000\m5\datasets\pk-pd\analysis\adam\datasets
popPK SOF	p12v1.xpt, p12v2.xpt, p123v3.xpt, p123v4.xpt, p123v5.xpt	\\Cdsesub1\evsprod\NDA204671\0000\m5\datasets\pop-pk-sof\analysis\legacy\datasets
popPK GS-331007	gs1.xpt, gs3.xpt, gs4.xpt, p123v1.xpt, p123v2.xpt	\\Cdsesub1\evsprod\NDA204671\0000\m5\datasets\pop-pk-gs-331007\analysis\legacy\datasets

4.3.2 Software

Estimation and simulation were performed NONMEM 7.2 on the Pharmacometrics Group Linux cluster using the front end manager Perl Speaks NONMEM (PsN). Diagnostic graphs, model comparison, and statistical analysis were performed in R (version 10.1).

4.3.3 Models

4.3.3.1 On-Treatment Virologic Response: Percentage of Subject with HCV RNA <25 IU/mL

On-treatment virologic time course for each Phase III study and each treatment arm was calculated based on the percentage of subjects with HCV RNA not detected at each visit. Divisions of on treatment virologic response were made for genotype 2 and

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genotype 3 in P7977-1231 and GS-US-334-0108. Similar, on treatment virologic response was separated for genotype 1 subjects based on fibrosis score and cirrhosis status from GS-US-334-0110.

4.3.3.2 Logistic Regression: Exposure- Safety Response Relationships

Logistic regression models for common adverse events were performed using the applicant's Phase III trial data in genotype 1, 2, and 3 subjects. Three independent variables were used for developing logistic regression plots: steady-state AUC for GS-331007 and SOF, and maximum concentration (C_{max}) for GS-331007 (the sparse sampling for SOF combined with its rapid elimination did not permit accurate assessment of SOF C_{max} in the Phase III subjects). AUC_T and C_{0h} were calculated for each subject using empirical Bayes' estimates from the population PK model.

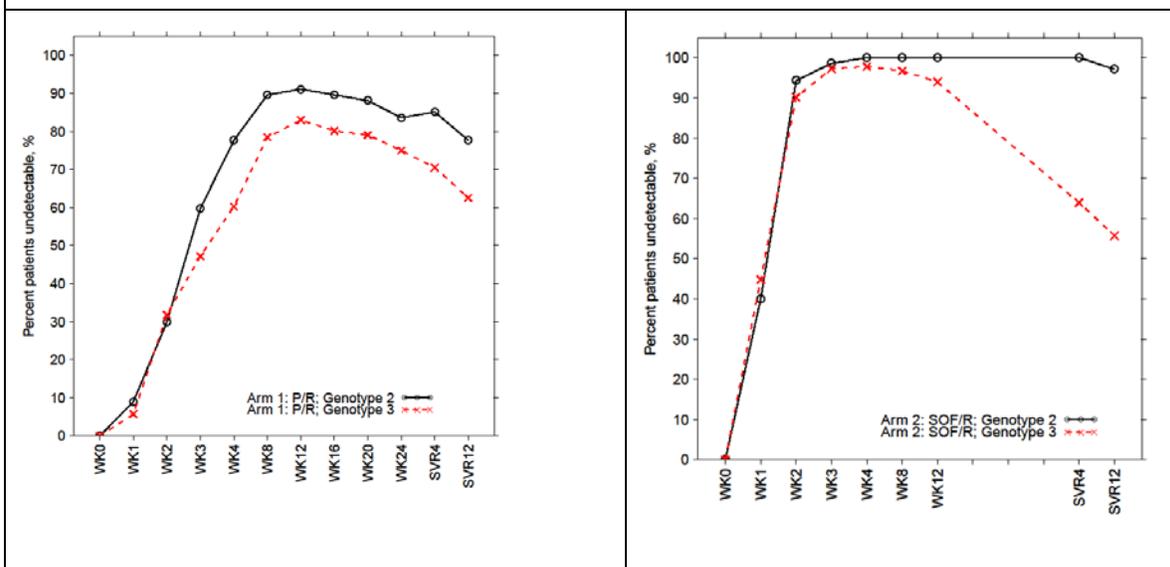
4.4 Results

4.4.1 On-Treatment Virologic Response: Percentage of Subject with HCV RNA <25 IU/mL

Genotype 2 and 3

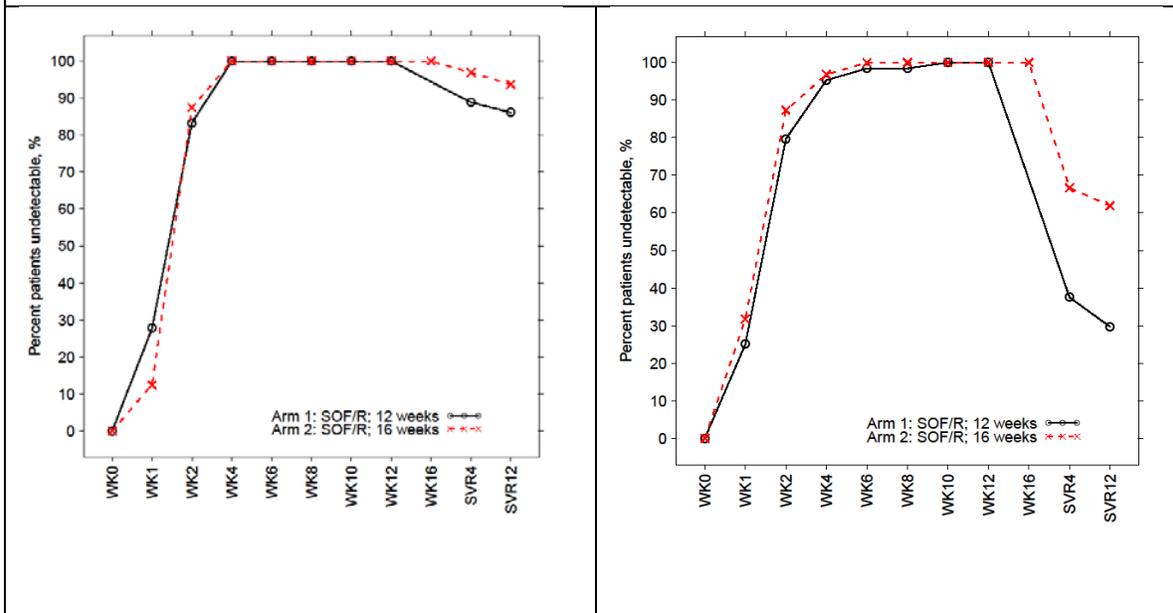
Time course for P7977-1231 genotype 2 and 3 subjects are shown below in Figure 9.. These results show that with PEG/RBV treatment, a slightly more favorable response in genotype 3 subjects compared to genotype 2 subjects with more subjects with HCV RNA not detected at week 3 onward, as well as a higher overall SVR12 rate (63% for genotype 3 versus 78% in genotype 2). For SOF/RBV, no discernible differences in on-treatment response could be identified between genotype 2 and genotype 3 subjects. Most subjects had HCV RNA not detected as early as week 1-2 of treatment. However, despite this on-treatment response, higher relapse rates were observed for 12-weeks of treatment in genotype 3 treatment-naïve subjects compared to genotype 2 treatment-naïve subjects.

Figure 9: Virologic Time Course for PEG/RBV (left) and SOF/RBV (right) Treatments Grouped by Genotype from P7977-1231



Time course for GS-US-334-0108 genotype 2 and 3 subjects are shown below in Figure 10. This study evaluated prior PEG/RBV-nonresponders and different SOF/RBV durations (12- and 16-weeks respectively). Similar to before, subjects with either genotype displayed rapid initial decline in HCV RNA with most subjects achieving HCV RNA not detected by week 2-4 of treatment. Also, genotype 3 subjects were more likely to relapse compared to genotype 2 subjects for either treatment duration. There was an increase in SVR12 response rate in both genotype 2 and genotype 3 subjects by extending the treatment duration from 12-weeks to 16-weeks, though the increase was lower in genotype 2 subjects (increase from 86% to 94% compared to 30% to 62% in genotype 3 subjects).

Figure 10: Virologic Time Course for Genotype 2 (left) and Genotype 3 (right) Subjects from GS-US-334-0108 Grouped by Treatment

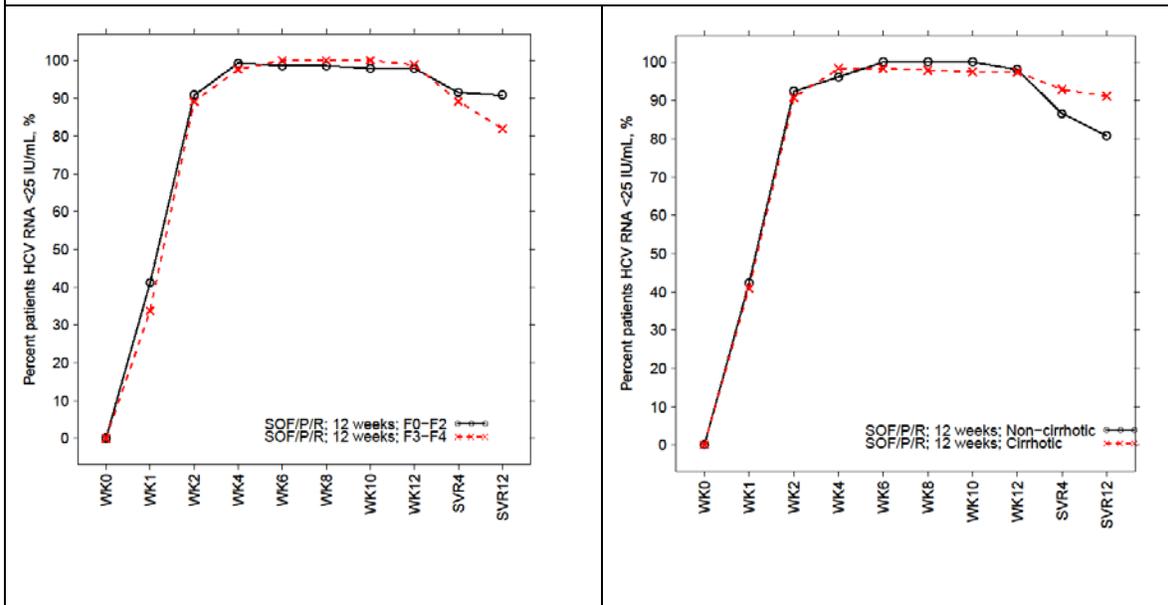


Genotype 1

The percentage of genotype 1 subjects with HCV RNA <25 IU/mL at each visit from GS-US-334-0110 is shown below in Figure 11. These analyses could not identify any difference in percentage of subjects with on-treatment HCV RNA >25 IU/mL between fibrosis score and cirrhosis status (68 subjects had missing fibrosis score data and 3 subjects had missing cirrhosis status are excluded from this analysis). The main difference between subjects with worse fibrosis score (F3-F4 compared to F0-F2) or cirrhosis status (presence of cirrhosis) was an increased likelihood of post treatment relapse in these subjects. The overall SVR rate in subjects with fibrosis score F3-F4 was 82.0% (68/83), of which 82 subjects had HCV target not detected at the end of treatment. Of these subjects, 14 relapsed during follow-up for a virologic relapse rate of 17.1% (14/82). In contrast, the SVR rate in subjects with fibrosis score F0-F2 was 90.8% (128/141). One hundred thirty nine subjects had HCV RNA target not detected at the end of treatment and 11 subjects relapsed during follow-up for a virologic relapse rate of 7.9% (11/139). A similar trend was observed from the cirrhotic versus non-cirrhotic analysis with cirrhotics having a relapse rate of 19.2% (10/52) compared to a relapse rate of 7.3% in non-cirrhotics (17/233).

Figure 11: Virologic Time Course for Genotype 1 fibrosis score (F0-F2 versus F3-

F4) (left) and cirrhosis status (cirrhotic versus non-cirrhotic) (right) from GS-US-334-0110



4.4.2 SVR Rate by Time to HCV RNA Target Not Detected

Genotype 3

Data from P7977-1231 and GS-US-334-0108 was summarized to determine if on treatment assessments at week 1, week 2, and week 4 (week 3 in P7977-1231) were predictive of whether a patient would achieve SVR. Summary results from these two trials are shown below in Table 17.

In general, a higher SVR rate in the subgroup of subjects with HCV RNA target not detected at each visit. By week 2, approximately half of the genotype 3 subjects in P7977-1231 achieved HCV RNA target not detected with an SVR rate of 64% compared to 49% in subjects with HCV RNA detectable. In addition, achieving HCV RNA target not detected at either week 2 or week 3 of treatment was identified as a predictive factor of achieving SVR12 in univariate analyses.

Similar trends of a higher SVR rate were observed in GS-US-334-0108 in subjects who achieved HCV RNA target not detected by week 2 or week 4 of treatment. However, the small number of genotype 3 subjects in each of the categories hinders interpretation of these results. The data does suggest that SVR rates were improved with the 16-week duration regardless of on treatment response at week 1, week 2, or week 4. The utility of on treatment response in genotype 3 treatment-experienced subjects should continue to be evaluated as additional data with 16-week or longer treatment durations becomes available.

Table 17: Summary of SVR Rates in Genotype 3 Subjects from P7977-1231 and GS-US-334-0108 Based on On-Treatment Virologic Response

P7977-1231				
P7977-1231	HCV RNA Assessment	Week 1 (# subjects [SVR])	Week 2 (# subjects [SVR])	Week 3 (# subjects [SVR])

S0000

Genotype 3 - 12 weeks	Not detected	15 [73%; 11/15]	86 [64%; 55/86]	134 [62%; 83/134]
	<25 IU/mL detected	67 [67%; 45/67]	80 [50%; 40/80]	41 [44%; 18/41]
	>25 IU/mL	88 [48%; 42/88]	11 [45%; 5/11]	2 [0%; 0/2]

GS-US-334-0108

GS-US-334-0108	HCV RNA Assessment	Week 1 (# subjects [SVR])	Week 2 (# subjects [SVR])	Week 4 (# subjects [SVR])
Genotype 3 - 12 weeks	Not detected	1 [0%; 0/1]	19 [37%; 7/19]	52 [35%; 18/52]
	<25 IU/mL detected	15 [40%; 6/15]	32 [34%; 11/32]	9 [11%; 1/9]
	>25 IU/mL	44 [30%; 13/44]	13 [8%; 1/13]	3 [0%; 0/3]
Genotype 3 - 16 weeks	Not detected	5 [60%; 3/5]	25 [72%; 18/25]	54 [65%; 35/54]
	<25 IU/mL detected	15 [73%; 11/15]	30 [53%; 16/30]	7 [57%; 4/7]
	>25 IU/mL	42 [60%; 25/42]	5 [60%; 3/5]	2 [0%; 0/2]

In addition, a summary table of SVR based on time to first HCV RNA target not detected was calculated for genotype 3 subjects from P7977-1231 and GS-US334-0108 (Table 18). In P7977-1231, those subjects with HCV RNA target not detected earlier on treatment (i.e., week 1) were more likely to achieve SVR than those with HCV RNA not detected at week 2 or later. This trend of higher SVR rate with earlier on treatment response was not observed in the 12-week treatment duration from GS-US-334-0108, but was observed for the 16-week treatment duration, lending further evidence that 12-week duration is not sufficient in genotype 3 subjects.

Table 18: Summary of SVR Rates in Genotype 3 Subjects from P7977-1231 and GS-US-334-0108 Based on First Assessment with HCV RNA <25 IU/mL

	GS-US-334-0108		
	P7977-1231	GS-US-334-0108	
	12-week SOF/RBV Treatment Naive	12-week SOF/RBV Treatment-Experienced	16-week SOF/RBV Treatment-Experienced

S0000

	N (%)	SVR	N (%)	SVR	N (%)	SVR
Week 1	81 [44%]	69%	16 [25%]	38%	19 [30%]	74%
Week 2	84 [46%]	48%	34 [53%]	35%	36 [57%]	56%
Week 3	13 [7%]	46%	-	-	-	-
Week 4	1 [0.5%]	0%	11 [17%]	9%	6 [10%]	83%
>Week4	1 [0.5%]	0%	3 [5%]	0%	2 [3%]	0%

Genotype 1

Data from GS-US-334-0110 was summarized to determine if on treatment assessments at week 1, week 2, and week 4 were predictive of whether a patient would achieve SVR (subjects with missing assessments were removed for that visit from this analysis). Summary results for SVR rate based on on-treatment virologic response and time to first HCV RNA target not detected are shown below in Table 19 and Table 20.

Similar to the observations from genotype 3 subjects, a higher SVR rate was observed in those subjects who achieved HCV RNA target not detected by week 2 or week 4 of treatment. Achieving HCV RNA target not detected by week 2 or week 4 of treatment was identified as a predictive factor for SVR, though the SVR rate in those subjects with HCV RNA detectable remained high (86%). In the subgroup of subjects with HCV RNA target not detected at each visit. It may be possible to further maximize SVR rate by using on-treatment response (HCV RNA >25 IU/mL at week 2 or HCV RNA detectable at week 4) to identify subjects who may benefit from longer SOF/PEG/RBV treatment duration as the primary reason for treatment failure in subjects with HCV RNA >25 IU/mL at week 2 or HCV RNA detectable at week 4 was relapse.

Table 19: Summary of SVR Rates in Genotype 1 Subjects from GS-US-334-0110 Based on On-Treatment Virologic Response

HCV RNA Assessment	Week 1 (# subjects [SVR])	Week 2 (# subjects [SVR])	Week 4 (# subjects [SVR])
Not detected	20 [95%; 19/20]	140 [94%; 131/140]	258 [91%; 236/258]
<25 IU/mL detected	100 [95%; 95/100]	124 [87%; 108/124]	27 [85%; 23/27]
>25 IU/mL	169 [85%; 144/169]	25 [80%; 20/25]	2 [0%; 0/2]

Table 20: Summary of SVR Rates in Genotype 1 Subjects from GS-US-334-0110 Based on First Assessment with HCV RNA <25 IU/mL

	Genotype 1	
	12-week SOF/PEG/RBV	
	n [%]	SVR
Week 1	114 [39%]	96%
Week 2	150 [51%]	87%
Week 4	25 [9%]	84%
>Week4	2 [1%]	50%

4.4.3 Exposure-Response for Safety: Other Adverse Events

Logistic regression models were evaluated for SOF and GS-331007 C_{max} , and AUC_T . Modeling results for adverse event rates versus GS-331007 AUC_T indicate no significant relationship with headache or fatigue, though likelihood of nausea increased with higher SOF or GS-331007 exposure. Exposure-response evaluation of cardiac events is described by the reviewer in Question 1.2.

5 LISTING OF ANALYSES CODES AND OUTPUT FILES

File Name	Description	Location	in
HemaBili_Analysis.R	Plots of hemoglobin versus bilirubin from the Phase III trials	Reviews\Ongoing Reviews\Sofosbuvir_NDA204671S000_J AF\ER Analyses	PM
ER_NEUTRINO.R	Data analysis codes for the NEUTRINO study	Reviews\Ongoing Reviews\Sofosbuvir_NDA204671S000_J AF\ER Analyses	PM
ER_Genotype23	Data analysis codes for P7977-1231, GS-US-334-0108, and GS-US-334-0107	Reviews\Ongoing Reviews\Sofosbuvir_NDA204671S000_J AF\ER Analyses	PM
Study_Data_Load.R	Loads Phase III study data and combines with post-hoc PK parameters from the popPK model	Reviews\Ongoing Reviews\Sofosbuvir_NDA204671S000_J AF\ER Analyses	PM
Renal_analysis.R	Evaluation of SOF and GS-331007 exposure with respect to renal function	Reviews\Ongoing Reviews\Sofosbuvir_NDA204671S000_J AF\ER Analyses	PM

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/s/

HUIMIN ZHENG
09/05/2013

SU-YOUNG CHOI
09/05/2013

SARAH E DORFF
09/05/2013

JEFFRY FLORIAN
09/05/2013

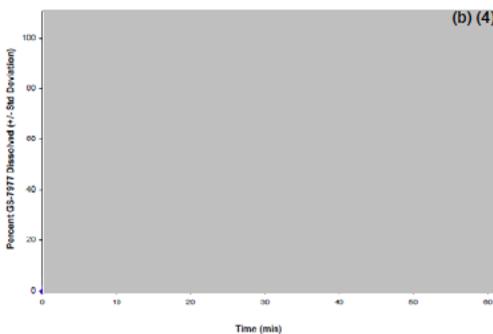
MICHAEL A PACANOWSKI
09/05/2013

YANING WANG
09/05/2013

SHIRLEY K SEO
09/05/2013

BIOPHARMACEUTICS REVIEW Office of New Drug Quality Assessment			
Application No.:	NDA 204671	Reviewer: Minerva Hughes, Ph.D.	
Submission Date:	6 April 2013		
Received Date:	8 April 2013		
Division:	Division of Anti-viral Products	Team Leader: Angelica Dorantes, Ph.D. Acting Supervisor: Richard Lostritto, Ph.D.	
Sponsor:	Gilead	Secondary Reviewer: Angelica Dorantes, Ph.D.	
Trade Name:	TBD	Date Assigned:	8 April 2013
		GRMP Date:	6 September 2013
		PDUFA Date:	8 December 2013 (orig)
Generic Name:	Sofosbuvir	Date of Review:	28 August 2013
Indication:	Hepatitis C infection	Type of Submission: Original NDA Review (NME – Priority)	
Formulation/strengths	Tablet, 400 mg		
Route of Administration	Oral		
Biopharmaceutics Review Topics:			
<ul style="list-style-type: none"> ○ Dissolution test method and acceptance criterion, ○ Formulation/process attributes impacting dissolution, and ○ Dissolution stability 			
<p>SUBMISSION: NDA 204671 was submitted in accordance with Section 505(b)(1) of the FDC Act for the use of sofosbuvir, 400 mg tablets, to treat chronic hepatitis C infection in adults with compensated liver disease, including cirrhosis. Sofosbuvir (SOF) is a novel nucleotide prodrug that potently inhibits genotype 1 to 6 HCV RNA replicons in vitro and has demonstrated sustained virologic response (SVR) rates when administered with ribavirin to subjects with chronic genotype 2 and 3 HCV infection and with pegylated interferon + ribavirin (PEG+RBV) to subjects with chronic genotype 1, 2, 3, 4, and 6 HCV infection.</p> <p>The Phase 3 program for SOF includes 4 clinical studies: P7977-1231 (FISSION), GS-US-334-0107 (POSITRON), GS-US-334-0108 (FUSION), and GS-US-334-0110 (NEUTRINO), which evaluated the efficacy and safety of SOF +RBV or SOF+PEG+RBV for various durations and genotypes.</p> <p>If approved, sofosbuvir will be the first nucleotide-analog HCV therapy.</p> <p>BIOPHARMACEUTIC INFORMATION: The proposed drug product is a film-coated tablet containing 400 mg SOF and the excipients mannitol, microcrystalline cellulose, croscarmellose sodium, colloidal silicon dioxide, and magnesium stearate, polyvinylalcohol, titanium dioxide, (b) (4), talc, and yellow iron oxide. The formulation and dose of the proposed commercial SOF product were selected based on two bioavailability and food effects studies and 1 pharmacokinetic study evaluating the effect of (b) (4) Form I and Form II on drug exposure. (b) (4)</p> <p>(b) (4)</p> <p>Dissolution is a critical quality attribute, which also is noted by the Applicant as critical for efficacy. A dissolution method (TM-195) was developed to assure the consistency of batch-to-batch dissolution performance.</p> <p>The Applicant's proposed dissolution method and acceptance criterion were as follows.</p> <ul style="list-style-type: none"> ▪ USP II, 900 mL 50 mM potassium phosphate, 75 rpm ▪ Q = (b) (4) 			

Lot DC1203B ((b) (4) Used in Fission-108 Phase 3 Study)



Product development incorporated design of experiment (DOE) studies and design space evaluation for which dissolution was used as a response factor in certain instances. Although design spaces were established for various parameters, the Applicant intends to operate only at the normal operating range. Any changes outside the normal operating range, even if within the design space, will adhere to current post approval guidelines. Nevertheless, dissolution was not appreciably influenced by parameters other than tablet compression force, and the proposed ranges support manufacturing product that meet the recommended regulatory specification.

CONCLUSIONS AND RECOMMENDATION:

1. Dissolution Method

The following dissolution method and acceptance criterion are acceptable.

Dissolution Method (TM-195)	
Parameter	
Apparatus	USP II
Medium	50 mM potassium phosphate buffer, pH 6.8, 900 mL
Temperature	37 °C
Paddle Speed	75 rpm
Analytical Assay	UV at 262 nm
Acceptance Criterion	Q = (b) (4) at 15 minutes

2. Biowaiver request

Bioavailability data from an in vivo study were submitted to satisfy the CFR requirement of characterizing the pharmacokinetics of the proposed product. The proposed commercial product was used in the pivotal clinical studies. Therefore, a biowaiver is not needed for this NDA.

3. Comments for Applicant

None. There are no outstanding Biopharmaceutics deficiencies.

4. Overall recommendation

From the perspective of Biopharmaceutics, NDA 204671 for Sofosbuvir Tablets is recommended for approval.

APPROVAL SIGNATURES: {see electronic signature page}

Minerva Hughes, Ph.D.
Biopharmaceutics Reviewer/ONDQA

Angelica Dorantes, Ph.D.
Biopharmaceutics Team Leader/ONDQA

BIOPHARMACEUTICS ASSESSMENT - REVIEW NOTES

1.0 GENERAL INFORMATION

1.1 Overview and Relevant Regulatory History

Original NDA 204671 was submitted in accordance with Section 505b of the FDC Act for the use of sofosbuvir (SOF), 400 mg tablets in the treatment of chronic hepatitis C virus (HCV). SOF, if approved, will be the first nucleotide prodrug for the treatment of chronic HCV infection. The tablets are intended for once daily administration, without regards to food.

HCV has significant genetic (RNA sequence) variability and is classified on this basis into at least 6 genotypes. The most common genotype in the US and in Europe is genotype 1 followed by genotype 2 and genotype 3. Genotype 4, 5, and 6 HCV infections are most prevalent in the Middle East, South Africa, and Southeast Asia, respectively.

The Phase 3 program for SOF includes 4 clinical studies: P7977-1231 (FISSION), GS-US-334-0107 (POSITRON), GS-US-334-0108 (FUSION), and GS-US-334-0110 (NEUTRINO). Studies P7977-1231, GS-US-334-0107, and GS-US-334-0108 evaluated the efficacy and safety of SOF+ ribavirin (RBV) in different genotype 2 and 3 HCV patient populations. Study P7977-1231 evaluated the efficacy and safety of SOF+RBV for 12 weeks versus the currently approved standard of care for genotype 2 or 3 HCV infection (PEG+RBV for 24 weeks) in treatment-naive subjects. GS-US-334-0107 evaluated the efficacy and safety of SOF+RBV for 12 weeks versus placebo in subjects with genotype 2 and 3 HCV infection who were interferon intolerant, interferon ineligible, or unwilling to take interferon. Study GS-US-334-0108 evaluated the efficacy and safety of SOF+RBV for 12 or 16 weeks in treatment-experienced subjects with genotype 2 and 3 HCV infection. Study GS-US-334-0110 evaluated the efficacy and safety of SOF+PEG+RBV for 12 weeks for treatment-naive subjects with genotype 1, 4, 5, and 6 HCV infection.

The clinical development program was conducted under IND 106,739 by Pharmasset, Inc., which was acquired on 17 January 2012, by Gilead Sciences, Inc. Several meetings were held between the Applicant and FDA to discuss the development program. The relevant Biopharmaceutics advice that was communicated under the IND is summarized below.

End of Phase 2 CMC Meeting – 11 October 2011

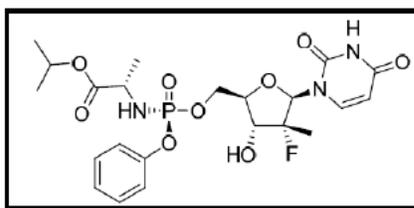
- [REDACTED] (b) (4)
- The dissolution method development report submitted for review lacked data evaluating the discriminating ability of the method. Therefore the reviewer (Dr. Angelica Dorantes) did not comment on the adequacy of the method, but communicated to the Applicant that the testing conditions seemed appropriate and that the specification should be where $Q =$ [REDACTED] (b) (4) occurs.

- The Sponsor was pursuing (b) (4) at this time and FDA requested assurance that adequate controls were in place to mitigate formation of the (b) (4). (b) (4) The Sponsor stated that if (b) (4)

1.2 Drug Substance Summary

The drug substance sofosbuvir is the nucleotide prodrug 2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate that is converted to the active uridine triphosphate form (GS-461203) within the hepatocyte and is a novel HCV NS5B-directed inhibitor that has displayed potent inhibition of HCV replicon (genotypes 1-6) RNA replication in vitro.

SOF has the following chemical structure and formula.



$C_{22}H_{29}FN_3O_9P$, MW = 529.45 g/mol

The drug substance has six chiral centers and is isolated as a single stereoisomer. (b) (4) have been isolated in laboratory studies. (b) (4)

(b) (4) The following illustration captures the timing of each use with respect to clinical studies.

The Applicant's rationale for the changes were as follows.

(b) (4) - To comply with regulatory and clinical preferences to have a (b) (4)



(b) (4)

A summary of the key physicochemical properties reported for (b) (4) is provided in supplemental tables appended to this report. The apparent solubility, intrinsic dissolution rates, and melting points are tabulated below.

	(b) (4)
Melting Point	
Apparent Solubility	
Intrinsic Dissolution Rate (mg/cm²/min)	

Reviewer's Evaluation:



(b) (4)

1.3 Drug Product Summary

The proposed drug product is a yellow, film-coated tablet (capsule shaped) comprised of the drug substance, and the excipients mannitol, microcrystalline cellulose, croscarmellose sodium, colloidal silicon dioxide, magnesium stearate, polyvinyl.

An (b) (4) for SOF tablets, 400 mg designated for Access countries differs only in the (b) (4) used. Access tablets will be (b) (4) whereas the commercial tablets will be yellow.

Several tablet formulations were used throughout development as the Applicant optimized the manufacturing process and tablet strengths, as illustrated below.

Comparison of Quantitative Compositions of SOF Clinical Trial Material with the To-Be-Marketed Formulation

	Phase 2	Phase 2, Phase 3	Phase 3	Phase 3 and Primary Stability	Designated Commercial (b) (4)
Sofosbuvir (Form I)	(b) (4)				
Sofosbuvir (Form II)					
Mannitol					
Microcrystalline Cellulose					
Croscarmellose Sodium					
Colloidal Silicon Dioxide					
Magnesium Stearate					
Total Tablet Core					
Tablet Core Weight (mg)					
(b) (4) (b) (4) (b) (4)					
Yellow					
Total Tablet Weight (mg) ^c	(b) (4)				
a					
b	(b) (4)				

With the exception of the (b) (4) the excipients were qualitatively the same throughout development. The transition from the (b) (4)

Minor changes in the (b) (4) manufacturing process were implemented when (b) (4) These changes included (b) (4)

(b) (4)

(b) (4)

Reviewer's comment: *The drug product development and manufacturing information were adequate to assess the impact of biopharmaceutics quality on overall product quality.*

1.4 Biopharmaceutics Classification System/Pharmacokinetics

A BCS Class 3 designation is proposed for the drug substance based on high solubility (dose solubility volume of less than 250 mL) and low apparent permeability. Therefore, the Applicant believes that the rate and extent of sofosbuvir dissolved is not likely to be solubility/dissolution-limited or impacted by gastrointestinal pH when formulated as an immediate release tablet formulation.

Solubility: Drug substance (b) (4) equilibrium solubility was determined at 37 °C across the pH range of 1.2 – 6.8. The solubility data are tabulated below.

Sofosbuvir Solubility at 37 °C

pH (Media)	Solubility (mg/mL) (b) (4)
1.2 ^a (HCl)	1.3
2.0 (HCl)	2.0
4.5 (Acetate Buffer)	2.1
6.8 (Phosphate Buffer)	1.9
FaSSIF (pH 6.5)	2.1
FeSSIF (pH 5.0)	1.8

Reference: PDM-1458

a (b) (4)

Sofosbuvir is a weak acid with a pK_a of 9.3 and exhibits pH-independent solubility over the physiological range. At pH 1.2 however, the Applicant notes that the drug substance (b) (4) (b) (4) and the apparent solubility is lower as a result.

Permeability: The permeability of sofosbuvir was determined using Caco-2 monolayers and 3 mM (1.6 mg/mL) sofosbuvir solutions. The permeability coefficients measured for apical to basolateral transport and basolateral to apical transport were 0.708×10^{-6} cm/s and 4.11×10^{-6} cm/s, respectively. The efflux ratio was 5.81. Sofosbuvir is considered to have low apparent permeability with efflux potential.

Reviewer's Evaluation: *A BCS 1 classification is not claimed. Therefore, an extensive review of the BCS classification information is not warranted for regulatory purposes (i.e., future BCS-based biowaiver eligibility). The Applicant also did not include extensive*

information for review. Regarding the solubility assessment, it is unclear what percentage of (b) (4) is contributing to the 1.3 mg/mL solubility limit at pH 1.2. A solubility limit of 1.3 mg/mL does not meet the BCS designation of high solubility (highest dose soluble in <250 mL). Approximately 308 mL of solution at pH 1.2 is required to dissolve the 400 mg tablet. Further, this Reviewer agrees with the 2004 CVM draft Guidance (#171) for Animal Drug Biowaivers position that a drug is highly soluble when it meets the USP solubility definition of at least soluble or 1 g is soluble in 10 to 30 mL of solution. The solubility data show that roughly 500 mL of solution is needed to dissolve 1 g, which puts this drug in the “slightly soluble” category. Thus, this reviewer is inclined to view the drug as a low soluble drug substance.

1.5 Biopharmaceutics Review Focus

This Biopharmaceutics review evaluates the following:

- (1) Dissolution method and acceptance criteria
- (2) Dissolution stability
- (3) Dissolution as a response variable in design of experiment studies

2.0 BIOPHARMACEUTICS REVIEW TOPICS

2.1 Dissolution Test Method

During the filing review, CMC requested clarification on whether different analytical procedures, or different versions of analytical procedures, than those submitted in this application were used for the NDA release and primary stability data in light of the Office of Regulatory Affairs (ORA) observations communicated in a form 483 dated 26 April 2013. In the 9 May 2013 amendment, the Applicant confirmed that some of the commercial analytical methods are indeed different from those used for release and stability testing during development. A tabular summary of the dissolution method changes throughout development is provided below.

	Development Method Test Parameters – 1	Development Method Test Parameters – 2	Commercial Method Test Parameters
Use Period:	(b) (4)		
References:			
Validation with IND/NDA reference:			
Dissolution Medium:			
Volume:			
Temperature:			
Paddle Speed:			
Determinant Step:			
HPLC Condition:			

The Applicant’s final proposed regulatory dissolution method is highlighted below.

Dissolution Method (TM-195)	
Parameter	
Apparatus	USP II
Medium	50 mM potassium phosphate buffer, pH 6.8, 900 mL
Temperature	37 °C
Paddle Speed	75 rpm
Analytical Assay	UV at 262 nm
Acceptance Criterion	Q = (b) (4)

2.1.1 Method Development Information (Report PDM-1524)

Drug pH Solubility

As noted previously, drug instability was noted at pH 1.2. Across the pH range of 2.0 – 6.8, and in FESSIF (Fed State Simulated Intestinal Fluid pH 5.0) and FaSSIF Fasted State Simulated Intestinal Fluid pH 6.5), the apparent solubility was approximately 2.0 mg/mL.

Reviewer’s Evaluation: Acceptable evaluation of drug solubility.

Apparatus Selection

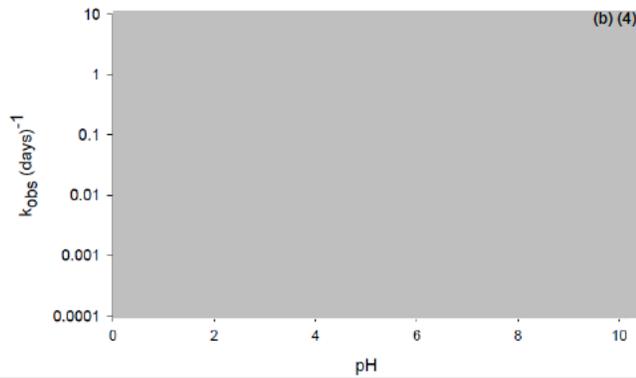
A USP 2 apparatus was selected (b) (4)

Reviewer’s Evaluation: The suitability of the apparatus selected should have been experimentally determined. However, a USP 2 apparatus is commonly used for tablet

products and is known to be amenable to developing a robust method. Therefore, its selection seems reasonable to this Reviewer.

Medium and Volume

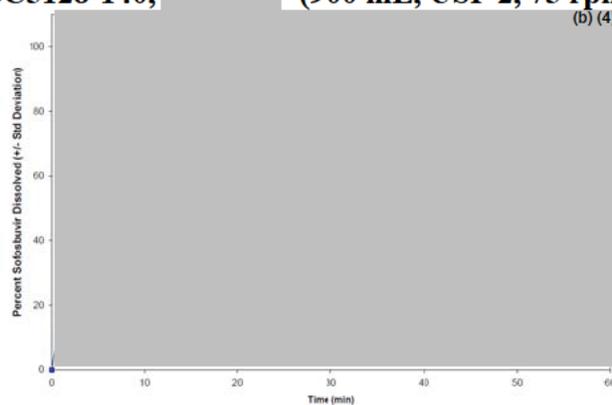
Dissolution was found to be rapid across the pH range (2.0 – 6.8). From the solution stability perspective, only the avoidance of acidic media was considered significant. The reported t_{95} at pH 2 is 0.6 days, confirming that low pH media is a poor choice for a robust QC dissolution test. There was acceptable sofosbuvir stability at pH 6.8 with the t_{95} at pH 6 and pH 7 reported to be 16.5 and 2.4 days. The pH-rate dependant profile is illustrated below.



[Redacted text block]

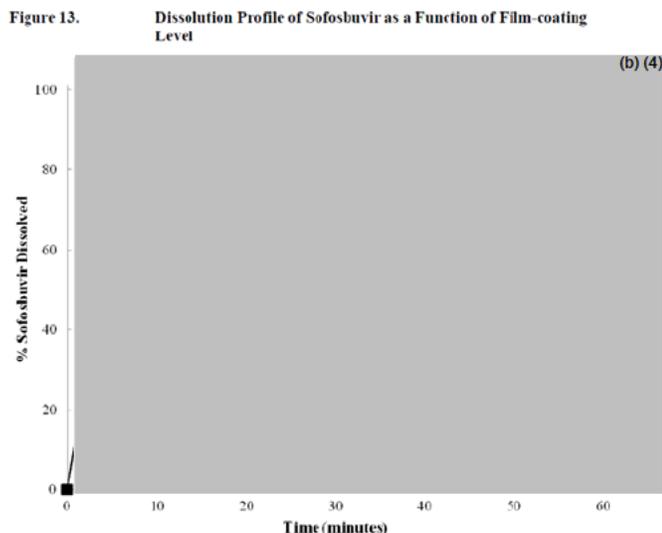
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Effect of Media on Dissolution of Sofosbuvir from Sofosbuvir Tablets, 400 mg (Lot# BC5128-140, (b) (4) (900 mL, USP 2, 75 rpm, 37°C)



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Reviewer's Evaluation: *The mean dissolution profiles were comparable across the range evaluated and adequately support a (b) (4) for the manufacturing process.*

Overall: *The Applicant completed a reasonable evaluation of the process parameters affecting dissolution. The impact of (b) (4) on dissolution was not clearly presented. However, since dissolution was not used as a response variable to define a design space, this deficiency is not critical for approval. Also, relying on a visual assessment of the dissolution profiles (i.e., individual data were not submitted) to ascertain differences was considered reasonable.*

3.0 INFORMATION REQUESTS DURING THE REVIEW

The following Biopharmaceutics Information Requests were communicated to the Applicant during the review.

12 July 2013 – IR

1. *In general, a drug substance's intrinsic dissolution rate correlates with the apparent dissolution of the drug product since the same solid state properties influence both dynamic processes (i.e., slow rate translates to slow dissolution). However, the characterization information on the major drug substance (b) (4)*

An adequate response was received on 23 July 2013.

2. *Your proposed dissolution acceptance criterion of $Q = (b) (4)$ minutes is not supported by the data and is not acceptable. The product performance data using the*

proposed method (USP 2, 50 mM potassium phosphate buffer at pH 6.8, 75 rpm) suggest that 15 minutes is a more appropriate sampling time point for routine quality control testing. Provide a revised drug product specification table reflecting a change in the dissolution acceptance criterion from $Q = \text{(b) (4)}$ minutes to $Q = \text{(b) (4)}$ at 15 minutes.

An inadequate response was received on 23 July 2013. The issue was followed up in the 8 August 2013 IR.

8 August 2013 - IR

- 1. FDA respectfully disagrees with your conclusion that the proposed QC dissolution method over discriminates at early time points and recommends a dissolution acceptance criterion of $Q = \text{(b) (4)}$ at 15 minutes. We acknowledge the relative bioavailability data submitted for finished tablets manufactured using (b) (4) and (b) (4) drug substance in Study GS-US-334-0131; however, these data are insufficient to support the conclusion that the two formulations are bioequivalent by current FDA standards. Moreover, your proposed commercial product is intended to use only (b) (4) drug substance and appropriate controls should be implemented to assure that the dissolution performance of future drug product (b) (4) lots is consistent with the observed performance of the (b) (4) product used in the pivotal clinical studies. It is not the general practice to establish quality controls that account for future unknown process or analytical variability. From FDA's perspective, a mean dissolution of (b) (4) at 15 minutes adequately supports an acceptance criterion of $Q = \text{(b) (4)}$ at 15 minutes and already accounts for reasonable process and analytical variability. Provide a revised drug product specification table reflecting a change in the dissolution acceptance criterion from $Q = \text{(b) (4)}$ to $Q = \text{(b) (4)}$ at 15 minutes on or before August 14, 2013.*

An adequate response was received in the 14 August 2013 amendment.

SUPPLEMENTAL TABLES AND FIGURES

Physicochemical Comparative Data for Drug Substance (b) (4)

Photostability of Sofosbuvir (b) (4) at 25°C Exposed for Visible and UV Light

Exposure Level	Sample	Total Degradation/Impurities (%)		
		Control	Exposed	Difference (Exposed - Control) (b) (4)
1.2 Million lx-hrs with UV energy of not less than 200 watt hrs/m ²	(b) (4)			(b) (4)
				(b) (4)

Solid-State Stability of Sofosbuvir (b) (4) Stored 30 Days at 40°C/75% RH (Hydrolytic Stability)

Sample	Initial	30 Days, Closed HDPE Container with Desiccant		30 Days, Closed HDPE Container		30 Days, Open HDPE Container	
	Total Deg. (%)	Total Deg. (%)	(b) (4)	Total Deg. (%)	(b) (4)	Total Deg. (%)	(b) (4)
a	(b) (4)						
b	(b) (4)						

Relative Physical Stability of Sofosbuvir (b) (4)

Attribute	(b) (4)
Hygroscopicity at 25 °C (cycled from 0 to 90% RH) ^a	(b) (4)
Physical Stability at 40 °C/75% RH (1 month, open container)	
Physical Stability when suspended in water (3 day slurry) ^a	
a	(b) (4)

Thermal Properties of Sofosbuvir (b) (4)

Attribute	(b) (4)
Melt Peaks	(b) (4)
Enthalpy of Melt	

Apparent Solubility of Sofosbuvir (b) (4) in 37 mM Acetate Buffer, pH 4.5 at 37°C

Aqueous Solubility (pH 4.5, 37 °C)	(b) (4)	(b) (4)
Dose Solubility Volume ^b	(b) (4)	(b) (4)
a	(b) (4)	(b) (4)
b	(b) (4)	(b) (4)

Intrinsic Dissolution Rates of Sofosbuvir (b) (4)

Intrinsic Dissolution Rate (mg/cm ² /min)	(b) (4)	(b) (4)

Phase 3 Study Design Overview

Phase 3 Studies				
P7977-1231 (FISSION)	SOF+RBV for 12 weeks or PEG+RBV 800 mg/day (2 divided doses) for 24 weeks	2, 3	Treatment-naive	Up to 20% of subjects may have had cirrhosis.
GS-US-334-0107 (POSITRON)	SOF+RBV or placebo for 12 weeks	2, 3	IFN intolerant, IFN ineligible, or unwilling to take IFN	Up to 20% of subjects may have had cirrhosis.
GS-US-334-0108 (FUSION)	SOF+RBV for 12 or 16 weeks	2, 3	Treatment-experienced	Up to 30% of subjects may have had cirrhosis.
GS-US-334-0110 (NEUTRINO)	SOF+PEG+RBV for 12 weeks	1, 4, 5, 6	Treatment-naive	Up to 20% of subjects may have had cirrhosis.

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/s/

MINERVA HUGHES
08/30/2013

ANGELICA DORANTES
08/30/2013

**CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS
FILING FORM/CHECKLIST FOR NDA/BLA or Supplement**

Office of Clinical Pharmacology

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA/BLA Number	204671	Brand Name	Not available
OCP Division (I, II, III, IV, V)	IV	Generic Name	Sofosbuvir (PSI-7977)
Medical Division	DAVP	Drug Class	HCV NS5B inhibitor
OCP Reviewer	Jenny H Zheng	Indication(s)	Treatment of chronic hepatitis C in adults in combination with other agents
OCP Team Leader	Shirley Seo	Dosage Form	400 mg tablets
Pharmacometrics Reviewer	Jeff Florian	Dosing Regimen	400 mg once daily with or without food - Genotype 1, 4, 5 and 6: SOF+Peg-interferon+ribavirin for 12 weeks - Genotype 2: SOF+ribavirin for 12 weeks - Genotype 3: SOF+ribavirin for 16 weeks
Pharmacogenomics Reviewer	Sarah Dorff		
Date of Submission	4/8/2013	Route of Administration	Oral
Estimated Due Date of OCP Review	9/6/2013	Sponsor	Gilead
Medical Division Due Date	12/08/2013	Priority Classification	Priority
PDUFA Due Date	12/8/2013		

Clin. Pharm. and Biopharm. Information

	“X” if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	x			
Tabular Listing of All Human Studies	x			
HPK Summary	x			
Labeling	x			
Reference Bioanalytical and Analytical Methods	x			
I. Clinical Pharmacology				
Mass balance:	x	1	1	
Isozyme characterization:	x	15	15	
Transporter characterization:	x	1	1	
Blood/plasma ratio:				
Plasma protein binding:		1	1	
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	x	1	1	
multiple dose:				
Patients-				
single dose:				
multiple dose:	x	1	1	
Dose proportionality -				
fasting / non-fasting single dose:	x			
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -		3	3	
In-vivo effects on primary drug:	x			
In-vivo effects of primary drug:	x			

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In-vitro:	x	12	12	
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				
geriatrics:				
renal impairment:	x	1	1	
hepatic impairment:	x	1	1	
PD -				
Phase 2:				
Phase 3:				
PK/PD -				
Phase 1 and/or 2, proof of concept:	x	7	7	
Phase 3 clinical trial:	x	4	4	
Population Analyses -	x	2	2	Both popPK reports include rich/sparse data
Data rich:				
Data sparse:				
II. Biopharmaceutics				
Absolute bioavailability				
Relative bioavailability -	x	3	3	
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:				
replicate design; single / multi dose:				
Food-drug interaction studies				
Bio-waiver request based on BCS				
BCS class				
Dissolution study to evaluate alcohol induced dose-dumping				
III. Other CPB Studies				
Genotype/phenotype studies	x			IL28B in Phase 3 trial reports
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies		53	53	18 in vivo PK studies +2 popPK/PD studies +29 in vitro studies +4 Phase 3 studies

On **initial** review of the NDA/BLA application for filing:

	Content Parameter	Yes	No	N/A	Comment
Criteria for Refusal to File (RTF)					
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?	x			
2	Has the applicant provided metabolism and drug-drug interaction information?	x			
3	Has the sponsor submitted bioavailability data satisfying the CFR requirements?	x			
4	Did the sponsor submit data to allow the evaluation of the validity of the analytical assay?	x			
5	Has a rationale for dose selection been submitted?	x			
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive	x			

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CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

	review to begin?				
7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can begin?	x			
8	Is the electronic submission searchable, does it have appropriate hyperlinks and do the hyperlinks work?	x			
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					
Data					
9	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?	x			
10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?	x			
Studies and Analyses					
11	Is the appropriate pharmacokinetic information submitted?	x			
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?	x			
13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?	x			
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?	x			
15	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?			x	
16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			x	
17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?	x			
General					
18	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	x			
19	Was the translation (of study reports or other study information) from another language needed and provided in this submission?			x	

IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE?

Yes

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

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

None.

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**CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS
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Reviewing Clinical Pharmacologist

Date

Team Leader/Supervisor

Date

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/s/

HUIMIN ZHENG
05/09/2013

SHIRLEY K SEO
05/10/2013