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APPLICATION NUMBER:

204671Orig1s000

MICROBIOLOGY REVIEW(S)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW ADDENDUM
NDA: 204671 **SDN**: 000 **DATE REVIEWED:** 11/08/2013
Virology Reviewer: Lisa K. Naeger, Ph.D.

NDA#: 204,671 **Serial #:** 000
Reviewer's Name(s): Lisa K. Naeger, Ph.D.

Sponsor's Name and Address:
Gilead Sciences, Inc
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SDN 031	09/17/2013	09/18/2013
SDN 033	09/26/2013	09/27/2013
SDN 038	10/07/2013	10/07/2013
SDN 039	10/08/2013	10/08/2013
SDN 040	10/08/2013	10/09/2013
SDN 041	10/09/2013	10/10/2013
SDN 043	10/11/2013	10/11/2013
SDN 047	10/16/2013	10/21/2013
SDN 052	11/01/2013	11/01/2013
SDN 055	11/12/2013	11/13/2013
SDN 058	11/18/2013	11/19/2013

Related/Supporting Documents: IND106739

Product Name(s)
Proprietary: TRADENAME
Non-Proprietary/USAN: Sofosbuvir
Code Name/Number: GS-7977, PSI-7977 (Pharmasset)

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

Molecular Weight: 529.46
Molecular Formula: $C_{22}H_{29}FN_3O_9P$

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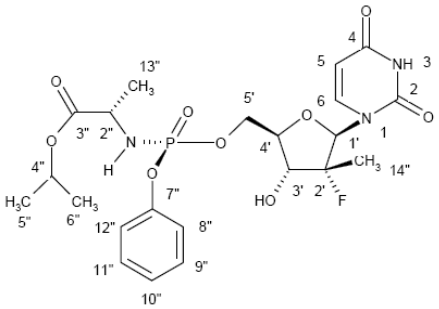
Structural Formula:



Sofosbuvir (SOF)

Dosage Form(s): tablet
Route(s) of Administration: Oral
Indication(s): nucleotide analog NS5B polymerase inhibitor indicated in combination with ribavirin (RBV) for the treatment of chronic genotypes 2 and 3 HCV infection and with pegylated interferon + RBV for the treatment of chronic genotype 1 HCV infection in adults.

Dispensed: Rx

Abbreviations: BL, baseline; DAA, direct acting antiviral; EC_{50}, effective concentration at 50%; FC, fold-change; FDA, Food and Drug Administration; GT, genotype; HCV, hepatitis C virus; HSA, human serum albumin; IC_{50}, inhibitory concentration at 50%; IFN, recombinant human interferon α; mt, mitochondria; NGS, next generation sequencing; NRTIs, nucleoside reverse transcriptase inhibitors; PBL, peripheral blood lymphocytes; PDVF, protocol defined virologic failure; PEG, pegylated human interferon; PR, protease; P/R, pegylated interferon/ribavirin; RBV, ribavirin; SDM, site-directed mutants; SOF, sofosbuvir; WT, wild-type; SVR, sustained virologic response

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EXECUTIVE SUMMARY

This NDA for sofosbuvir (SOF) is approvable with respect to virology for the treatment of HCV genotypes 2 and 3 in combination with ribavirin (RBV) or genotype 1 in combination with pegylated recombinant human interferon α + RBV. Sofosbuvir is a nucleotide prodrug of 2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate that is converted by cellular enzymes to the active uridine triphosphate form (GS-461203) within the hepatocyte and inhibits HCV replication. Subsequent to the original NDA review of SOF, further analyses were performed and additional studies were reviewed in this addendum virology review. This addendum also documents label negotiations with the sponsor.

1.1 Recommendations on Approvability

This NDA for sofosbuvir (SOF) is approvable with respect to virology for the treatment of HCV in combination with ribavirin (RBV) for Genotype 2 and 3 or with pegylated interferon + RBV for Genotype 1.

- Sofosbuvir in combination with pegylated interferon and ribavirin is indicated in adults with HCV genotype 1 or 4 infection.
- Sofosbuvir in combination with ribavirin is indicated in adults with HCV genotype 2 or 3 infection.
- Sofosbuvir in combination with ribavirin is indicated in adults with hepatocellular carcinoma meeting Milan criteria and awaiting liver transplantation.
- Monotherapy of sofosbuvir is not recommended for treatment of CHC.
- Treatment response to sofosbuvir varies based on baseline host and viral factors (See 14. Clinical Trials).

1.2 Recommendation on Post-Marketing Requirements

1. Determine the phenotypic susceptibility of sofosbuvir against:
   GT1a HCV replicons with
   - L159F
   - L159F+L320F
   - L159F+C316N
   - C316N, H, and F
   - L320F, S282R, and L320F+S282R
   - D61G
   - D61G+N62H, D and N

   GT1b HCV replicons with
   - L159F
   - L159F+L320F
   - L159F+C316N
   - C316N, H, and F
   - E440G

   GT2b HCV replicons with
   - L159F
   - L159F+L320F

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- L159F+C316N

GT3a HCV replicons with

- L159F
- L159F+L320F
- L159F+C316N
- K211R
- V321A
- P540L
- T542A

2. Clinical Virology

ON-TREATMENT FAILURE

Substitutions T84S+A150T+E202D emerged in one subject who experience on-treatment failure in Study P7977-1231(FISSION). The subject (Subject 1224-310357) was assessed for study drug adherence by plasma drug level analysis. At the Week 8 and EOT visits, the concentration of GS-331007 (the predominant metabolite of SOF) was below the assay lower limit of quantitation (i.e., < 10 ng/mL), which is indicative of noncompliance with study drug. The HCV from this subject also demonstrated these 3 NS5B substitutions as mixtures with wildtype at baseline. Phenotypic analysis demonstrated no change in EC_{50} value to SOF at either the baseline or Week 8 on-treatment virologic failure sample (Table 1; 1.11.3 Clinical Information Amendment, September 18, 2013, page 5). Additional analyses found that all 3 of these NS5B positions 84, 150, and 202 are highly polymorphic. Among the SOF Phase 3 studies GS-US-334-0107 (POSITRON), GS-US-334-0108 (FUSION) and P7977-1231 (FISSION), 19 additional subjects had triple substitutions A84S+A150T+E202D in NS5B at baseline. The presence of these triple substitutions at baseline did not appear to have a negative impact on the treatment outcome with 14/15 subjects (93%) who were treated with SOF+RBV and who completed follow-up visits achieving SVR12. An additional subject from the Phase 2 Study P7977-0523 (ELECTRON) had the T84S+E202D double substitutions at baseline and Week 4 post-treatment with phenotypic data available. Phenotypic analysis showed no change in susceptibility to SOF with the T84S+E202D variants (Table 1).

Table 1. Phenotypic Susceptibility of NS5B Substitutions at 84, 150 and 202

Subject ID	Study	GT	TimePoint	NS5B Substitutions at 84, 150 and 202	Fold Change in SOF EC_{50} from Reference
310357	P7977-1231	3a	Baseline	T84T/S, A150A/T, E202E/D	0.72
310357	P7977-1231	3a	Week 8 post-treatment	T84S, A150T, E202D	0.70
5062	P7977-0523	2b	Baseline	T84S, E202D	0.61
5062	P7977-0523	2b	Week 4 post-treatment	T84S, E202D	0.60

GT = genotype

EMERGENT SUBSTITUTIONS IN PHASE 3 CLINICAL TRIALS

For the analysis of SOF resistant substitution emergence, next generation nucleotide sequencing (NGS) data were submitted for 4 Phase 3 clinical trials. Of the 982 subjects in SOF+RBV or SOF/PEG/RBV groups of the Phase 3 Studies P7977-1231, GS-US-334-0107, GS-US-334-0108 and GSUS-334-110, a total of 224 subjects had NGS data with 676 files (Table 1). The raw sequence data were submitted in fastq format and resistance analysis tables were populated at a 15% cutoff by Gilead. The number of subjects with NGS data for Studies P7977-1231, 107, 108 and 110 were 78, 41, 76, and 29, respectively (Table 2). The FDA virology analyses of the NGS data was conducted using an internal NGS analysis pipeline that is described in detail in the NDA 204671 SDN002 review by Clinical Virology Reviewer Eric Donaldson, Ph.D. Briefly, the NGS analysis pipeline mapped the NGS sequence reads for each subject and timepoint to an appropriate reference sequence and used two variant detection methods (i.e., PVD75 and QbVD) to call variants. The variants called by these algorithms were then converted to amino acid frequency tables and resistance analysis tables to facilitate bioinformatics analysis and interpretation of the NGS data. The FDA analysis used a cutoff of 10% for emergence of substitutions (i.e., substitutions were not present at baseline but were present at a frequency of at least 10% at failure).

Table 2. Summary of Available Next Generation Sequencing Data

Clinical Trial	Subjects with NGS data	NGS files
P7977-1231 (FISSION)	**78**	**308**
GS-US-334-0107 (POSITRON)	**41**	**115**
GS-US-334-0108 (FUSION)	**76**	**189**
GS-US-334-0110 (NEUTRINO)	**29**	**64**
Totals	**224**	**676**

Summary of Emergent Substitutions in GT3 HCV-Infected Subjects

The substitutions listed in Table 3 were detected in at least 2 subjects and detected by 2 or 3 of the detection algorithms (QbVD, PVD75 and Gilead's algorithm). There were numerous other subjects whose virus had substitutions at these positions identified by only one algorithm.

Table 3. Summary of Treatment-Emergent Substitutions in GT3 HCV Identified with 2 or 3 Algorithms in 2 or more Subjects

NS5A POS	No. Subjects	SUB10 (+/-)	Ratio	Conservation	SOF EC_{50} FC from REF	SOF EC_{50} FC from BL
341	13	E341D		99.87%		
116	11	V116I/I116V/T116I	8-2-1	67%	1.23	1.1

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542	10	T542A/A542T	6-4	97%		
517	7	R517K/K517R	6-1	84%	0.6/0.7	0.9/1.1
535	7	K535N/N535K	5-2	52%		
11	6	V11I		78%	0.92	1.04
159	6	L159F		100%	0.71	0.91
250	6	K250R/R250K	5-1	86%		
114	5	K114R/R114K	4-1	62%	1.08	1.26
321	5	V321A		100%	1.2	0.75
106	4	K106R/R106K	3-1	96%		
113	4	G113S/S113G	3-1	93%		
156	4	A156P/P156S	3-1	96.5%		
520	4	I520V/V520I	3-1	76%	1.3	1.2
147	3	M147V		93%	1.4	1.55
438	3	V438I		89.6%		
519	3	R519K		84%	1.2	0.75

Highlighted positions are potential treatment-emergent substitutions that may be resistance-associated; these substitutions at conserved amino acid positions were not detected at baseline

The Subject IDs of subjects who had the key treatment-emergent substitutions L159F, V321A, E341D or T542A are listed in Table 4. These sites were conserved and occurred in multiple subjects across studies. Substitution E341D was a treatment-emergent substitution that developed in 13 subjects infected with GT3a HCV in two clinical trials (P7977-1231, n=10; GS-US-334-0107, n=3) and it was only found in subjects who relapsed. This substitution was reassessed by the sponsor and they concluded that the substitution was introduced as an artifact during PCR amplification and through cross-contamination during multiplexing. The Division agrees with the sponsor that the detection of emergent E341D was an artifact (please see Dr. Eric Donaldson's virology addendum review for a detailed explanation).

Table 4. Subjects with Key Emergent Substitutions detected by NGS

Substitution	Subject IDs	Genotype/Subtype	N
L159F	GS-US-334-0108-1055-1438 **GS-US-334-0108-5868-1526** **P7977-1231-1017-310257** P7977-1231-1140-310566 **P7977-1231-1228-310637** P7977-1231-1241-310504	3a	6
V321A	GS-US-334-0107-5665-7226 GS-US-334-0108-4238-1508 P7977-1231-1036-310286 P7977-1231-1220-310625 P7977-1231-1230-310369	3a	5

E341D	P7977-1231-310006 P7977-1231-310079 P7977-1231-310013 P7977-1231-310167 GS-US-334-0107-0529-7295 GS-US-334-0107-4262-7294 GS-US-334-0107-5730-7477 P7977-1231-1001-310307 **P7977-1231-1017-310257** P7977-1231-1031-310385 P7977-1231-1042-310013 P7977-1231-1079-310167 P7977-1231-1140-310563 P7977-1231-1216-310472 P7977-1231-1216-310488 P7977-1231-1224-310678 P7977-1231-1225-310195	1a (98.59% conserved) 1b (99.75% conserved) 3a (99.87% conserved)	N=13 15% (2/13) cirrhotics IL28B 15% CC (2/13) 62% CT (8/13) 23% CT (3/13)
T542A	GS-US-334-0108-0057-1482 GS-US-334-0108-0071-1518 **GS-US-334-0108-5868-1526** GS-US-334-0108-6214-1435 P7977-1231-1170-310428 P7977-1231-1171-310448 P7977-1231-1171-310470 P7977-1231-1192-310518 **P7977-1231-1228-310637** P7977-1231-1235-310549	3a (96.79% conserved)	**10**

Bolded subject ID had multiple substitutions

The L159F substitution emerged in 6 subjects infected with GT3a HCV in two clinical studies (P7977-1231, n=4; GS-US-334-0108, n=2) and is a previously identified NS5B substitution which with L320F was been shown to confer 3- to 5.5-fold decreased susceptibility to mericitabine and SOF (Tong X et al., 2013). The phenotypic susceptibility of subject isolates with L159F or V321A was tested by the applicant and the results showed no significant shift in SOF susceptibility. (b) (4)

Additional Baseline Response Analysis

SVR12 response in the presence of baseline NS5B substitutions T390I and F415Y (genotype 1 RBV-resistance associated substitutions) was analyzed. Overall response rates were 71% when these substitutions were present at baseline (Table 5). However, the numbers in each of the genotype/subtype subgroups were too small to make conclusions.

Table 5. Response by Baseline Substitutions T390I and F415Y

Baseline Substitution	Genotype	SVR12
F415Y	1a	4/4
	2b	1/2
	3a	0/1
Total		5/7 (71%)
T390I or V	1a	2/2
	1b	3/3
	2a/2c	0/1
	2b	3/3
	3a	4/8
Total		12/17 (71%)

PRE-TRANSPLANT STUDY P7977-2025

P7977-2025 is an ongoing Phase 2, open-label trial that evaluates the efficacy of SOF+RBV administered to subjects awaiting liver transplant prior to receiving the transplant in preventing HCV recurrence post liver transplant in subjects with GT1 through 6 HCV infection and hepatocellular carcinoma (HCC) meeting the Milan criteria prior to undergoing liver transplantation with an anticipated time until transplantation within one year. Treatment duration was for a maximum of 24 weeks before a protocol amendment or 48 weeks after protocol amendment or until time of transplant, whichever comes first. A total of 11 of 15 subjects (73%) who completed 24 weeks of SOF+RBV treatment relapsed in the pre-transplant phase. This finding led to the protocol amendment to extend the treatment duration from 24 weeks to 48 weeks or the time of transplant. Prevention of post-transplant reinfection is determined by a sustained post-transplant virological response (HCV RNA <lower limit of quantitation [LLOQ]) at 12 weeks post-transplant (pTVR12).

Thirty-six subjects reached post-transplantation week 12 and 64% (23/36) attained a 12 week post-transplant virologic response (pTVR) (Table 6). Subject 6928-7702, previously noted by the site investigator to have been transplanted with an HCV^+ donor liver was found to have been transplanted with a HCV^- liver, had HCV recurrence post-transplant and is now counted in the denominator as a failure. The majority of the subjects had HCV GT1 infection (72%; 36% GT1a and 36% GT1b) while 14%, 11%, and 3% had GT 2, 3, and 4 HCV infection, respectively. The pTVR12 rates for GT2 (n=5) and GT4 (n=1) were 100% and were 75% for GT3 (n=4) (Table 6). Response rates were lower for the subjects with GT1a and GT1b HCV with pTVR12 rates of 62% and 46%, respectively, consistent with SOF+RBV being a suboptimal regimen for GT1 subjects.

Table 6. Study P7977-2025: Post-transplant Virologic Response Rates at Week 12 by HCV Genotype

HCV Genotype	SOF+RBV, (N=36)
Overall pTVR12 90% CI	23/36 (63.9) 48.8-77.1
1a, n (%) 90% CI	8/13 (61.5) 35.5-83.4
1b, n (%) 90% CI	6/13 (46.2) 22.4-71.3
2, n (%) 90% CI	5/5 (100.0) 54.9-100.0
3, n (%) 90% CI	3/4 (75.0) 24.9-98.7
4, n (%)	1/1 (100.0)

pTVR = post-transplant virologic response
Source: Ad Hoc 6218

Summary of Resistance Analysis in Study 2025

Baseline Sanger population nucleotide sequencing of NS5B was performed for all 61 subjects enrolled in study P7977-2025. A full NS5B population sequence was obtained for the virus from 59 subjects and a short NS5B fragment population sequence (amino acids 270-311) for 2 subjects' virus.

In the pre-transplant phase, 17 of 61 subjects qualified for resistance testing. Of these 17 subjects, on-treatment HCV breakthrough was observed in 3 subjects (Subjects 0773-7734, 0585-7751, and 0451-7732), slower response to treatment was observed in 2 subjects (Subjects 0773-7712 and 1249-7720), early treatment termination occurred in 1 subject (Subject 0585-7758), and post-treatment relapse in 11 subjects (Subjects 0522-7707, 1028-7706, 1657-7704, 1657-7715, 6927-7713, 7585-7710, 0773-7717, 6927-7721, 1028-7724, 1657-7722, and 7585-7731) .

Seven of 11 subjects who experienced viral relapse after 24 weeks of SOF+RBV treatment were retreated with the same regimen (Subjects 1028-7706, 0522-7707, 7585-7710, 6927-7721, 1028-7724, 1657-7722, and 7585-7731). The sponsor stated that 1 to 6 substitutions were detected in NS5B at relapse in 6 of these subjects before retreatment. HCV RNA was re-suppressed in all 7 retreated subjects indicating that longer treatment may lead to viral clearance.

Full length NS5B amino acid sequences at baseline and virologic failure timepoints were derived by NGS with an assay cut-off of 1% in 16 of 17 subjects. Subject 0451-7732 did not qualify for resistance testing prior to transplantation due to HCV RNA levels <1,000 IU/mL. The sponsor provided resistance data via email correspondence for one additional subject (Subject 7702). The sponsor informed us that this subject had emergent low-level V321G, which did not persist. However, the NGS data for these 18

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relapse subjects were not submitted to the FDA. Thus, we have not been able to confirm the sponsor's resistance analysis for these subjects.

Five Subjects with On-Treatment Virologic Failure

Five subjects had on-treatment virologic failure (3 breakthroughs and 2 non-response) (Table 7). One subject with HCV RNA ≥LLOQ on the day of transplantation was not discontinued from the study. Four of these subjects had GT1 HCV (3 with GT1a and 1 with GT1b) and one subject had GT2b HCV. We requested the NGS data for these 5 subjects who experienced on-treatment virologic failure in order to confirm the sponsor's findings. The FDA analysis of the NGS data from these 5 subjects is described in detail in Dr. Eric Donaldson's secondary virology addendum review.

Table 7. Study P7977-2025: Summary of Subjects with On-Treatment Virologic Failure

Subject ID	GT	Age	Baseline Weight (kg)	Baseline HCV RNA (IU/mL)	Baseline Platelets ($x10^3/\mu l$)	Sex	Race	Baseline CPT Score	Prior Treatment (Response)	Ribavirin Dose[1] (mg/day)	Type of VF	Timing of VF
0451-7732	1a	73	91.2	2,350,000	91	M	W	7	Yes (Unknown)	600	Breakthrough	Week 16 (day of transplant)
0585-7751	1b	61	85.9	700,000	80	M	W	5	Yes (Null Responder)	1200	Breakthrough	Week 8
0773-7712	1a	52	98	3,760,000	60	M	W	7	No	1200	Nonresponse	Week 12
0773-7734	2b	56	64.7	14,900,000	134	M	W	5	No	1000	Breakthrough	Week 12
1249-7720	1a	55	74.5	1,620,000	89	M	W	5	Yes (Partial Responder)	1200	Nonresponse	Week 8

GT=genotype; VF = virologic failure; CPT – Child-Turcotte-Pugh score, w = white
1. Indicates ribavirin dose at the time of virololgic failure

- **Subject 0451-7732** (GT1a) had a baseline serum HCV RNA value of 2,350,000 IU/mL. At Week 8, the subject still had detectable HCV RNA at 42 IU/mL. A confirmatory HCV RNA measurement 7 days later reported <25 IU/mL. By Week 12, the subject's HCV RNA was TND; this increased to 215 IU/mL at Week 16, the day of transplantation. Therefore, the subject underwent a transplantation with HCV RNA >LLOQ. At Posttransplant Week 24, the subject had HCV RNA of 21,800,000 IU/mL. The subject is continuing in the follow-up phase of the study. Substitutions S/N62S/H, K/E124E, G/S166S and A421A/V were emergent at post-transplant Week 1 and 2. Plasma concentrations of GS-331007 (the predominant metabolite) were comparable to mean concentrations observed in Phase 3 studies.
- **Subject 0585-7751** (GT1b) had a baseline serum HCV RNA value of 700,000 IU/mL. By Week 3, the subject's HCV RNA was <25 IU/mL, which increased to 232 IU/mL at Week 8. The subject discontinued study treatment and HCV RNA was 1510 IU/mL at Posttreatment Week 4. The subject discontinued study treatment and the study due to efficacy failure. Substitutions S300S/T, Q514Q/R, K517R/K and S556S/G were emergent at Follow-up Week 4. Plasma concentrations of GS-331007 (the predominant metabolite) were comparable to mean concentrations observed in Phase 3 studies.

- **Subject 0773-7734** (GT2b) had a baseline serum HCV RNA value of 14,900,000 IU/mL. By Week 2, the subject's HCV RNA was TND. At Week 12, the subject's HCV RNA was 447 IU/mL. A confirmatory HCV RNA measurement 6 days later reported 6200 IU/mL. The subject discontinued study treatment for efficacy failure and discontinued study by withdrawal of consent. Substitutions A80A/T, P/Q130Q, T/A483A, R/H523H and G554G/A were emergent at Week 12. At the Week 1, 4, and 8 visits, plasma concentrations of GS-331007 were comparable to the mean concentrations observed in the Phase 3 studies. However, at the Week 12 visit (sample drawn 26 hours post dose), the GS-331007 plasma concentration was below the quantification limit (i.e., < 10 ng/mL), which is substantially lower than the mean GS-331007 plasma concentration in the Phase 3 population 24 hours post dose [160 ng/mL) indicative of noncompliance.
- **Subject 1249-7720** (GT1a) had a baseline serum HCV RNA value of 1,620,000 IU/mL, which decreased to 268 IU/mL at Week 8. The subject discontinued treatment at Week 8 due to non-response (efficacy failure). The subject's HCV RNA increased to 1,330,000 IU/mL by post-treatment Week 12. Due to progressive liver disease the subject was no longer a transplantation candidate and therefore discontinued the study. No substitutions emerged at the Week 8 timepoint, but S/N189S was present at the post-treatment pre-transplant Week 4 timepoint. Plasma concentrations of GS-331007 (the predominant metabolite) were comparable to mean concentrations observed in Phase 3 studies.
- **Subject 0773-7712** (GT1a) had a baseline serum HCV RNA value of 3,760,000 IU/mL, which decreased to 1490 IU/mL at Week 8. The subject discontinued treatment at Week 12 due to non-response (efficacy failure). The subject's HCV RNA increased to 4,680,000 IU/mL by posttreatment Week 12. The subject discontinued the study due to efficacy failure. Substitutions A252A/V and N273N/S were emergent at Week 8, N273S at Week 12 and I405V/I and F575F/L at post-treatment pre-transplant Week 4. Plasma concentrations of GS-331007 (the predominant metabolite) were comparable to mean concentrations observed in Phase 3 studies.

The presence of nucleotide resistance-associated variants was evaluated at virologic failure by population and deep sequencing with a 1% assay cut-off for the 5 subjects who experienced breakthrough or had a slower response (Table 8). No S282T was detected at virologic failure among these subjects. However, S282R and L320F NS5B variants were detected at 1.1% and 1.8%, respectively at Week 4 post-treatment in Subject 0773-7712 (genotype 1a) who experienced slow-response to treatment. Previous phenotypic analyses of an S282R site-directed mutant were not successful due to the lack of replication of this mutant in cell culture. L320F site-directed mutants of genotype 1a or 1b replicons were successful and had <2.5-fold increase in SOF EC_{50} value. The combined substitutions have not been assessed phenotypically.

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Table 8. Study P7977-2025: Nucleotide Resistance-Associated Variants at Baseline and Virologic Failure Timepoints by Population and Deep Sequencing

Subject ID	GT	Baseline NI RAVs		Post-Baseline NI RAVs		Post-Baseline Changes from Baseline at Polymorphic Sites
		Population Sequencing	Deep Sequencing[1]	Population Sequencing	Deep Sequencing[1]	Population Sequencing
0773-7712	1a	None	None	None	S282R (1.1%) L320F (1.8%)	A252A/V N273N/S I405V/I F574F/L
1249-7720	1a	None	None	None	None	S/N189S
0451-7732	1a	None	None	None	L159F (9.5%)	S/N62S/H K/E124E G/S166S A421A/V
0585-7751	1b	L159F	L159F	L159F	L159F	S300S/T Q514Q/R K517R/K S556S/G
0773-7734	2b	None	None	None	L159F (2.1%)	A80A/T P/Q130Q T/A483A R/H523H G554G/A

GT = genotype, NI = nucleoside inhibitor, RAVs = resistance-associated variants
1. Deep sequencing conducted with a 1% assay cut-off

At baseline, the HCV isolate from Subject 0585-7751 with GT1b HCV, had L159F which is a polymorphic site among genotype 1b isolates. This subject also had the substitutions C316N at baseline. In addition, 3 genotype 1b infected subjects (Subjects 6437-7723, 6437-7735 and 6437-7736) who experienced post-transplant relapse had the L159F+C316N substitutions at baseline. In GT1b, L159 is 91% conserved and C316 is 82% conserved. Baseline prevalence of L159F across treatment-naïve genotype 1b subjects is approximately 9%. Subject 0451-7732 (genotype 1a) and Subject 0774-7734 (genotype 2b) had detectable L159F at breakthrough at 9.5% and 2.1%, respectively. In GT1a, L159 and C316 are 99% conserved. In GT2b, L159 and C316 are 100% conserved. In contrast to the transplant study, baseline analysis across all Phase 3 SOF treatment studies did not show a negative impact of the L159F substitution on viral response. In the sponsor's phenotypic assays, L159F site-directed mutants in genotype 1a or 1b replicons had <2.5-fold increase in SOF EC_{50} values. However, the L159F substitution with L320F has been shown to confer 3- to 5.5-fold decreased susceptibility to mericitabine and SOF by Tong et al. (Tong X et al., 2013).

Summary of Resistance Findings in the Pre-Transplant Study 2025

The resistance data from both population and deep sequencing of subjects who had on-treatment failure (n=5) and subjects who relapsed (n=20) in the Pre-transplant study P7977-2025 provided supportive information for the FDA resistance findings in the SOF Phase 3 studies. The presence of substitution L159F at baseline was associated with SOF breakthrough and relapse in 4 subjects infected with GT1b HCV, and this substitution emerged on treatment in 2 subjects who were infected with GT1a HCV (one breakthrough and one relapse) and 1 subject infected with GT2b HCV (breakthrough) (Table 9). These 4 subjects' virus also had the C316N substitution along with the L159F substitution at baseline. A total of 6 subjects' virus had the C316N substitution in their GT1b HCV at baseline in this trial and all were post-transplant relapsers. In addition, S282R and L320F substitutions were detected by deep sequencing in the on-treatment sample from a subject infected with GT1a HCV who did not respond to SOF.

Table 9. Summary of Resistance Data from Pre-Transplant Study P7977-2025

PID	GT	IL28B	Reason for Failure	Prior Experience	Baseline NS5B Substitutions	Emergent NS5B Substitutions
P7977-2025-0451-7732	1a	CT	DAA BT			A421A/V **L159F (9.5% NGS)**
P7977-2025-0773-7734	2b	CC	DAA BT Week 12	NAIVE		A80A/T P130Q T483A/T R523H G554A/G **L159F (2.1% NGS)**
P7977-2025-0773-7712	1a	CT	DAA NR	NAIVE		A252A/V N273N/S **S282R (1.1% NGS) L320F (1.8% NGS)**
P7977-2025-1028-7706	1a	CT	Relapse FU4 Retreated but died	P/R NULL		**L159L/F** E86E/K A486A/V
P7977-2025-0585-7751	1b		DAA BT	P/R NULL RESPONDER	**L159F** C316N	Q514Q/R
P7977-2025-6437-7723	1b	CT	PTX Relapser after 12 weeks trt	NAIVE-ALL	**L159F** C316N	
P7977-2025-6437-7735	1b	CT	PTX Relapser After 4 weeks trt	P/R PARTIAL RESPONDER	**L159F** C316N	T77T/A
P7977-2025-6437-7736	1b	CT	PTX Relapser after 16 weeks trt	P/R PARTIAL RESPONDER	**L159F** C316N	I323I/V
P7977-2025-0522-7737	1a	TT	PTX Relapser after 20 weeks trt	P/R NULL		
P7977-2025-1028-7705	1a	CT				
P7977-2025-1249-7720	1a	TT	DAA NR	P/R + PI PARTIAL RESPONDER		N189S
P7977-2025-1651-7748	1a	CT	PTX relapse after 28 weeks trt	P/R NULL	415Y	V131V/I T235T/V E237E/G Q309R S335S/N A487T K523K/R
P7977-2025-1657-7715	1a	CT	DAA Relasper	P/R EXP		I178I/L

P7977-2025-6926-7762	1a	CC	DC while suppressed	P/R EXP		
P7977-2025-6927-7713	1a	CT	DAA relapser	NAIVE		S189S/N
P7977-2025-0585-7758	1a	CT	Relapsed after ET Week 4			
P7977-2025-0522-7707	1b	TT	Relapse after 24 Weeks trt; Retrt	P/R EXP		
P7977-2025-0773-7717	1b	CT	DAA relapse; PTX relapser	IFN		
P7977-2025-0773-7730	1b	CT	PTX relapse after 4 weeks trt	P/R RELAPSER		K124E Q514R
P7977-2025-1028-7724	1b	CT	Relapse after 24 Weeks trt; Retrt	P/R PARTIAL RESPONDER		
P7977-2025-1657-7704	1b	TT	DAA Relapser; PTX relapser	P/R NULL RESPONDER		E440E/G
P7977-2025-1657-7722	1b	TT	DAA relapse after 24 wks trt; Retrt		C316N	
P7977-2025-6437-7718	1b	TT	PTX relapse after 16 weeks trt	P/R RELAPSER	C316N	L68F L392F
P7977-2025-6928-7702	1b	TT	PTX Relapser after 2 weeks trt	OTHER UNKNOWN		V321G via email correspondence
P7977-2025-7585-7710	1b	CT	DAA relapse after 24 weeks trt; retrt 8 weeks; PTX not detectable at wk 12			
P7977-2025-7585-7731	1b	CT	DAA Relapser	P/R UNKNOWN		
P7977-2025-6927-7721	2b	CC	DAA Relapser	NAIVE		D213N
P7977-2025-0585-7752	3a	CT	DAA Relapser after 4 weeks trt	OTHER NON-RESPONDER		I85I/V R203C 250K

All these subjects had cirrhosis; Bolded PIDs are post-treatment relapsers according to the sponsor.

The sponsor argues the presence at only ~1% of the HCV population cannot explain the observed slow-response or breakthrough to SOF with >99% wild-type HCV. However, these substitutions are unfit and may not persist long enough to be detected at a high frequency, yet may still contribute to viral breakthrough and relapse. Many of the GT3a relapser samples were collected weeks after termination of treatment, so it is possible that F159 was present in the relapse samples while on-treatment, but rapid displacement would result in no detectable F159 in samples that were taken too long after relapse (>2 weeks).

There are limited data on the persistence of these substitutions following treatment, but some data are available from two subjects. In the GT2b SOF monotherapy subject, the S282T substitution emerged at Week 4 post-treatment and was no longer detected at Week 12 post-treatment. In one breakthrough subject from the pre-transplant study 2025, L159F was present at a frequency of 9.5% at Post-Transplant Week 1 and this dropped to 1.2% by Post-Transplant Week 2. The long term clinical consesquences of the emergence of these substitutions is not known, but retreatment studies may provide informative data.

Phylogenetic Analysis of NS5B Baseline Nucleotide Sequences

Gilead conducted a phylogenetic analysis of baseline NS5B sequences and evaluated nucleoside inhibitors resistance-associated variants and other NS5B substitutions for the 5 subjects that exhibited on-treatment virologic failure. Phylogenetic analysis showed no apparent clustering of baseline NS5B sequences from the 5 subjects who experienced on-treatment virologic failure as compared with all other subjects in the pre-transplant study P7977-2025 (Figure 1). This result is consistent with the observation that virologic failures were seen across different HCV genotypes.

Summary of Available Preliminary Pharmacokinetic Data

Single PK samples were collected at all study visits and are being analyzed on an ongoing basis. Per protocol, plasma concentrations of the predominant circulating metabolite, GS-331007 were measured and are available for the 5 subjects experiencing on-treatment virologic failure (Table 10). In an effort to provide exposure metrics, individual GS-331007 exposures were estimated using the established population PK model for GS-331007 included in NDA 204671.

For Subject 0773-7734, specifically, Gilead has noted that although the population PK estimate of GS-331007 is comparable to the mean Phase 3 exposure, examination of the GS-331007 plasma concentrations from each visit suggested non-compliance with study drugs at or around the week 12 study visit coincident with the increase in HCV RNA (Refer to page 19 NDA 204671 Sequence No. 0012, dated 29 July 2013). The population PK estimate of exposure in this subject is derived from measurable concentrations at earlier study visits and as such, is not negatively impacted by the non-compliance at or around the week 12 study visit. None of the other subjects had evidence of non-compliance based on examination of the GS-331007 concentration data. In addition, a review of concomitant medications in these 5 subjects did not identify use of any agents that may have resulted in potential drug-drug interactions.

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Figure 1. Study P7977-2025: Phylogenetic Analysis of NS5B Baseline Nucleotide Sequences

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1. The phylogenetic tree was constructed using the Neighbor-joining method with 500 bootstrap replications.
2. The tree is constructed based on n=39 baseline sequences from Study P7977- 2025 (two subjects were not included in the analysis because only partial sequences were obtained at baseline).

Table 10. Preliminary Population PK Estimates of GS-331007 Exposure in Subjects Receiving SOF+RBV

Subject ID	GS-331007 AUC_{tau} (ng*hr/mL)
0451-7732	8260
0585-7751	7250
0773-7712	4060
0773-7734	10,000
1249-7720	8940
Population Mean (%CV) from Phase 3* (n=986) [Min, Max]	7200 (30.5) [1780, 17,400]

*Population PK derived GS-331007 AUC in HCV infected subjects following multiple dose administration of SOF 400 mg + ribavirin± PEG-IFN

POTENTIAL IMPACT OF C316N ON THE EFFICACY OF SOFOSBUVIR IN SUBJECTS INFECTED WITH HCV GT1B

In the Phase 3 Study 110, GT1b subjects showed an overall SVR12 rate 10% lower than GT1a Subjects (82% vs. 92%). The lower SVR12 rates for subjects with GT1b HCV were also seen in the PHOTON study where GT1b subjects receiving 24 weeks of SOF+RBV had overall response rates of 54% (13/24) compared to response rates of 82% (74/90) for GT1a subjects. Therefore, Dr. Eric Donaldson performed a sequence and structural bioinformatics analyses to determine if amino acid differences between these 2 genotypes in the NS5B polymerase could account for this efficacy difference (Please see Dr. Donaldson's Addendum Virology review). Dr. Donaldson generated a structural comparison using PyMol to model the X-ray crystal structures of NS5B for GT1a with the C316 residue and GT1b with the N316 residue using structures with the following accession numbers: 3HKW (GT1a - H77) and 3HHK (GT1b - Con1). He found a notable difference at position 316, which is near the catalytic triad, where C316 was present in the GT1a structure and N316 was present in the GT1b structure. N316 found in the HCV GT1b polymerase structure is a larger and more bulky amino acid with two additional side chains. Given the increased bulk present with this amino acid, it is possible that N316 or additional substitutions that add bulk (i.e., histadine, phenylalanine), could sterically interfere with the ability of sofosbuvir to interact with to the active site to inhibit the HCV polymerase.

Data from the Phase 3 trial GS-US-334-0110 and from the pre-transplant study P7977-2025 were analyzed to determine the potential impact of substitutions at the 316 position in GT1a and GT1b HCV. C316 is 99.89% conserved in the GT1a NS5B and is 81.83% conserved in GT1b polymerase.

In study GS-US-334-0110, all of the subjects infected with HCV GT1a had virus with C316 in the polymerase at baseline. One GT1a subject, 0530-6412, in this study had emergent C316F detected at relapse at Week 4 follow-up timepoint. Of note, development of a C316N substitution is unlikely as it requires 2 mutations in the cysteine codon.

Among subjects in study GS-US-334-0110 infected with HCV GT1b, no subjects developed substitutions at position C316 over the course of treatment. However, 6 GT1b subjects had C316N (n=4) or C316H (n=2) at baseline and in both of these subgroups the SVR12 rate was 50% (Table 11). Of note, among GT1b subjects viruses that had C316 in NS5B at baseline, the SVR12 rate was 83% (52/63).

Table 11. SVR12 Rates for GT1a and GT1b Subjects with Substitutions at 316

	Study 110	Study 2025
GT1b	82% (55/67)	26% (5/19)
C316	85% (51/60)	38% (5/13)
C316N	50% (2/4)	0% (0/6)
C316H	50% (1/2)	na
GT1a		
C316	92% (207/226)	42% (8/19)

An examination of each of the GT1b subjects with C316N or H showed that most of the subjects who had virologic failure also had other baseline factors such as non-CC IL28B genotypes, high viral loads and cirrhosis (Table 12). The presence of a bulky amino acid at C316 may act as another baseline factor that predicts poor response, but additional data will be needed to answer this question.

Table 12. GT1b Subjects with C316N or H

PID	316	SVR12	IL28B	Cirrhosis	Baseline Viral Load
0110-0532-6602	N	n	TT	Yes	1,440,000
0110-0532-6672	N	y	CC	No	1,220,000
0110-5586-6473	N	n	CC	No	1,710,000
0110-7864-6536	N	y	CC	No	12,200,000
0110-3060-6450	H	y	CT	No	4,770,000
0110-4308-6656	H	n	TT	No	1,210,000
2025-0585-7751	N	n	Prior Null	Yes	700,000
2025-1657-7722	N	n	TT	Yes	8,610,000
2025-6437-7718	N	n	TT	Yes	2,850,000
2025-6437-7723	N	n	CT	Yes	3,180,000
2025-6437-7735	N	n	CT	Yes	405,000
2025-6437-7736	N	n	CT	Yes	6,880,000

In summary, C316N is the only difference between HCV GT1a and GT1b polymerase in the active site. Substitutions that add bulk to this position are predicted to interfere with sofosbuvir's ability to enter the active site and inhibit HCV RNA replication. C316 is highly conserved in GT1a (99.89%) but not in GT1b (81.83%). The presence of C316N/H/F at baseline has been associated with sofosbuvir failure in 10 subjects infected with HCV GT1b in studies P7977-2025 and GS-US-334-0110. Subjects infected with HCV GT1b with C316N/H at baseline had SVR12 rates of 0% (0/6) and 50% (3/6) in studies P7977-2025 and GS-US-334-0110, respectively. One subject infected with HCV GT1b who

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relapsed after treatment with sofosbuvir had emergent C316F. Therefore, substitutions at C316, which is a polymorphic site in the GT1b NS5B polymerase, may contribute to reduced efficacy among subjects infected with this genotype of HCV, but additional future resistance and efficacy data will be needed to address the affect of C316 substitutions.

SUBJECTS WITH MISCLASSIFIED HCV

The Division performed their analyses using the Intent-to-Treat approach and included the 9 subjects who were found by NS5B sequencing to be infected with GT1 HCV although they had been screened as GT2 by the LiPA assay. Gilead performed additional analysis on these 9 subjects' viruses which were misclassified based on the LiPA assay (Table 13).

Table 13. LiPA and NS5B Sequencing Results from the Nine Genotype 2 Subjects in P7977-1231 (FISSION) and GS-US-334-0108 (FUSION)

Study	Patient ID	Subtype by LiPA	Subtype by Core/E1 Sequencing	Subtype by NS5B Sequencing	Treatment Arm	SVR
FUSION	1500	2b	2b	1a	SOF+RBV 16 wks	Yes
FUSION	1405	2b	2b	1b	SOF+RBV 12 wks	Yes
FUSION	1412	2b	2b	1a	SOF+RBV 16 wks	No
FUSION	1542	2b	2b	1a	SOF+RBV 12 wks	No
FUSION	1507	2a/2c	1b/2k	1b	SOF+RBV 12 wks	No
FUSION	1480	2b	2b	1b	SOF+RBV 16 wks	No
FISSION	310006	2b	2b	1a	SOF+RBV 12 wks	No
FISSION	310079	2b	2b	1b	SOF+RBV 12 wks	No
FISSION	310114	2[a]	na	1a	SOF+RBV 12 wks	Yes

[a] = LIPA assay failed to determine the genotype at screening and historical genotype data indicated genotype 2; na = Not Applicable

In 2 of the sofosbuvir (SOF) Phase 3 studies, 9 genotype 2 subjects' viruses, determined by LiPA, were found to have genotype 1 NS5B sequences. In order to determine if the discordance resulted from an assay error or from intergenotypic recombination of the viruses, HCV core sequences were amplified and sequenced from 8 of the 9 subjects. The core sequences from all eight subjects' viruses were genotype 2, consistent with the LiPA assay. This result confirmed that the LiPA assay was correct and indicates that these eight subjects have intergenotypic recombinant HCV viruses. For the one remaining subject, the sequencing analysis of the core gene is still ongoing. Although the core sequence is not available from this subject (Subject 310114), it is possible that Subject 310114 also has an intergenotypic recombinant HCV virus based on the

historical LiPA data of genotype 2 for this subject. Further sequencing analysis is ongoing to determine the recombination points for these 9 viruses. Previously, several other natural intergenotypic recombinant HCV strains have been described in the literature (V. Morel et al., 2011). In these studies, the 5' part of the genome was always related to GT2, similar to the cases in this study.

We looked more closely at these 9 subjects, including 3 additional subjects from the HIV/HCV co-infected Study 123 (identified as GT2 at screening by LiPA but were found to have GT1 NS5B), by region and investigator to see if these recombinant viruses were specific to a given region (Table 14). All these subjects except one were from the USA, but come from all over the US. One subject was from Puerto Rico.

Table 14. Subjects from the HIV/HCV Co-Infected Study 123 Identified as GT2 at Screening by LiPA but GT1 by NS5B Sequencing

PID	Screening Genotype	Analysis Genotype	IL28B	Arm	SVR12	Region	Demo	Investigator
0108-0057-1480	2b	1b	CC	SOF+RBV 16 Weeks	N	USA	White 62 yr male	Jay Lalezari SF
0108-0451-1507	2a/2c	1b	CT	SOF+RBV 12 Weeks	N	USA	White 53 yr male	Eugene Schiff Miami
0108-0530-1405	2b	1b	CC	SOF+RBV 12 Weeks	Y	USA	White 68 yr female	Tarek Hassanein San Diego
1231-1004-310079	2b	1b	CC	SOF+RBV	N	USA	White Hispanic 36 yr male	Maribel Rodriguez Torres Florida
0108-0521-1542	2b	1a	CT	SOF+RBV 12 Weeks	N	USA	White 63 yr male	Michael Bennett San Diego
0108-0535-1412	2b	1a	CC	SOF+RBV 16 Weeks	N	USA	White 56 yr male	Kris V Kowdley Seattle Wash
0108-5852-1500	2b	1a	CC	SOF+RBV 16 Weeks	Y	USA	White 53 yr male	Keyur Patel NC
1231-1019-310006	2b	1a	CT	SOF+RBV	N	USA	White 61 yr male	Tarek Hassanein San Diego
1231-1239-310114	2	1a	CC	SOF+RBV	Y	USA	White 55 yr male	Zeid Kayali Las Vegas
0123-0734-8815	2	1a	CT	SOF+RBV 24 weeks	Y	USA	Black 58 yr male	Daar, Eric CA
0123-4262-8738	2	1a	CT	SOF+RBV 24 weeks	Y	PRI	White Hispanic 50 yr male	Rodriguez-Torres NC

0123-7446-8910	2	1a	CC	SOF+RBV 24 weeks	N	USA	White 52 yr male	Bansal NY

STUDY 110 GT1 BASELINE FACTOR SUBGROUPS ANALYSIS

Analysis of GT1 subgroups with baseline factors that typically predict poor response showed that subjects with multiple baseline factors such as cirrhosis, GT1b and IL28 non-CC alleles had lower SVR12 rates. SVR12 rates ranged from 67% to 78% when subjects had two of the following baseline factors: GT1b, non-CC IL28B genotype, or cirrhosis (Table 15). When subjects had all 3 of these baseline factors, the response rate was 57%. The numbers of subjects in some of these subgroups were small.

Table 15. Response by Baseline Factors: Percentage of GT1 Subjects Receiving 12 weeks SOF +P/R Achieving SVR12 in Study 110

	SVR12 after 12 Weeks SOF+PEG+RBV N=327
GT1a	92% (207/226)
GT1b	82% (55/67)
IL28 CC	98% (85/87)
IL28 CT/TT	86% (177/207)
No Cirrhosis	91%(217/238)
Cirrhosis	81% (42/52)
GT1b CT/TT	78% (42/54)
GT1b Cirrhotics	67% (6/9)
CT/TT Cirrhotics	72% (26/36)
GT1b CT/TT Cirrhotic	57% (4/7)

STUDY 1231 (FISSION) RESULTS IN GT3 SUBJECTS BY GEOGRAPHICAL REGION

In the Phase 3 studies of GT2 and GT3 subjects, Study 1231 (FISSION) was the only study that included treatment-naïve subjects from the EU. The non-EU subjects had numerically higher SVR12 rates than the EU subjects in both arms (Table 16). This is relevant, because the VALENCE study was only done in the EU and the VALENCE results are being used to support 24-week duration of SOF+RBV for GT3 and GT1 interferon-ineligible or intolerant.

The SVR12 rates were analyzed by IL28B genotype for the GT3 subjects from both the non-EU and EU regions (Table 16). The SVR12 rates were higher for IL28B CC genotypes in EU GT3 subjects but were still lower than the SVR12 rates in non-EU GT3 subjects. The proportion of IL28B subgroups does not appear to explain the lower SVR12 rates in the EU. Although there were a lower number of subjects in the SOF+RBV arm with IL28B CC genotype in the EU population than in the non-US

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population, the numerical difference does not seem high enough to explain the difference in SVR12 rates.

Table 16. SVR12 Rates for GT3 Subjects in Study 1231 by Region

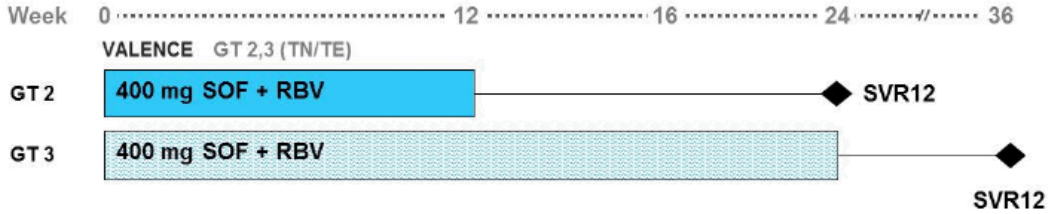
	12-week SOF+RBV	Proportion	24-week PEG+RBV
North America, Australia/New Zealand	58% (98/168)		63% (105/167)
IL28b CC	59% (41/70)	42% 70/166	75% (52/69)
IL28b CT	63% (50/80)	48% 80/166	61% (44/72)
IL28b TT	44% (7/16)	10% 16/166	32% (8/25)
EU	27% (4/15)		56% (5/9)
IL28b CC	40% (2/5)	33% 5/15	67% (2/3)
IL28b CT	22% (2/9)	60% 9/15	50% (2/4)
IL28b TT	0/1	7% 1/15	50% (1/2)

EU=NLD (n=4), SWE (n=8) and ITA (n=12)

VALENCE STUDY GS-US-334-0133

Study 133 is an ongoing Phase 3, randomized, double-blind, placebo-controlled study, which examined the safety, tolerability, and antiviral efficacy of SOF and RBV compared with SOF placebo and RBV placebo in treatment-naive or treatment-experienced subjects with chronic GT2 or 3 HCV infection in Europe (Figure 2). Initially, eligible subjects were randomized in a 4:1 ratio to receive either SOF 400 mg administered once daily + RBV total daily dose of 1000 or 1200 mg administered in a divided daily dose for 12 weeks or SOF placebo administered once daily + RBV placebo twice daily (BID) for 12 weeks. However, during the treatment phase of the study, changes were made to the protocol based on emerging clinical data from Study GS-US-334-0108 (FUSION) indicating that subjects with GT3 HCV infection could benefit from longer treatment duration. The protocol was amended to extend the treatment duration of SOF+RBV from 12 to 24 weeks for patients with GT3 HCV infection, provided they had not completed the original 12-week treatment course.

Figure 2. GS-US-334-0133: Amended Study Design



In the SOF+RBV 12 Week GT2 group, SVR12 rates of >90% were achieved in most subgroups in both treatment-naive and treatment-experienced subjects (Table 17). SVR12 rates were also high in the genotype 2 HCV-infected subjects who were treatment-experienced with or without cirrhosis (88% (7/8) and 91% (30/33), respectively). SVR12 rates for treatment-experienced GT2 HCV-infected subjects who were IFN-intolerant, prior nonresponders, and those who had prior relapse/breakthrough were also high (100%, 90% and 89%, respectively) and consistent with that observed in subjects with GT2 HCV infection receiving SOF+RBV 12-week treatment in the SOF+RBV registrational Phase 3 studies.

In the SOF+RBV 12 Week GT3 group, response rates in subgroups were generally low, consistent with the overall SVR12 rate in this small group of subjects (Table 17). In the SOF+RBV 24 Week GT3 group, treatment-naive subjects had higher SVR12 rates than subjects who had previously received treatment (93% and 77%, respectively) (Table 17). Subgroups with multiple baseline factors such as treatment-experienced, cirrhosis and non-CC IL28B genotype had SVR12 rates >80%, but the numbers of subjects in these subgroups were small (Table 17).

Table 17. Proportion of Subjects Achieving SVR12 (n=334)

	GT2 SOF+RBV 12 Weeks N=73	**GT3 SOF +RBV 12 Weeks N=11**	**GT3 SOF + RBV 24 weeks N=250**
Overall	93% (68/73)	27% (3/11)	84% (210/250)
GT2	89% (24/27)		
GT2a/2C	93% (26/28)		
GT2b	100% (18/18)		
GT3		27% (3/11)	86% (6/7)
GT3a			84% (204/243)
IL28B CC	100% (24/24)	50% (2/4)	87% (75/86)
IL28B non-CC	90% (44/49)	14% (1/7)	82% (135/164)
Cirrhosis	90% (9/10)	0% (0/2)	67% (39/58)
No Cirrhosis	94% (59/63)	33% (3/9)	89% (171/192)
IFN eligible	96% (26/27)	0% (0/2)	93% (87/94)
IFN ineligible	100% (5/5)		11/11
IFN intolerant	100% (3/3)		10/10
Naïve	97% (31/32)	0% (0/2)	93% (98/105)
Experienced	90% (37/41)	33% (3/9)	77% (112/145)

Experienced Cirrhosis IL28B non-CC	80% (4/5)	na	56% (18/32)
Experienced IL28B non-CC	87% (27/31)	na	76% (78/102)
Cirrhosis IL28B non-CC	83% (5/6)	na	61% (23/38)
Experienced Cirrhosis	88% (7/8)	na	60% (27/45)

When comparing these data to those generated in the registrational Phase 3 studies with 12 or 16 weeks of SOF+RBV in treatment-naive and treatment-experienced GT3 subjects, SVR12 rates were higher following 24 weeks of treatment (Table 18). In GT3 HCV-infected subjects treated with 24 weeks of SOF+RBV, response rates were similar for subjects with IL28B CC and non-CC genotypes (87% vs. 82%, respectively). In GT3 HCV-infected subjects treated with 24 weeks of SOF+RBV, SVR12 rates were higher in subjects without cirrhosis (89%) compared with subjects with cirrhosis (67%) (Table 18).

Table 18. Response by Baseline Factors in GT3 Subjects

	Study 108		**Study 133**	
	SOF + RBV 12 Weeks	**SOF + RBV 16 Weeks**	**SOF+RBV 12 Weeks**	**SOF+ RBV 24 Weeks**
GT3			27% (3/11)	84% (210/250)
Naïve	58% (162/281)		0% (0/2)	93% (98/105)
Experienced	30% (19/64)	62% (39/63)	33% (3/9)	77% (112/145)
IL28B CC	39% (9/23)	63% (10/16)	50% (2/4)	87% (75/86)
IL28B CT/TT	24% (10/41)	62% (29/47)	14% (1/7)	82% (135/164)
Cirrhosis Yes	19% (5/26)	61% (14/23)	0% (0/2)	67% (39/58)
No	38% (14/37)	63% (25/40)	33% (3/9)	89% (171/192)

For treatment-experienced GT2 subjects, data from subgroups in the Phase 3 registrational Study 108 indicated that a a 16-week duration might provide higher SVR12 rates for subgroups with baseline factors such as cirrhosis, but the number of subjects in the subgroups was small. However, data from these subgroups in Study 133 do not support a longer duration given the higher SVR12 rates seen for 12 weeks of SOF + RBV (Table 19).

Table 19. Response by Baseline Factors in Treatment-Experienced GT2 Subjects

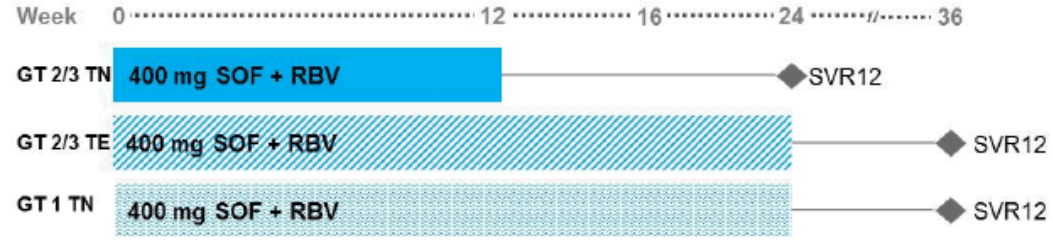
	Study 108		**Study 133**
	SOF + RBV 12 Weeks	**SOF + RBV 16 Weeks**	**SOF+ RBV 12 Weeks**
GT2	82% (32/39)	89% (31/35)	90% (37/41)
P/R Breaktrhough or Relapser	86% (25/29)	89% (24/27)	89% (25/28)
P/R Null or Partial Responder	70% (7/10)	88% (7/8)	90% (9/10)

IL28B CC	88% (7/8)	71% (10/14)	100% (14/14)
IL28B CT/TT	86% (25/29)	100% (21/21)	87% (27/31)
Cirrhosis Yes	60% (6/10)	78% (7/9)	88% (7/8)
No	90% (26/29)	92% (24/26)	91% (30/33)

PHOTON STUDY GS-US-334-0123

In Study 123, subjects were treatment-naive or treatment-experienced with chronic genotype 1, 2, or 3 HCV infection and confirmed HIV-1 coinfection. Subjects who were on ARV treatment were required to have been on a stable regimen for >8 weeks prior to screening, have an HIV-1 RNA <50 copies/mL, and have a $CD4^+$ T-lymphocyte count >200 cells/mm^3. ARV regimens containing emtricitabine/tenofovir, atzanavir/ritonavir, darunavir/ritonavir, raltegravir, efavirenz, or rilpivirine were permitted. Subjects not on ARV treatment must have had a CD4 T^+-lymphocyte count > 500 cells/mm^3 at screening. The study design for Study 123 is shown in Figure 3. Subjects were treated with SOF 400 mg once daily + RBV total daily dose of 1000 or 1200 mg administered in a divided daily dose for 12 or 24 weeks based on genotype and prior treatment history. Treatment duration was 12 weeks for treatment-naive subjects with GT2 or 3 HCV infection and 24 weeks for treatment-naive subjects with GT1 HCV infection and treatment-experienced subjects with GT2 or 3 HCV infection.

Figure 3. GS-US-334-0123: Study Design



GT = genotype; TE = treatment experienced; TN = treatment naive

N=115 (1:1)

For subjects in the SOF+RBV 12 Week treatment-naive GT2/3 group, the SVR12 rate was 75% (Table 20); 17 subjects did not achieve SVR12, of whom 13 had virologic failure (2 subjects were lost to follow-up, 1 subject died, and 1 subject withdrew consent before having a posttreatment Week 12 assessment). Among the 26 treatment-naive GT2 HCV-infected subjects, the SVR12 rate was 89% with no subjects who relapsed; 1 subject had on-treatment virologic failure likely due to study drug nonadherence as assessed by plasma SOF levels, hematologic parameters (as a surrogate for RBV use), and investigator report. Among the 42 treatment-naive GT3 HCV-infected subjects, the SVR12 rate was 67% with no on-treatment virologic failures; 12 subjects relapsed. Two of the 12 subjects relapsed after SVR4.

For subjects in the SOF+RBV 24 Week treatment-experienced GT2/3 group, the SVR12 rate was 93% (Table 20). Among the 15 treatment-experienced GT2 HCV-infected subjects, the SVR12 rate was 93%. One subject did not achieve SVR12 and relapsed after SVR4 (GT2b HCV infection assessed by LiPA 2.0 assay, but GT1a HCV infection per NS5B sequencing). Among the 13 treatment-experienced GT3 HCV-infected subjects, the SVR12 rate was 92%. One subject did not achieve SVR12 due to virologic relapse at the post-treatment Week 4 visit.

For subjects in the SOF+RBV 24 Week GT1 group, the SVR12 rate was 76% (Table 20). One subject with GT1 HCV infection had on-treatment virologic failure with hematologic parameters indicative of inconsistent RBV adherence; this subject also exhibited undetectable plasma concentrations of SOF and its metabolite, GS-331007, at the Week 4 and 6 visits, indicative of nonadherence at or around the time of the study visits. A total of 27 subjects (24%) did not achieve SVR12, of whom 26 had virologic failure and 1 subject withdrew consent before the Week 20 visit. Twenty-two relapsed before or at post-treatment Week 4 and 5 subjects relapsed after SVR4.

Table 20. GS-US-334-0123 Virologic Outcome

	Group 1	Group 2	Group 3:
	SOF+RBV 12 Weeks GT2/3 TN (N = 68)	SOF+RBV 24 Weeks GT 2/3 TE (N = 28)	SOF+RBV 24 Weeks GT1 TN (N = 114)
SVR12	51/68 (75.0%)	26/28 (92.9%)	87/114 (76.3%)
Overall Virologic Failure	13/68 (19.1%)	2/28 (7.1%)	26/114 (22.8%)
Relapse	12/67 (17.9%)	2/28 (7.1%)	25/113 (22.1%)
Study Drug Completer	11/61 (18.0%)	1/27 (3.7%)	19/103 (18.4%)
Study Drug Non-Completer	1/6 (16.7%)	1/1 (100.0%)	6/10 (60.0%)
On-Treatment Virologic Failure	1/68 (1.5%)	0/28	1/114 (0.9%)
Other	4/68 (5.9%)	0/28	1/114 (0.9%)

GT= genotype; TE = treatment experienced; TN = treatment naive
Source: Appendix 2, Table 5

In the SOF+RBV 12 Week treatment-naive GT2/3 group, subjects with GT2 HCV infection had a higher SVR12 rate than subjects with GT3 HCV infection (86% vs 67%) (Table 21). SVR12 rates were similar in subjects with or without cirrhosis (71% and 75%, respectively). One cirrhotic subject had GT2 HCV infection and achieved SVR12 and the other 6 cirrhotic subjects had GT3 HCV infection, 4 of whom achieved SVR12 (67%).

For the SOF+RBV 24 Week treatment-experienced GT2/3 group, SVR12 rates were ≥80% in all subgroups (Table 21). Subjects with GT2 HCV infection had an SVR12 rate similar to subjects with GT3 HCV infection (93% and 92%, respectively). Subjects without cirrhosis had a higher SVR12 rate than subjects with cirrhosis (95% vs 86%). Two cirrhotic subjects had GT2 HCV infection and achieved SVR12 and the other 5 cirrhotic subjects had GT3 HCV infection, 4 of whom achieved SVR12 (80%).

For the SOF+RBV 24 Week GT1 group, subjects without cirrhosis had a higher SVR12 rate than subjects with cirrhosis (77% vs 60%) (Table 21). Subjects with GT1a HCV infection had a higher SVR12 rate (82%) compared to subjects with GT1b HCV infection (54%).

Table 21. Proportion of Subjects Achieving SVR12

	GT2/3 TN SOF+RBV 12 Weeks N=68	GT2/3 TE SOF +RBV 24 Weeks N=28	GT1 TN SOF + RBV 24 weeks N=114
Overall	75% (51/68)	93% (26/28)	76% (87/114)
GT2a	83% (5/6)	2/2	na
GT2b	90% (18/20)	10/10	na
GT3a	67% (28/42)	92% (12/13)	na
GT1a	na	67% (2/3)	82% (74/90)
GT1b	na		54% (13/24)
IL28B CC	68% (17/25)	92% (11/12)	80% (24/30)
IL28B non-CC	79% (34/43)	94% (15/16)	75% (62/83)
Cirrhosis	71% (5/7)	86% (6/7)	60% (3/5)
No Cirrhosis	75% (46/61)	95% (20/21)	77% (84/109)
IFN eligible	73% (36/49)	na	76% (65/85)
IFN ineligible	79% (15/19)	na	76% (22/29)
IFN intolerant	na	80% (4/5)	
Naïve	75% (51/68)	na	76% (87/114)
Experienced	na	93% (26/28)	na
Breakthrough/Relapser	na	94% (16/17)	na
Null	na	100% (6/6)	na

Overall, subjects with GT2 HCV infection, both treatment-naive and treatment-experienced, had similar SVR12 rates after treatment with SOF+RBV for 12 weeks and 24 weeks (89% and 93%, respectively). Subjects with GT3 HCV infection who were treated with SOF+RBV for 24 weeks had a higher SVR12 rate than GT3 subjects who were treated with SOF+RBV for 12 weeks (92% vs 67%). No notable differences in SVR12 rates were observed for subjects who were interferon eligible and ineligible. The SVR12 rates for cirrhotic subjects were lower than for noncirrhotic subjects across all treatment groups.

EFFECTS OF SOF+RBV ON HIV

HIV Virologic Rebound

There were 2 subjects (0.9%) on antiretroviral therapy who had HIV-1 virologic rebound during SOF+RBV treatment. Subject 4262-8725 (SOF+RBV 12 Week treatment-naive GT2/3 group) was receiving ARV treatment with raltegravir and emtricitabine/tenofovir. HIV-1 RNA was not detected from baseline through Week 8 in the subject, but was detected at Week 12. Per the investigator (on file), this subject had poor adherence to HIV-1 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 during SOF+RBV treatment. Subject 0843-8852 (SOF+RBV 24 Week GT1 group) was receiving antiretroviral treatment with atazanavir/ritonavir and emtricitabine/tenofovir. HIV-1 RNA was <20 copies/mL at baseline, ranged between <20 and 75 copies/mL during SOF+RBV treatment, and was 491 copies/mL and 32 copies/mL at the posttreatment Week 4 and 12 visits, respectively. Per the investigator (on file), this subject also had poor adherence with antiretroviral treatment and had completely stopped antiretroviral treatment along with insulin, temporarily due to depression, which led to an elevation in HIV-1 RNA of 491 copies/mL in addition to hospitalization for diabetic ketoacidosis. The subject had HCV RNA <LLOQ from Week 8 of SOF+RBV treatment through the post-treatment Week 12 visit.

Of the 11 subjects who were not on antiretroviral treatment upon enrollment, 8 had detectable HIV-1 RNA at baseline. These 8 subjects did not have any clear evidence of HIV-1 specific antiviral effect from study drugs. The remaining 3 subjects have maintained control of HIV-1 RNA to below 100 copies/mL for the duration of the study.

$CD4^+$ T-Lymphocytes

As previously described with RBV-containing therapy, median $CD4^+$ T-lymphocyte counts gradually decreased during SOF+RBV treatment in all treatment groups (range, -73 to 87 cells/mm^3 at the end of treatment). There were no clinically significant sequelae of these decreases reported in any treatment group. Despite the decrease in total $CD4^+$ T-lymphocyte count during SOF+RBV treatment, the median $CD4^+$ T-lymphocyte percent remained relatively stable, with percentages that ranged from 30.6% to 36.7% across the 3 treatment groups during SOF+RBV treatment.

3. CONCLUSION

The new DAA, sofusbuvir (SOF), offers a new treatment option for patients with chronic HCV with genotypes 1, 2, 3 and 4. The efficacy and baseline response data from the VALENCE study support a 24-Week duration of SOF+RBV for all subjects infected with GT3 HCV. Data from the PHOTON study also support the longer 24-week duration in subjects with GT3 HCV. Additional data and subgroup analyses from the VALENCE study did not confirm that a longer duration (>12 weeks) of SOF + RBV in treatment-experienced cirrhotic subjects with GT2 is necessary.

In addition, the efficacy and safety data from the PHOTON study support the use of SOF+RBV for HIV-1/HCV coinfected subjects with GT1 HCV. However, the optimal duration of this treatment has not been determined.

In the NEUTRINO study, further analysis of response by baseline factors in subjects with GT1 identified some groups of subjects with poor baseline predictors that will have lower response rates to 12 weeks SOF+P/R than the overall 90% SVR12 rate. Analysis of GT1 subgroups with multiple baseline factors that predict poor response such as cirrhosis, GT1b and IL28B non-CC alleles had SVR12 rates that ranged from 57% to 78%.

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The resistance data from both population and deep sequencing of subjects who had on-treatment failure (n=5) and subjects who relapsed (n=20) in the Pre-transplant study P7977-2025 provided supportive information for the FDA resistance findings in the SOF Phase 3 studies. NGS data were provided to the FDA for the 5 subjects who had on treatment failure and we were able to confirm the presence of nucleotide inhibitor resistance-associated substitutions. However, we did not have NGS data from the remaining 20 relapse subjects and were not able to perform an independent resistance analysis.

The presence of substitution L159F at baseline was associated with SOF breakthrough and relapse in 4 subjects infected with GT1b HCV, and this substitution emerged on treatment in 2 subjects who were infected with GT1a HCV (one breakthrough and one relapse) and 1 subject infected with GT2b HCV (breakthrough). In addition, S282R and L320F substitutions were detected by deep sequencing in the on-treatment sample from a subject infected with GT1a HCV who did not respond to SOF. Overall, these resistance results indicate that in cases where SOF is not used in an optimal regimen or duration, resistance may emerge. There is evidence of genotypic resistance in some subjects who experienced on treatment breakthrough or relapse.

- SOF Monotherapy Relapse: S282T with mean 13.5-fold reduced susceptibility to SOF
- SOF+RBV GT1a nonresponder: S282R+L320F
- Breakthroughs/Relapses in multiple studies and genotypes: L159F and V321A (no detectable shift in phenotypic susceptibility to sofosbuvir)

The long term consesquence of the emergence of these substitutions is not known. There are limited data on the persistence of these substitutions.

- S282T (GT2b SOF monotherapy subject) emerged at Week 4 post-treatment and was no longer detected at Week 12 post-treatment.
- In one breakthrough subject, L159F was present at a frequency of 9.5% at Post-Transplant Week 1 and this dropped to 1.2% by Post-Transplant Week 2
  - Many of the GT3a relapser samples were collected weeks after termination of treatment, so it is possible that F159 was present in the relapse samples while on-treatment, but rapid displacement would result in no detectable F159 in samples that were taken too long after relapse (>2 weeks).

Retreatment studies are being done and these data will hopefully provide information on whether the emergenct of NI substitutions on SOF-containing regimens contribute to lower response rates for future treatments.

Additional analyses were performed by FDA virology reviewer, Dr. Eric Donaldson, Ph.D. to examine why subjects with GT1b HCV had lower SVR12 rates than subjects with GT1a HCV. C316N is the only difference between HCV GT1a and GT1b polymerase in the active site. Substitutions that add bulk to this position are predicted to interfere with sofosbuvir's ability to enter the active site and inhibit HCV RNA replication. C316 is highly conserved in GT1a (99.89%) but not in GT1b (81.83%). Subjects infected with HCV GT1b with C316N/H at baseline had SVR12 rates of 0% (0/6) and 50% (3/6) in studies P7977-2025 and GS-US-334-0110, respectively. One subject infected with HCV GT1b who relapsed after treatment with sofosbuvir had emergent

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C316F. Therefore, substitutions at C316, which is a polymorphic site in the GT1b NS5B polymerase, may contribute to reduced efficacy among subjects infected with this genotype of HCV, but additional future resistance and efficacy data will be needed to address the affect of C316 substitutions on response to sofosbuvir.

4. PACKAGE INSERT

FDA PROPOSED PACKAGE INSERT

[TRADENAME] is an HCV nucleotide analog NS5B polymerase inhibitor indicated in combination with other agents for the treatment of chronic hepatitis C (CHC) in adults.

12.1 Mechanism of Action

Sofosbuvir is a direct-acting antiviral agent against the hepatitis C virus *[See Microbiology (12.4)].*

12.4. Microbiology

Mechanism of Action

Sofosbuvir is an inhibitor of the HCV NS5B RNA-dependent RNA polymerase, which is essential for viral replication. Sofosbuvir is a nucleotide prodrug that undergoes intracellular metabolism to form the pharmacologically active uridine analog triphosphate (GS-461203), which can be incorporated into HCV RNA by the NS5B polymerase and acts as a chain terminator. In a biochemical assay, GS-461203 inhibited the polymerase activity of the recombinant NS5B from HCV genotype 1b, 2a, 3a and 4a with IC_{50} values ranging from 0.7 to 2.6 μM. GS-461203 is not an inhibitor of human DNA and RNA polymerases nor an inhibitor of mitochondrial RNA polymerase.

Antiviral Activity

In HCV replicon assays, the EC_{50} values of sofosbuvir against full-length replicons from genotype 1a, 1b, 2a, 3a and 4a, and chimeric 1b replicons encoding NS5B from genotype 2b, 5a or 6a ranged from 0.014 to 0.11 μM. The median EC_{50} value of sofosbuvir against chimeric replicons encoding NS5B sequences from clinical isolates was 0.062 μM for genotype 1a (range 0.029-0.128 μM; N=67), 0.102 μM for genotype 1b (range 0.045-0.170 μM; N=29), 0.029 μM for genotype 2 (range 0.014-0.081 μM; N=15) and 0.081 μM for genotype 3a (range 0.024-0.181 μM; N=106). In infectious virus assays, the EC_{50} values of sofosbuvir against genotype 1a and 2a were 0.03 and 0.02 μM,

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respectively. The presence of 40% human serum had no effect on the anti-HCV activity of sofosbuvir. Evaluation of sofosbuvir in combination with interferon alpha or ribavirin showed no antagonistic effect in reducing HCV-RNA levels in replicon cells.

Resistance

In Cell Culture

HCV replicons with reduced susceptibility to sofosbuvir have been selected in cell culture for multiple genotypes including 1b, 2a, 2b, 3a, 4a, 5a and 6a. Reduced susceptibility to sofosbuvir was associated with the primary NS5B substitution S282T in all replicon genotypes examined. An M289L substitution developed along with the S282T substitution in genotype 2a, 5 and 6 replicons. Site-directed mutagenesis of the S282T substitution in replicons of 8 genotypes conferred 2- to 18-fold reduced susceptibility to sofosbuvir and reduced the replication viral capacity by 89% to 99% compared to the corresponding wild-type. In biochemical assays, recombinant NS5B polymerase from genotypes 1b, 2a, 3a and 4a expressing the S282T substitution showed reduced susceptibility to GS-461203 compared to respective wild-types.

In Clinical Trials

In a pooled analysis of 982 subjects who received [TRADENAME] in Phase 3 trials, 224 subjects had post-baseline NS5B genotypic data from next generation nucleotide sequencing (assay cutoff of 1%).

Treatment-emergent substitutions L159F (n= 6) and V321A (n= 5) were detected in post-baseline samples from GT3a-infected subjects across the Phase 3 studies. No detectable shift in the phenotypic susceptibility to sofosbuvir of subject isolates with L159F or V321A substitutions was seen. The sofosbuvir-associated resistance substitution S282T was not detected at baseline or in the failure isolates from the Phase 3 trials. However, an S282T substitution was detected in one genotype 2b subject who relapsed at Week 4 post-treatment after 12 weeks of sofosbuvir monotherapy in the Phase 2 Study P7977-0523 [ELECTRON]. The isolate from this subject displayed a mean 13.5-fold reduced susceptibility to sofosbuvir. For this subject, the S282T substitution was no longer detectable at Week 12 post-treatment by next generation sequencing with an assay cut off of 1%.

(b) (4)

the L159F substitution emerged in multiple subjects with GT1a or GT2b HCV who experienced virologic failure (breakthrough and

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relapse). Furthermore, the presence of substitutions L159F and/or C316N at baseline was associated with sofosbuvir breakthrough and relapse post-transplant in multiple subjects infected with GT1b HCV. In addition, S282R and L320F substitutions were detected on-treatment by next generation sequencing in a subject infected with GT1a HCV with a partial treatment response.

The clinical significance of these substitutions is not known.

Cross-Resistance

HCV replicons expressing the sofosbuvir-associated resistance substitution S282T were susceptible to NS5A inhibitors and ribavirin. HCV replicons expressing the ribavirin-associated substitutions T390I and F415Y were susceptible to sofosbuvir. Sofosbuvir was active against HCV replicons with NS3/4A protease inhibitor, NS5B non-nucleoside inhibitor and NS5A inhibitor resistant variants.

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/s/

LISA K NAEGER
11/20/2013

JULIAN J O REAR
11/20/2013

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.

NDA#: 204671 **SDN** 016, 027, 030, 031, 040, 047 **DATES REVIEWED:** 9/16/13, 10/04/13, 10/10/13, 10/21/13

Reviewer's Name: Eric F. Donaldson, Ph.D.

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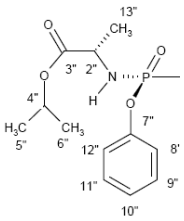
N204671 SDN 031	09/17/2013	09/18/2013
N204671 SDN 040	10/09/2013	10/10/2013
N204671 SDN 047	10/16/2013	10/21/2013

Product Names: GS-7977, PSI-7977, Sofosbuvir
Chemical Name: (S)-Isopropyl 2-((*S*)-(((2*R*,3*R*,4*R*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy) phosphorylamino) propanoate

Structure:



GS-7977

Molecular formula: $C_{22}H_{29}FN_3O_9P$
Molecular weight: 529.46
Drug category: Antiviral against Hepatitis C Virus
Indication: Treatment of chronic HCV infection
Dosage Form/Route of administration: Capsules/Oral
Supporting documents: IND106739; IND106739 SDN 286; NDA 204671 SDN 002
Abbreviations: DAA, HCV direct antivirals; GT, genotype; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IFN, interferon; LLOQ, lower limit of quantification; LOD, limit of detection; NGS, next generation sequencing; PEG, pegylated interferon alpha; RBV, ribavirin; SOC, standard-of-care; SOF, sofosbuvir; SVR, sustained virologic response

Reference ID: 3409507

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EXECUTIVE SUMMARY

The original review of the Next Generation Sequencing (NGS) data and a detailed analysis of resistance for sofosbuvir (SOF) was completed and archived on September 6, 2013 (NDA 204671 SDN 002). This is an addendum to the original review describing analyses of additional data for the transplant population, including a structural comparison of NS5B of GT1a and GT1b, and data provided by the sponsor to address the E341D emergent substitution that was identified in the NGS analysis.

The sponsor submitted the original NDA 204671 SDN 000 on April 08, 2013 and it received priority review status. In the original NDA the sponsor provided resistance data for 5 Phase 2 and 4 Phase 3 clinical trials, including next generation sequencing (NGS) data. Given that NGS is an emerging technology and there is currently no standard analysis pipeline available for the analysis of these data, DAVP performed independent analyses of the NGS data submitted for the 4 pivotal Phase 3 clinical trials and compared the results to those reported by the sponsor. In general, there was good agreement between these results. Overall, the DAVP assessment of the NGS data from subjects who relapsed in the 4 pivotal Phase 3 trials determined that there were insufficient data to draw any conclusions on resistance-associated treatment-emergent substitutions for HCV GT1 and GT2. In contrast, polymorphisms at C316 of the HCV GT1b NS5B at baseline were associated with lower SVR12 rates in this genotype.

However, in contrast to the conclusions drawn by the sponsor who stated that there were no clinically relevant sofosbuvir resistance-associated substitutions identified, DAVP identified three treatment-emergent substitutions among subjects infected with HCV GT3a that occurred frequently at conserved amino acid positions in the virus of subjects who relapsed, including E341D, L159F, and V321A. Of note, L159 is present inside the polymerase synthesis channel and V321 is proximal to the catalytic site (Bressanelli et al., 2002). Substitution E341D emerged in 13 relapse subjects infected with HCV GT3a in two clinical trials (FISSION, n=10; POSITRON, n=3) and it was only found in the virus of subjects who relapsed. The L159F substitution emerged in 6 relapse subjects infected with HCV GT3a and is a previously identified NS5B substitution shown, along with L320F, to confer reduced susceptibility to HCV nucleotide inhibitors (Tong X et al., 2013). The L159F and E341D substitutions emerged together in one HCV GT3a relapse subject. V321A emerged in 5 subjects infected with GT3a who relapsed. Phenotypic data for the L159F and V321A substitutions alone did not show detectable decreased susceptibility to SOF. The E341D substitution has not been analyzed phenotypically. Therefore, the Division had requested that the Applicant characterize the E341D substitution and the combination of L159F + E341D in phenotypic assays.

In submission NDA 204671 SDN 040, the sponsor provided additional information about their deep sequencing protocol indicating that the E341D substitution was an artifact introduced into several NGS samples by a pan-genotypic nested-PCR primer. After careful review of the NGS data for all for all samples from all subjects that had detectable E341D, Clinical Virology agrees with the conclusions reached by the sponsor. Substitution E341D is no longer considered to be resistance-associated.

In submission NDA 204671 SDN 016, the sponsor provided summary resistance data for all treatment failures in Study P7977-2025, an ongoing Phase 2, open-label trial that evaluates the efficacy of SOF+RBV administered to subjects awaiting liver transplant prior to receiving the transplant in preventing HCV recurrence post liver transplant in subjects with genotype 1 through 6 HCV infection and hepatocellular carcinoma (HCC) meeting the Milan criteria prior to undergoing liver transplantation with an anticipated time until transplantation within one year. In submission NDA 204671 SDN 030, raw NGS data (fastq files) were provided for five subjects who experienced breakthrough (n=3) or were partial responders (n=2).

The available resistance data from both population and deep sequencing of subjects who had on-treatment failure (N=5) and subjects who relapsed (N=20) in the pre-transplant trial P7977-2025 provided supportive

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information for the FDA resistance findings in the sofosbuvir Phase 3 trials. The L159F substitution emerged on-treatment in 2 subjects who were infected with HCV GT1a (one breakthrough and one relapser) and 1 subject infected with HCV GT2b (breakthrough). The presence of substitution L159F at baseline was also associated with sofosbuvir breakthrough and relapse post-transplant in 4 subjects infected with HCV GT1b. In addition, S282R and L320F substitutions were detected by deep sequencing in the on-treatment sample from a subject infected with HCV GT1a who did not respond to sofosbuvir. Importantly, L159F and L320F impacted resistance to another NS5B nucleotide analog polymerase inhibitor, mericitabine; however L320F+L159F in combination had a greater impact than L159F or L320F alone (Tong X et al., 2013). S282T substitutions developed in replicons from multiple genotypes in cell culture selection studies and developed in 1 relapse subject from a Phase 2 study. In addition, D61G emerged in three on-treatment failures in P7977-2025, and all three of these had variable substitutions at position 62 (N62H, G62D/N, and S62N). Among GT1b, E440G was detected as treatment emergent in three subjects.

Baseline analysis showed that six subjects infected with HCV GT1b had C316N at baseline and all six of these subjects experienced post-transplant relapse. Four of the six subjects also had L159F at baseline. By comparison, six GT1b subjects had C316N (n=4) or C316H (n=2) at baseline in the Phase 3 trials and in both of these subgroups the SVR12 rate was 50%. In addition, C316F emerged in one subject infected with HCV GT1a who was a relapser. Of note, C316 is highly conserved in GT1a (99.89%) but polymorphic in GT1b (81.83%).

Structural bioinformatics analyses performed by DAVP showed that the only difference within 8 angstroms of the catalytic triad between the HCV GT1a and GT1b NS5B polymerases occurred at position 316. Nucleoside analogs are known to directly interact with the active site to facilitate incorporation into the nascent RNA template leading to chain termination. Importantly, C316 is the only variable amino acid position in the active site that differs between the polymerases of GT1a and 1b, which indicates that substitutions at C316, particularly substitutions that add bulkier amino acids, may interfere with the ability of sofosbuvir to access the active site. Substitutions at position C316, which is a polymorphic site in the GT1b NS5B polymerase, may contribute to reduced efficacy among subjects infected with this genotype of HCV.

Taken together, the assessment of resistance data from the 4 Phase 3 clinical trials and the Phase 2b liver transplant study (P7977-2025) have indicated that L159F (sometimes in combination with L320F or C316N) and V321A contribute to resistance to sofosbuvir. Substitutions at the polymorphic GT1b NS5B amino acid position 316 at baseline were associated with reduced SVR12 rates of 50% or less. In addition, other treatment-emergent substitutions were detected in either the Phase 3 trials (V321A) or in study P7977-2025 (GT1a: D61G + variants at position 62 and GT1b: E440G). Based on these findings, the following post-marketing requirements will be communicated to the sponsor:

1. Determine the phenotypic susceptibility of SOF against HCV GT1a, GT1b, GT2b, GT3a replicons bearing L159F and L159F+L320F (Note: The sponsor reported a 1.2 fold change in EC_{50} value for V321A).
2. Determine the phenotypic susceptibility of SOF against HCV GT1a replicons with L320F and S282R individually and in combination.
3. Determine the phenotypic susceptibility of SOF against HCV GT1a and GT1b replicons with C316N/H/F.
4. Determine the phenotypic susceptibility of SOF against HCV GT1b replicons with E440G and HCV GT1a replicons with D61G (with itself and with N62H, G62D/N, and S62N).

RECOMMENDATIONS

Recommendation and Conclusion on Approvability

This NDA is approvable with respect to Clinical Virology (Please see the original review and addendum review by the primary Clinical Virology reviewer, Dr. Lisa Naeger (NDA 204671 SDN 000) and review of the NGS data for the 4 pivotal Phase 3 clinical trials performed by Dr. Eric Donaldson (NDA 204671 SDN 002).

Recommendation PMCs, PMAs, and REMS, if Approvable.

Final Virology Related PMRs

1. Determine the phenotypic susceptibility of SOF against HCV GT1a, GT1b, GT2b, GT3a replicons bearing L159F and L159F+L320F.
2. Determine the phenotypic susceptibility of SOF against HCV GT1a replicons with L320F and S282R individually and in combination.
3. Determine the phenotypic susceptibility of SOF against HCV GT1a and GT1b replicons with C316N/H/F.
4. Determine the phenotypic susceptibility of SOF against HCV GT1b replicons with E440G and HCV GT1a replicons with D61G (with itself and with N62H, G62D/N, and S62N).
5. Three substitutions at highly conserved positions (T84S, E202D, and T473S) were enriched in the one subject infected with HCV GT3a who experienced breakthrough in clinical trial P7977-1231. Assess T84S+E202D+T473S in combination in a GT3a phenotypic assay.
6. Additional substitutions at highly conserved positions may be associated with resistance to SOF and should be further characterized in a GT3a phenotypic assay: K211R, V321A, P540L, and T542A.

BACKGROUND AND SUMMARY

The sponsor submitted the original NDA 204671 SDN 000 on April 08, 2013 and it received priority review status. In the original NDA the sponsor provided resistance data for 5 Phase 2 and 4 Phase 3 clinical trials, including next generation sequencing (NGS) data. Given that NGS is an emerging technology and there are currently no standard analysis pipeline available for the analysis of these data, DAVP performed an independent analysis of the NGS data submitted for 4 pivotal Phase 3 clinical trials and compared the results to those reported by the sponsor. In general, there was good agreement between these results. Overall, the DAVP assessment of the NGS data from subjects who relapsed in the 4 pivotal Phase 3 trials determined that there were insufficient data to draw any conclusions on resistance-associated treatment-emergent substitutions for HCV GT1 and GT2. By contrast, polymophisms at C316 of the HCV GT1b NS5B at baseline were associated with lower SVR12 rates in this genotype. In addition, only one subject infected with HCV GT3a experienced on-treatment failure, and while there were no clear resistance-associated substitutions, three substitutions, T84S, E202D, and T473S, were enriched. We recommended assessing T84S+E202D+T473S in combination in a GT3a phenotypic assay. Moreover, the sponsor reported that there were no clinically significant resistance-associated substitutions detected for any Phase 2 or 3 clinical trials. Clinical Virology discussed the results of the independent analysis of the NGS data and it was agreed that the following modifications should be made to the label and/or proposed as post-marketing actions:

1. The L159F substitution is a previously identified resistance-associated NS5B substitution which with L320F has been shown to confer resistance to HCV nucleotide inhibitors (Tong X et al., 2013). L159F emerged in 6 GT3a infected relapse subjects.

2. In the Phase 3 trials, six GT1b subjects had C316N (n=4) or C316H (n=2) at baseline and in both of these subgroups the SVR12 rate was 50%. In addition, C316F emerged in one subject infected with HCV GT1a who was a relapser. Determine the phenotypic susceptibility of SOF against HCV GT1a and GT1b replicons with C316N/H/F.
3. Three substitutions at highly conserved positions (T84S, E202D, and T473S) were enriched in the one subject infected with HCV GT3a who experienced breakthrough in clinical trial P7977-1231. We recommend assessing T84S+E202D+T473S in combination in a GT3a phenotypic assay.
4. Additional substitutions at highly conserved positions may be associated with resistance to SOF and should be further characterized in a GT3a phenotypic assay: K211R, V321A, P540L, and T542A.
5. While S282T was not detected in Phase 3 virologic failures, it did arise in at least one GT2a infected subject in a Phase 2 trial. Given that this substitution is selected in cell culture, it should be included in the resistance section of the label.
6. Substitution E341D was a treatment-emergent substitution at a highly conserved position that developed in 13 subjects infected with HCV GT3a in two clinical trials (P7977-1231, n=10; GS-US-334-0107, n=3) and it was only found in subjects who relapsed. However, the sponsor provided additional information about their deep sequencing protocol indicating that the E341D substitution was an artifact introduced into the NGS sample by a pan-genotypic nested-PCR primer. Upon review of these data, Clinical Virology agrees that E341D is no longer considered a substitution of interest.

In submissions NDA 204671 SDNs 016 and 030, the sponsor submitted resistance data and raw NGS data, respectively, from ongoing study P7977-2025. P7977-2025 is an ongoing Phase 2, open-label trial that evaluates the efficacy of SOF+RBV administered to subjects awaiting liver transplant prior to receiving the transplant in preventing HCV recurrence post liver transplant in subjects with genotype 1 through 6 HCV infection and hepatocellular carcinoma (HCC) meeting the Milan criteria prior to undergoing liver transplantation with an anticipated time until transplantation within one year. Treatment duration is for a maximum of 24 weeks (before a protocol amendment) or 48 weeks (after protocol amendment) or until time of transplant, whichever comes first. Treatment is discontinued within 24 hours prior to liver transplant if it occurs before the subject has completed their 24- or 48-week treatment course. Prevention of post-transplant reinfection is determined by a sustained post-transplant virological response (HCV RNA <lower limit of quantification [LLOQ]) at 12 weeks post-transplant (pTVR12).

The majority of the subjects had HCV GT1 infection (73.8%, GT1; 39.3%, GT1a and 34.4%, GT1b) while 13.1%, 11.5%, and 1.6% had GT 2, 3, and 4 HCV infection, respectively. A total of 61 subjects received at least one dose of study drugs at the time of the data cutoff for this submission. The trial status of these subjects is as follows:

- 9 subjects (14.8%) were on treatment in the pre-transplant phase
- 29 subjects (47.5%) have undergone liver transplantation while on treatment
- 8 subjects (13.1%) completed 24 weeks of treatment and subsequently have undergone a liver transplantation
- 2 subjects (3.3%) completed 24 weeks of treatment and were prematurely terminated from the trial due to disease progression
- 7 subjects (11.5%) relapsed during post-treatment follow-up and were retreated with SOF+RBV in the retreatment substudy
- 6 subjects (9.8%) prematurely discontinued treatment

A total of 11 of 15 subjects (73%) who completed 24 weeks of SOF+RBV treatment relapsed in the pre-transplant phase. This finding led to a protocol amendment to extend the treatment duration from 24 weeks to 48 weeks or the time of transplant. The rate of virologic relapse after 24 weeks of treatment in this patient

population and the need for HCV RNA to be <LLOQ at the time of transplant indicates that subjects should continue on SOF+RBV treatment until the time of transplant.

A total of 41 subjects with any SOF+RBV treatment duration have undergone liver transplantation at the time of the updated submission. Thirty-seven subjects had HCV RNA <LLOQ at the time of liver transplantation and 36 have been followed to post-transplant Week 12. Of those subjects, 63.9% achieved sustained pTVR12. GT2 and GT3 subjects had improved virologic outcomes (75-100%) compared with GT1 subjects (54%). Of the approximately two-thirds of subjects reaching post-transplant Week 24, 71% (17/24) achieved sustained pTVR24. The sponsor submitted summary data in submission NDA 204671 SDN 016 and provided NGS data for all five on-treatment failures in submission NDA 204671 SDN 030.

CLINICAL STUDIES

Pre-Transplant Study P7977-2025 Resistance Analysis

Baseline Sanger population nucleotide sequencing of NS5B was performed for all 61 subjects enrolled in study P7977-2025. A full NS5B population sequence was obtained for the virus from 59 subjects and a short NS5B fragment population sequence (amino acids 270-311) for 2 subjects' virus. Sanger population DNA sequencing was used to verify (n=58 subjects) or identify (n=3) the HCV genotypes of each subject's virus.

In the pre-transplant phase, 17 of 61 subjects qualified for resistance testing (Table 1). Of these 17 subjects, on-treatment HCV breakthrough was observed in 3 subjects (Subjects 0773-7734, 0585-7751, and 0451-7732), slower response to treatment was observed in 2 subjects (Subjects 0773-7712 and 1249-7720), early treatment termination occurred in 1 subject (Subject 0585-7758), and post-treatment relapse in 11 subjects (Subjects 0522-7707, 1028-7706, 1657-7704, 1657-7715, 6927-7713, 7585-7710, 0773-7717, 6927-7721, 1028-7724, 1657-7722, and 7585-7731) (Table 2).

Table 1. Subjects who failed prior to transplant (compiled by DAVP).

PRE TRANSPLANT						
SUBJID	**NONRECAT**	**RETREAT FL**	**2nd NONRECAT**	**Outcome**	**FAIL TIMEPOINT**	**HCV_GT**
P7977-2025-0773-7734	VBT	N		Efficacy failure	Week 12	2b
P7977-2025-0585-7751	VBT	N		Efficacy failure	Week 8 first FU-4 above 1000	1b
P7977-2025-0451-7732	VBT	N		Transplantation with HCV RNA >LLOQ. At Posttransplant Week 24HCV RNA of 21,800,000 IU/mL.	Week 16/PTX Week 1	1a
P7977-2025-0773-7712	Partial	N		Efficacy failure	Week 12	1a
P7977-2025-1249-7720	Partial	N		Efficacy failure	Week 8/FU-Week 4	1a
P7977-2025-0585-7758	Relapser	N		Relapser	FU-Week 4	1a
P7977-2025-0522-7707	Relapser	Y	SVR	SVR on retreatment	FU-Week 4	1b
P7977-2025-1028-7706	Relapser	Y		Death	FU-Week 4	1a
P7977-2025-1657-7704	Relapser	N		Relapser, remained eleveated post transplant, removed from study by sponsor	FU-Week 3 or 4	1b
P7977-2025-1657-7715	Relapser	N		relapser	FU-Week 4	1a
P7977-2025-6927-7713	Relapser	N		relapser	FU-Week 4	1a
P7977-2025-7585-7710	Relapser	Y	SVR	SVR on retreatment/TX	FU-Week 4	1b
P7977-2025-0773-7717	Relapser	N		Relapser, remained eleveated post transplant, removed from study by sponsor	FU-Week 4	1b
P7977-2025-6927-7721	Relapser	Y	SVR	SVR on retreatment	FU-Week 4	2b
P7977-2025-1028-7724	Relapser	Y		Death	FU-Week 4	1b
P7977-2025-1657-7722	Relapser	Y	SVR	SVR on retreatment	FU-Week 8	1b
P7977-2025-7585-7731	Relapser	Y	Relapser	Relapse on retreatment	FU-Week 4/PTX Week 4-8	1b

VBT, viral breakthrough**; SVR**, sustained virologic rate at week 12 post treatment or post-transplant**; FU**, follow up**; PTX**, post-transplant**; Death**, subject died during retreatment

Full length NS5B amino acid sequences at baseline and virologic failure timepoints were derived by deep sequencing with an assay cut-off of 1% in 16 of 17 subjects; however, the NGS data were not submitted to the FDA in submission NDA 204671 SDN 016. Subject 0451-7732 did not qualify for resistance testing prior to transplantation due to HCV RNA levels <1,000 IU/mL.

Table 2. Subjects who failed post-transplant (compiled by DAVP).

POST TRANSPLANT					
SUBJID	**NONRECAT**	**Outcome**	**FAIL TIMEPOINT**	**HCV GT**	**Days of SOF pre-TX**
P7977-2025-0773-7730	PTX REC	Recurrent HCV infection posttransplant Week 1 through posttransplant Week 12	PTX Week 1	1b	35
P7977-2025-6437-7718	PTX REC	PTX relapser	PTX Week 1	1b	108
P7977-2025-6437-7723	PTX REC	PTX relapser	PTX Week 4	1b	91
P7977-2025-6437-7735	PTX REC	PTX relapser	PTX Week 4	1b	29
P7977-2025-6437-7736	PTX REC	PTX relapser	PTX Week 2	1b	118
P7977-2025-0522-7737	PTX REC	PTX relapser	PTX Week 1	1a	145
P7977-2025-0585-7752	PTX REC	PTX relapser	PTX Week 12	3a	35
P7977-2025-1651-7748	PTX REC	PTX relapser	PTX Week 2	1a	201

PTX, post-transplant; **REC,** recurrence

Seven of 11 subjects who experienced viral relapse after 24 weeks of SOF+RBV treatment were retreated with the same regimen (Subjects 1028-7706, 0522-7707, 7585-7710, 6927-7721, 1028-7724, 1657-7722, and 7585-7731). The sponsor stated that 1–6 substitutions were detected in NS5B at relapse in 6 of these subjects before retreatment. HCV RNA was successfully re-suppressed in all 7 retreated subjects, which they interpreted to mean that the substitutions observed at the relapse timepoints were not associated with resistance. However, FDA interprets resistance based upon failure to achieve SVR with a regimen demonstrating efficacy in other subjects with the same HCV genotype/subtype.

A total of 41 subjects received a liver transplantation. One subject received an HCV-infected liver and was excluded from post-transplantation sequencing analysis. Recurrent HCV infection was reported in 9 of 37 subjects who had HCV RNA <LLOQ at last observed measurement prior to transplantation. NS5B deep sequencing was completed at baseline and the post-transplantation virologic failure timepoint in 8 of these subjects (Subjects 0773-7730, 6437-7718, 6437-7723, 6437-7735, 6437-7736, 0522-7737, 0585-7752, and 1651-7748)(Table 2). NS5B amplification from 1 subject failed (Subject 7585-7731) due to very low HCV RNA at the reoccurrence timepoint sample. Recurrent HCV infection was imputed for 3 subjects because no samples for resistance testing were available due to death (Subjects 1028-7705, 1028-7706, and 1028-7724).

During the Antiviral Drugs Advisory Committee for sofosbuvir held on October 25, 2013, the sponsor provided further data that showed that post-transplant recurrence of HCV infection was more frequent among subjects with HCV viral RNA still detectable at the time of transplant. However, only 1 subject out of 41 experienced recurrence after being HCV RNA ≤25 IU/mL target not detected (TND) for ≥30 days prior to transplant.

Baseline Resistance Analysis

Baseline resistance analysis was performed by DAVP by analyzing the summary tables provided by the sponsor. Substitutions that occurred at baseline were compared between subjects who achieved pTVR12 and those who failed treatment with SOF either by viral breakthrough or relapse. Substitutions of interest were identified by looking at the baseline sequences of all subjects and identifying substitutions that occurred in 2 or more subjects who failed (Table 3). There were a total of 21 subjects infected with HCV GT1b in trial P7977-

2025 and of these, 8 subjects failed treatment prior to transplant and 5 subjects experienced recurrence of HCV infection after transplant. Of note, all six HCV GT1b infected subjects whose virus expressed a C316N substitution at baseline failed treatment. While the virus from 4 of these subjects expressed an L159F substitution, the others did not have this sofosbuvir resistance-associated substitution. C316N is a known resistance-associated substitution that emerges during treatment with non-nucleotide NS5B polymerase palm inhibitors (Mo et al., 2011; Hang et al., 2009; Howe et al., 2008). By contrast, C316N was not detected at baseline in any subjects infected with HCV GT1b who achieved pTVR12. In the Phase 3 trials, 4 of 74 subjects infected with HCV GT1b had C316N at baseline and 2 of 4 failed to achieve an SVR. In addition, two subjects infected with HCV GT1b had C316H at baseline, with 1 of 2 achieving SVR (see C316N Analysis below).

Table 3. Baseline substitutions in P7977-2025 (DAVP summary).

RESIST	GT	NS5B POS	SUBSTITUTIONS	No.
BASELINE	1a	77	K77R/K77T	2
		130	S130N	2
		231	S231A/S231N	2
		330	Q330P/Q330Q/P	2
		517	R517K/R517R/K	2
		520	I520F/I520T	2
	1b	316	C316N	6
		159	L159F	4
		90	K90A/K90M/K90K/M	3
		181	T181K/T181N/T181T/S	3
		5	T5S/T5T/S	2
		130	T130N/T130N/S	2
		135	D135N/D135D/N	2
		190	S190A	2
		206	N206N/D/N206N/K	2
		212	K212R/K212K/R	2
		357	E357A	2
		461	Q461L/Q461P	2
		506	S506A	2
		517	R517K	2
	2b	49	A49M	2

Four subjects' GT1b virus had an L159F substitution at baseline (Table 3), a known resistance-associated substitution of mericitabine (b) (4) Tong X et al., 2013) that was associated with sofosbuvir virologic failure in the Phase 3 trials (Clinical Virology review of NDA 204678 SDN 002). L159F was present in baseline isolates from 3 subjects who had recurring HCV infection after transplant and developed in the virus of 1 subject who experienced viral breakthrough prior to transplant. All four subjects with L159F also had C316N; however, in Phase 3 studies, L159F developed in the absence of C316 substitutions. In addition, C316N was detected at baseline in 1 subject who relapsed prior to transplant and 1 subject with recurring HCV GT1b infection post-transplant (see C316N Analysis below).

There were no novel substitutions present at baseline in the virus from the 1 subject infected with HCV GT3a who failed post-transplant. No subjects infected with HCV GT4 (n=1) failed in this trial. S282T was not observed at baseline for any subject in this trial.

Treatment-Emergent Resistance Analysis

The summary data provided by the sponsor were also analyzed to determine if any treatment-emergent substitutions could be associated with resistance. The baseline sequence was compared to the sequence derived from the sample closest to the time of failure for each subject who failed treatment with SOF (Table 4).

Table 4. Treatment-emergent substitutions in P7977-2025 (DAVP summary).

RESIST	GT	NS5B POS	SUBSTITUTIONS	No.
EMERGENT	1a	86	E86E/K	1
		159	L159L/F	1
		252	A252A/V	1
		273	N273N/S	1
		421	A421A/V	1
		486	A486A/V	1
		574	F574F/L	1
	1b	**440**	**E440G/E440E/G**	**3**
		68	L68F	1
		77	T77T/A	1
		124	K124E	1
		323	I323I/V	1
		392	L392F	1
		514	Q514Q/R	1
		541	A541A/V	1
	2b	213	D213N	1

Given the small size of this clinical trial, the number of failures was limited. Therefore, while substitutions did occur at later timepoints that were not present at baseline among treatment failures, only one emerging substitution, E440G (n=3; one subject's virus had E440G and two had E440E/G) was observed in the virus from multiple subjects infected with HCV GT1b and this site was 91.23% conserved for this genotype. One out of three of these virologic failure subjects had virus expressing a C316N substitution at baseline. For subjects infected with HCV GT1a and GT2a, specific substitutions were identified in the virus of only 1 subject (Table 4). Of note, one GT1a subject's virus developed an L159L/F substitution, previously associated with sofosbuvir virologic failure in GT3a subjects in the Phase 3 trials and associated with failure of GT1b subjects in this study when present at baseline. In addition, there were no novel substitutions present at later timepoints that were not present at baseline in the 1 subject infected with HCV GT3a who failed post-transplant. No subjects infected with HCV GT4 (n=1) failed in this trial. S282T was not detected in any post-baseline sequences.

Next Generation Sequencing Analysis of the Five On-Treatment Failures

In the updated response (NDA 204671 SDN 016), the sponsor provided summary data regarding potential resistance to SOF among subjects who failed treatment, and these data were analyzed in the previous section using the sponsor's Sanger population sequencing and NGS data (summarized in Table 5).

Table 5. Summary of resistance data from Pre-Transplant Study P7977-2025.

PID	GT	IL28GEN	NONRECAT	EXPERTCAT	Baseline NS5B Substitutions	Emergent NS5B Substitutions
P7977-2025-0451-7732	1a	CT	DAA BT			A421A/V **L159F (9.5%)**
P7977-2025-0522-7720	1a	TT	DAA NR	P/R PARTIAL RESPONDER		
P7977-2025-0522-7737	1a	TT		P/R null		
P7977-2025-1028-7705	1a	CT				
P7977-2025-1028-7706	1a	CT	Relapse FU4 Retreated but died	P/R null		**L159L/F** E86E/K A486A/V
P7977-2025-1651-7748	1a	CT		P/R null		
P7977-2025-6926-7762	1a	CC				
P7977-2025-0773-7712	1a	CT	DAA NR	Naive		A252A/V N273N/S **S282R (1.1% NGS)** **L320F (1.8% NGS)**
P7977-2025-0585-7751	1b		DAA BT	P/R NULL RESPONDER	**L159F** C316N	Q514Q/R
P7977-2025-0773-7730	1b	CT		P/R RELAPSER		K124E Q514R
P7977-2025-1657-7704	1b	TT	DAA Relapser	P/R NULL RESPONDER		E440E/G
P7977-2025-6437-7718	1b	TT		P/R RELAPSER		L68F L392F
P7977-2025-6437-7723	1b	CT	PTX Relapser after 12 weeks trt	NAIVE-ALL	**L159F** C316N	
P7977-2025-6437-7735	1b	CT	PTX Relapser after 4 weeks trt	P/R PARTIAL RESPONDER	**L159F** C316N	T77T/A
P7977-2025-6437-7736	1b	CT	PTX Relapser after 16 weeks trt	P/R PARTIAL RESPONDER	**L159F** C316N	I323I/V
P7977-2025-6928-7702	1b	TT	PTX Relapser after 2 weeks trt	OTHER UNKNOWN		
P7977-2025-7585-7731	1b	CT	DAA Relapser	P/R UNKNOWN		
P7977-2025-0585-7759	2b	CT		NAIVE		
P7977-2025-0773-7734	2b	CC	DAA BT	NAIVE		A80A/T P130Q T483A/T R523H G554A/G **L159F (2.1% NGS)**
P7977-2025-6927-7721	2b	CC	DAA Relapser	NAIVE		D213N
P7977-2025-0585-7752	3a	CT	DAA Relapser after 4 weeks trt	Other nonresponder		I85I/V R203C 250K

All subjects had cirrhosis

In addition, DAVP requested that the sponsor provide the fastq files for all subjects who failed, and in response the sponsor submitted 14 fastq files for HCV NS5B sequences derived from baseline and timepoints close to the time of failure for the five subjects who experienced on-treatment failure, including two partial responders and three subjects who experienced virologic breakthrough (Table 6).

Table 6. NGS data submitted for the five on-treatment failures of SOF+RBV in P7077-2025.

HCV_GT	USUBJID	NONRECAT	Sample Sequenced	Day of Sample (Post-dose)	First Dose	Last Dose	Days on Drug	Time of Fail - Week (days)
1a	p7977-2025-0451-7732	VBT	Baseline	1	8/8/2012	11/23/2012	108	Between Week 12 (85) and 16 (109)
			PTXW1	118 (+10)				
			PTXW2	125 (+17)				
	p7977-2025-0773-7712	Partial	Baseline	1	6/8/2012	8/31/2012	85	Between Week 12 (71) and Week 12 (85)
			W8	56				
			FU4	112 (+27)				
	p7977-2025-1249-7720	Partial	Baseline	1	6/27/2012	8/24/2012	59	Between Week 8 (57) and Week 9 (63)
			W8	57				
			FU4	87 (+28)				
1b	p7977-2025-0585-7751	VBT	Baseline	1	9/27/2012	12/6/2012	71	Between Week 4 (29) and Week 8 (55)
			FU4	79 (8+)				
2b	p7977-2025-0773-7734	VBT	Baseline	1	8/10/2012	11/15/2012	98	Between Week 4 (29) and Week 12 (71)
			W12-1	92				
			W12-2	98				

VBT, viral breakthrough; **Partial**, partial responder; **Post-dose,** number of days after dosing was stopped

DAVP analyzed the NGS data using the Next Generation Sequencing Analysis Pipeline described in detail in the initial review of this NDA (NDA204671 SDN 002). Briefly, the NS5B gene sequence from the GT1a (H77), GT1b (Con1), or GT2b (HC−J8) reference sequence was used as a template to map the short NGS sequence reads from subjects infected with these genotypes/subtypes using CLC Genomics Workbench. Two variant detection methods (Quality-based Variant detection [QbVD] and Probabilistic Variant detection using a 75% threshold [PVD75]) were used to detect nucleotide variants above a threshold of 1%. Non-synonymous mutations were then translated into amino acids and compared to determine if substitutions were present at baseline and to identify substitutions that emerged during treatment. The SUBS10+- criteria were used to call emerging substitutions by identifying those substitutions that were not present at baseline (<1%) but were present at ≥10% at later timepoints.

Baseline NGS Analysis

The data generated by the sponsor were compared to the data generated by DAVP, and for the most part, there was good agreement between the two analysis approaches for baseline sequences. In all cases, the substitutions and frequencies were comparable. In summary of the results, there was one subject infected with HCV GT1b whose virus expressed L159F and C316N at high frequency at baseline and at failure (Table 7). This subject experienced virologic breakthrough by Week 8 on treatment (Table 7). None of the additional 4 subjects had virus with baseline substitutions that appeared to be associated with later treatment failure.

Table 7. Baseline substitutions identified by NGS sequencing for one subject among the 5 on-treatment failures of P7977-2025.

USUBJID	SUBTYPE	NONRECAT	Time of Failure	VISIT	NS5B AAPOS	TCOV	AAREF	AASUB	VCOV	AAFREQ	SUBS
P7977-2025-0585-7751	1b	VBT	Week8	Baseline	159	6945	L	F	6900	0.994	L159F
P7977-2025-0585-7751	1b	VBT	Week8	FU4	159	5257	L	F	5234	0.996	L159F
P7977-2025-0585-7751	1b	VBT	Week8	Baseline	316	6499	C	N	6453	0.993	C316N
P7977-2025-0585-7751	1b	VBT	Week8	FU4	316	3425	C	N	3417	0.998	C316N

Of note, L159F was also detected in three relapsers in P7977-2025 and in all three cases, C316N was also present (Tables 3 and 5). NGS data was not provided for subjects who relapsed. C316N was also present in two additional relapsers lacking the L159F substitution (see the analyses above, Table 3 and the C316N Analysis below). No additional substitutions were identified at baseline by NGS that were not detected by the sponsor (Table 3).

Emergent NGS Analysis

In addition to the baseline analysis, DAVP also analyzed the NGS data using the SUBS10+- criteria described in detail in the original review of the NGS data for NDA 204671 SDN 002. Briefly, substitutions that occurred at later timepoints at a frequency of greater than or equal to 10% but that were not present at baseline were identified and compared to determine which NS5B amino acid positions contained substitutions that emerged with treatment. In three of the subjects who experienced on-treatment failure, substitutions emerged at positions L159, L320 or S282, albeit at low frequency (Table 8).

Table 8. Low frequency treatment-emergent substitutions associated with failure. Table based on the frequency table provided by the sponsor as there was complete agreement between the NGS analysis pipelines employed by DAVP and the sponsor.

USUBJID	SUBTYPE	NONRECAT	Time of Failure	VISIT	NS5B AAPOS	TCOV	AAREF	AASUB	VCOV	AAFREQ	SUBS
P7977-2025-0773-7734	2b	VBT	Week 12	Week 12	159	7324	L	F	152	0.021	L159F
P7977-2025-0773-7712	1a	Null	Week 12	Week 8	282	542	S	R	6	0.011	S282R
P7977-2025-0773-7712	1a	Null	Week 12	Week 8	320	5754	L	F	102	0.018	L320F
P7977-2025-0451-7732	1a	VBT	Week16	PTX Week 1	159	6652	L	F	631	0.095	L159F
P7977-2025-0451-7732	1a	VBT	Week16	PTX Week 2	159	5129	L	F	59	0.012	L159F

Of note, 4 of the 5 on-treatment failures had virus with substitutions at L159 or S282. The fact that these were low frequency may be related to the timing of the last sample, as in all cases treatment had stopped prior to collection of the timepoint closest to failure. The NGS data for subject 0451-7732 showed that L159F was present at 9.5% at post-transplant week and this dropped to 1.2% by post-transplant week 2, indicating that the viral population rapidly shifted from F159 to L159 when treatment drug was stopped at the time of transplant.

In addition to the substitutions shown above wherein there was complete agreement between the analysis approaches used by DAVP and Gilead, there were also treatment-emergent substitutions detected by DAVP that were not detected by Gilead (Table 9).

Table 9. Treatment-emergent substitutions detected by DAVP that were not detected by Gilead.

HCV GT	NS5B AAPOS	USUBJID	SUBS10+-	DETECT	PVD75	QbVD	GIL
1a	61	P7977-2025-0451-7732	D61G	2	X	X	
1a	61	P7977-2025-0773-7712	D61G	1		X	
1a	61	P7977-2025-1249-7720	D61G	1	X		
1a	62	P7977-2025-0451-7732	N62H	1	X		
1a	62	P7977-2025-0773-7712	G62D/N	2	X	X	
1a	62	P7977-2025-1249-7720	S62N	2	X	X	

Interestingly, three of the on-treatment failures' virus had emerging D61G (position 61 was 99.62% conserved among GT1a NS5B sequences), including subject P7977-2025-1249-7720 who was a partial responder with no substitutions at L159, C316, S282, L320, or V321. Of note, both partial responders had the D61G substitution along with one subject who experienced virologic breakthrough. In addition, the same three subjects' virus had substitutions at position 62, although these were more variable (Table 9) and this position was polymorphic with 63.74% conservation.

Summary of NGS Analysis of Five On-Treatment Failures

The sponsor concluded that after reviewing the clinical, pharmacokinetic, and virology data for the 5 subjects in Study P7977-2025 who were on-treatment virologic failures, one subject (0773-7734) was suspected to be non-compliant to study treatment and experienced virologic breakthrough. The sponsor further concluded that in the remaining four subjects, there was no consistent pattern or apparent reason for the on-treatment failures observed. Therefore, they claimed that no clinically relevant resistance-associated substitutions were identified.

In contrast, DAVP concluded that there were treatment-emergent substitutions that could be related to resistance to sofosbuvir. Of the five on treatment failures, one subject's GT1b virus had L159F and C316N at baseline at high frequency. This subject had breakthrough at Week 8. The other two subjects experiencing breakthrough (1 GT1a and 1 GT2b) both had emerging L159F in their viruses, although this was at low frequency in the sample taken a couple of weeks after breakthrough. There was evidence of a ~9-fold reduction in the frequency of this substitution in one week after treatment ceased in subject P7977-2025-0451-7732 who experienced breakthrough after liver transplant. This observation indicates that L159F disappeared rapidly after the cessation of treatment. One of the partial responders' virus had emerging S282R+L320F at the timepoint prior to ceasing treatment. The other partial responder did not have detectable substitutions at previously identified treatment-emergent sites. In addition, both partial responders and one subject who experienced virologic breakthrough had emerging D61G (and emerging substitutions at position 62) near the time of failure.

Overall Summary of Resistance for P7977-2025

Every subject whose virus had L159F at baseline in this study failed, including 3 subjects infected with HCV GT1b who had recurring HCV infection after transplant and 1 subject infected with HCV GT1b who failed prior

to transplant. In addition, 1 subject infected with HCV GT1a who relapsed had emergent L159F at the first timepoint after relapse. L159F was not observed at baseline in any subject who achieved SVR in this study.

C316N was present at baseline in the virus from 6 of 13 subjects infected with HCV GT1b who failed treatment with SOF in this study, and this substitution was not present at baseline in the virus of any subject who achieved SVR. Of note, C316N was present in the virus from all four subjects infected with HCV GT1b who failed treatment with SOF and had L159F at baseline. In addition to these four subjects described above, C316N in the absence of an L159F substitution was found in the virus from 1 subject who relapsed prior to transplant and from 1 subject who had recurring HCV GT1b infection post-transplant. C316N was found at baseline in one subject with treatment-emergent substitution E440G.

The emerging substitutions identified in this study, L159F and S282R, were compared to those identified in the four pivotal Phase 3 trials used to support NDA 204671 for SOF. A novel emergent substitution identified in this study, D61G, was detected by the NGS using the DAVP analysis pipeline. This substitution was found in the virus from three subjects, along with substitutions at position 62. Two of these subjects were partial responders. None of the additional treatment-emergent substitutions found in this clinical trial to date were identified as treatment-emergent in the Phase 3 trials.

L159F emerged in the Phase 3 trials in the virus from 6 subjects infected with HCV GT3a and is a previously identified NS5B substitution which with L320F was been shown to confer resistance to HCV nucleotide inhibitors (Tong X et al., 2013). In this study, L159 was present at baseline in 4 subjects who failed treatment (along with C316N) and emerged (detected at low frequency) in both subjects who experienced virologic breakthrough.

In the Phase 3 trials, six GT1b subjects had C316N (n=4) or C316H (n=2) at baseline and in both of these subgroups the SVR12 rate was 50%. In addition, C316F emerged in one subject infected with HCV GT1a who was a relapser. Of note, C316N and C316H substitutions are unlikely in GT1a subjects as each of these requires 2 mutations to occur. In total, C316N/H/F has been associated with sofosbuvir failure in 10 subjects infected with HCV GT1b in studies P7977-2025 and GS-US-334-0110 (NEUTRINO).

GILEAD'S RESPONSE TO THE ADVISORY COMMITTEE BACKGROUND PACKAGE

Comments and Analysis from Gilead

With submission NDA 204671 SDN 040, the sponsor provided a response to information provided by the division in the FDA Antiviral Drugs Advisory Committee Background Package. The background package included a section from Clinical Virology highlighting the resistance findings for SOF.

> *Gilead has determined that the apparent development of the E341D substitution may be an artifact of the deep sequencing assay as the base change associated with the substitution is consistently observed at the end of the sequencing read.*
>
> *Further analysis was performed and a total of 16 subjects from FISSION (P7977-1231) and FUSION (GS-US-334-0108) were identified to have the E341D substitution in post-baseline samples. The results indicated that the E341D NS5B substitution was authentically developed in only one subject across the SOF Phase 2 and 3 studies. In contrast, the substitution E341D in the other 15 subjects, including the subject with L159F+E341D, was derived from the primer used for amplification of the short NS5B fragment.*

During next generation or population sequencing of NS5B gene sequences for resistance analysis, if the full-length NS5B amplification was weak or failed, Gilead amplified a short fragment (220-340) of NS5B and sequenced it with NGS in order to maximize the detectability of the S282T substitution. The sponsor now

reports that the nested-PCR primers used for the short fragment amplification included a reverse primer that crossed through NS5B at position 341. The sponsor explains:

> *NS5B amino acid position 341 is conserved in genotype 1-5 (E341) and is polymorphic in genotype 6 (E/D341). The reverse primers were designed by* (b) (4) *to amplify samples of all genotypes for NS5B genotyping/subtyping and included a primer with 341D/E mixture. Due to unavailability of primer sequences to Gilead, FASTQ files for the short fragment were not "cleaned" to exclude primer sequences. In the deep sequencing analysis pipeline at Gilead, the terminal 15 nucleotides of each aligned read were excluded from consensus generation for each sample and therefore no E341D mutants originating from primer sequences were included in the consensus sequences and ADVR files.*

The sponsor reports that 18 post-baseline samples from 16 subjects and one baseline sample from a non-GT6 subject appear to have E341D/E mixtures in the fastq files (Table 10).

Table 10. Samples with E341D/E mixtures in the FASTQ files (Table 2, page 7, Efficacy Virology Report).

P7977

GS-US-
010

Comment: Table 2 from page 7 of the Efficacy Virology Report submitted on 10/10/2013 contains errors (not all STUDY IDs and SUBJIDs are correct). Please send a corrected version of this table and provide a second table that indicates all subjects and samples by HCV genotype for which the short NS5B fragment was generated using the nested-primer sets.

The sponsor reported that the results of their data analysis indicated that 1/16 subjects (Subject 310625) developed the E341D NS5B substitution at a frequency of 7.7%. That was evident from the deep sequencing reads containing this substitution at random positions of the reads as expected (Figure 1).

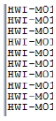

Figure 1. E341D reads of Subject 310625 aligned to the baseline population sequence (Figure 1, page 9, Efficacy Virology Report).

The sponsor reported that for 15 subjects, all samples had limited coverage of full-length NS5B and that E341D was only observed at the ends of the sequencing reads, which were derived from the shorter NSB5 fragment (Figure 2).

Figure 2. Schematic representation of the L159F and E341D/E substitutions across NS5B PCR products in Subject 310257 at the Follow-up Week 4 Visit (Figure 2, page 10, Efficacy Virology Report).

The sponsor performed sequence alignments of the short DNA sequence reads from samples that had the short PCR fragment and they claim that the alignments showed that the E341D substitution originated from the primer sequence used for the short amplification (Figure 3) because it occurred in the exact same position of several sequence reads. They reported that this was due to the fact that the substitution was incorporated into the 3' end of the short fragment being sequenced. Theoretically, if a site of interest was greater than ~150 nucleotides interior to the ends of the fragment being sequenced, the size and position of each read containing that site would be random because the various DNA fragments being sequenced in the NGS reaction would be of random sizes, resulting in variable sequence reads.

The sponsor reported that there were two scenarios for short fragment E341E/D primer-originated calls in these samples:

1. When the weak full-length NS5B PCR product and short NS5B PCR fragment from the same plasma sample were analyzed, the results were pooled together resulting in a false call of the E341D variants due to the primer used for the short fragment containing the 341E/D mixture. This happened in 7/15 subjects, including the subject with L159F detected (Table 10, Figure 2, and Figure 3).
2. When the full-length weak PCR product was deep sequenced for a given sample, due to the paucity of reads that aligned to HCV in those samples, cross-contamination of multiplexed NGS short PCR products from different subjects on the same deep sequencing run resulted in false detection of E341D calls in these low coverage samples. This scenario was observed in 8/15 subjects (Table 10 and Figure 4).

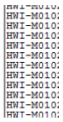

Figure 3. E341D reads from Subject 310257 at Follow-up Week 4 Visit are always from short PCR product (Figure 3, page 10, Efficacy Virology Report).

Cross-contamination of sequencing samples accounted for false enrichment of E341D in one subject (GS-US-334-0107-7234) due to several amplified fragments that derived from the PCR amplification using the reverse primer that was designed to amplify all 6 HCV GTs (Figure 4).

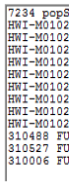

Figure 4. Example of cross-contamination of multiplexed short PCR products from different subjects on the same NGS run resulted in false enrichment of E341D in Subject 7234 (Figure 4, page 11, Efficacy Virology Report).

The sponsor also analyzed two subjects infected with HCV GT6 and showed that these subjects' virus had E341D at baseline at high frequency (>90%). They presented an alignment that showed that the reads associated with this baseline sample in the region of 341 were of variable length as expected. Given these data, the sponsor concluded that the E341D NS5B substitution developed only in one subject across the SOF Phase 2 and 3 studies and was not developed alone or in combination with L159F in any other subject.

Nested-PCR Primer Analysis

During the Late Cycle Meeting, the sponsor commented on the E341D substitution and explained that it was due to a nested-PCR artifact that was generated and pooled with failed NS5B gene amplification prior to NGS sequencing. The sponsor reported that the contractor who performed the nested-PCR amplification employed pan-genotypic HCV primers that contained the nucleotide variation that led to the E341D translation. DAVP asked the sponsor to provide the PCR primer sequences for validation. In submission NDA 204671 SDN 047 the sponsor provided these primer sequences (Table 11).

Table 11. PCR primer sequences used for the short NS5B amplification (Table 1, page 4, Virology Communication from 10/16/21).

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

1. The s

2. The p

In addition, the sponsor mapped these primer sequences on a cartoon of the HCV genome to show how these primers contributed to the PCR artifact containing the E341D substitution (Figure 5).

(b) (4)

Figure 5. PCR primers containing E341D substitutions used for the short NS5B amplification (Figure 1, page 5, Virology Communication from 10/16/21).

DAVP's Response to Gilead

Differences in analysis approaches employed by the sponsor and the division resulted in different subjects being identified as having HCV NS5B sequences bearing the E341D substitution. For example, Gilead indicated that they removed 15 terminal nucleotides from each sequence read during the trimming step. DAVP used the CLC Genomics Workbench, which performed trimming of the ends of reads based upon quality score. This trimming difference would eliminate many of the false reads from the Gilead dataset containing the E341D substitution derived from the PCR amplification of the short fragment.

In the initial analysis, DAVP observed higher levels of coverage at position 341 and noticed that many, but not all, of the reads bearing this substitution occurred near the sequence read ends. However, given that these were: 1) detected by at least two algorithms, 2) were also reported by the sponsor as developing, 3) were observed in two clinical trials, 4) were only found in a subset of relapsers, and 5) were not reported at baseline in any GT3 subject, we classified E341D as a treatment-emergent substitution of interest.

The results of this study by the sponsor indicate that:

1. E341D is an artifact that did not develop as a result of treatment with SOF
2. That E341D was introduced into some samples that did not have robust full-length NS5B NGS results that were pooled together with the amplified PCR product bearing E341D
3. That E341D was introduced into other samples that did not have robust full-length NS5B NGS results or that had low coverage via cross-contamination of sequences

To validate the results reported by the sponsor, DAVP reanalyzed the sequences bearing E341D.

E341D Data Analysis and Summary

To assess the presence of E341D in post-baseline samples, Clinical Virology reevaluated all NGS sequence reads for all samples from subjects in P7977-1231 and GS-US-334-0107 who were infected with HCV GT3a. This analysis included 74 subjects infected with HCV GT3a from P7977-1231 and 35 subjects with HCV GT3a infection in study GS-US-334-0107 (Figure 6, Table 12, Appendix 2).

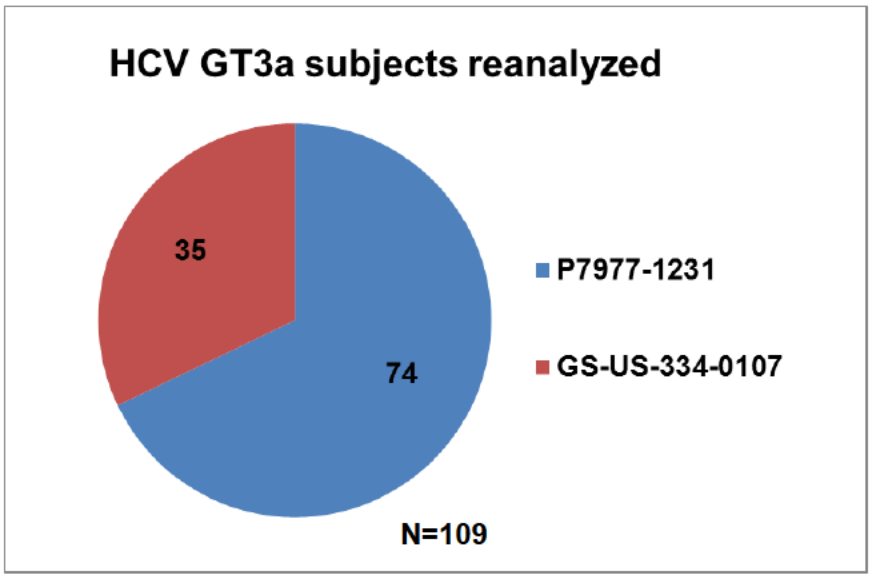

Figure 6. The number of HCV GT3a subjects reanalyzed.

At amino acid position 341, four codons were possible when changing the nucleotide in the third position (the wobble position): (b) (4)

(b) (4) (b) (4)

(b) (4) (reverse): (b) (4)

(reverse):

Sequences amplified (position (b) (4) only):

(b) (4)

Figure 7. Primer analysis for codons at amino acid position 341 (DAVP analysis). (b) (4) Provided the reaction pooled a 1:1 ratio of the two primers, GAT would be the predominant codon amplified by this reaction, followed by nearly equal proportions of GAA and GAG. GAC would not be amplified by this nested-PCR reaction.

To assess the composition of the codons described in Figure 7, DAVP determined the codon frequencies at position 341 for all subjects infected in HCV GT3a who had any sample that contained the substitution E341D at any frequency. A total of 393 samples were analyzed from 109 subjects (Appendix 2). Of these, 46 samples from 35 subjects contained E341D at a frequency greater than 1% and the codon frequencies were assessed for these samples (Table 12).

In general, the samples that had codon **GAT** at position 341 also had codons **GAA** and **GAG**. As expected, **GAT** had the highest frequency (median 86.14%, range 77.9% to 90.1%), whereas **GAA** (median 10.0%, range 7.0% to 12.4%) and **GAG** (median 3.4%, range 2.5% to 11.5%) were both present but at frequencies that were much lower than the frequency observed for codon **GAT**. This was observed in 24 subjects infected with HCV GT3a who had samples containing the E341D substitution (Table 12, subjects highlighted in yellow), and this observation was consistent with the primer analysis shown in Figure 7. The E341D substitution in these subjects was classified as a PCR artifact in agreement with the observation reported by the sponsor. Of note, the frequency of **GAT** indicates that the ratio of primers was not 1:1. By contrast, one subject (P7977-1231-310005) had E341D that was encoded by codon **GAT** at a frequency of 1.9% at FU12 with greater than 98% frequency of **GAG**

(Table 12). While this could be a potential E341D emergent substitution, it was not detected in the FU4 sample or at any other timepoint and therefore, is unlikely to be a real treatment-emergent substitution (Table 12, Appendix 2).

Table 12. Codon frequencies for subjects infected with HCV GT3a who had E341D at >1%. Only timepoints that had evidence of E341D at or greater than 1% are shown.

SUBJID	VISIT	Amino Acid 341 codons				Total	Codon Frequencies				GAT +3	Explanation
		GAG [E]	GAA [E]	GAT [D]	GAC [D]	Reads	GAG [E]	GAA [E]	GAT [D]	GAC [D]		
GS-US-334-0107-7234	BL	**35**	3	3	0	41	**85.37**	**7.32**	**7.32**	0.00	0	Coverage too low
GS-US-334-0107-7234	FU4.7	5	0	4	1	10	**50.00**	0.00	**40.00**	**10.00**	0	
GS-US-334-0107-7238	BL	**25**	1	3	0	29	**86.21**	**3.45**	**10.34**	0.00	0	Coverage too low
GS-US-334-0107-7269	FU4.11	**97**	1	5	0	103	**94.17**	0.97	**4.85**	0.00	0	Coverage too low
GS-US-334-0107-7295	FU4.14	10	**17**	**99**	0	126	**7.94**	**13.49**	**78.57**	0.00	13	Coverage too low
GS-US-334-0107-7295	FU4-3	**17**	**33**	**127**	0	177	**9.60**	**18.64**	**71.75**	0.00	31	
GS-US-334-0107-7445	FU4	**60**	0	1	0	61	**98.36**	0.00	**1.64**	0.00	0	Coverage too low
GS-US-334-0107-7476	BL-2	0	1	3	0	4	0.00	**25.00**	**75.00**	0.00	0	Coverage too low
P7977-310005	FU12	**1050**	2	**20**	1	1073	**97.86**	0.19	**1.86**	0.09	0	Potential emergence
P7977-310111	FU12	**73**	1	**18**	0	92	**79.35**	**1.09**	**19.57**	0.00	0	Coverage too low
P7977-310199	FU12	**78**	0	8	0	86	**90.70**	0.00	**9.30**	0.00	0	Coverage too low
P7977-310286	FU12	**101**	1	9	0	111	**90.99**	0.90	**8.11**	0.00	3	Coverage too low
P7977-310286	FU4.3	**35**	0	1	1	37	**94.59**	0.00	**2.70**	**2.70**	0	
P7977-310286	FU4	**42**	0	0	1	43	**97.67**	0.00	0.00	**2.33**	1	
P7977-310625	FU4	**685**	0	0	**62**	747	**91.70**	0.00	0.00	**8.30**	0	Potential emergence
GS-US-334-0107-7294	BL-2	**21**	2	2	0	25	**84.00**	**8.00**	**8.00**	0.00	0	Coverage too low
GS-US-334-0107-7294	FU4-2	**2171**	**2645**	**28213**	**106**	33135	**6.55**	**7.98**	**85.15**	0.32	16	PCR artifact
GS-US-334-0107-7294	FU4.9	**59**	1	4	0	64	**92.19**	**1.56**	**6.25**	0.00	0	Coverage too low
GS-US-334-0107-7307	FU4-4	**1810**	**3039**	**22672**	**87**	27608	**6.56**	**11.01**	**82.12**	0.32	17	PCR artifact
GS-US-334-0107-7477	FU4.18	**207**	2	**19**	0	228	**90.79**	0.88	**8.33**	0.00	0	Coverage too low
GS-US-334-0107-7477	FU4-4	**4275**	**5641**	**35364**	**139**	45419	**9.41**	**12.42**	**77.86**	0.31	31	PCR artifact
P7977-310002	BL-2	**218**	**192**	**1475**	7	1892	**11.52**	**10.15**	**77.96**	0.37	9	PCR artifact
P7977-310002	FU12	**20**	0	9	0	29	**68.97**	0.00	**31.03**	0.00	0	Coverage too low
P7977-310013	FU12-3	**816**	**2404**	**25957**	**101**	29278	**2.79**	**8.21**	**88.66**	0.34	0	PCR artifact
P7977-310013	FU12.4-2	**764**	**2201**	**21431**	**93**	24489	**3.12**	**8.99**	**87.51**	0.38	0	
P7977-310061	FU16	**822**	**3455**	**29062**	**105**	33444	**2.46**	**10.33**	**86.90**	0.31	0	PCR artifact
P7977-310167	FU12	**680**	**1813**	**22267**	**76**	24836	**2.74**	**7.30**	**89.66**	0.31	0	PCR artifact
P7977-310195	FU12.5-2	**652**	**2181**	**16956**	**62**	19851	**3.28**	**10.99**	**85.42**	0.31	0	PCR artifact
P7977-310252	FU4.7	**1363**	**2507**	**19284**	**81**	23235	**5.87**	**10.79**	**83.00**	0.35	3	PCR artifact
P7977-310257	FU4-2	**837**	**2341**	**21858**	**100**	25136	**3.33**	**9.31**	**86.96**	0.40	0	PCR artifact
P7977-310307	FU4-2	**693**	**2666**	**24166**	**149**	27674	**2.50**	**9.63**	**87.32**	0.54	3	PCR artifact
P7977-310369	FU8-1	**820**	**2369**	**17962**	**78**	21229	**3.86**	**11.16**	**84.61**	0.37	0	PCR artifact
P7977-310371	FU8-1	**1181**	**2147**	**21464**	**94**	24886	**4.75**	**8.63**	**86.25**	0.38	2	PCR artifact
P7977-310382	FU4-2	**349**	**896**	**7786**	**19**	9050	**3.86**	**9.90**	**86.03**	0.21	0	PCR artifact
P7977-310385	FU4-1	**672**	**1809**	**23164**	**73**	25718	**2.61**	**7.03**	**90.07**	0.28	0	PCR artifact
P7977-310436	FU4-2	**93**	**310**	**2733**	10	3146	**2.96**	**9.85**	**86.87**	0.32	0	PCR artifact
P7977-310472	FU4-2	**1768**	**4747**	**33159**	**138**	39812	**4.44**	**11.92**	**83.29**	0.35	0	PCR artifact
P7977-310488	FU8-1	**1878**	**2669**	**20576**	**84**	25207	**7.45**	**10.59**	**81.63**	0.33	10	PCR artifact
P7977-310488	FU8-2	**162**	0	9	0	171	**94.74**	0.00	**5.26**	0.00	0	Coverage too low
P7977-310527	FU8	**1078**	**2390**	**16616**	**51**	20135	**5.35**	**11.87**	**82.52**	0.25	0	PCR artifact
P7977-310528	FU4-3	**877**	**2707**	**21957**	**116**	25657	**3.42**	**10.55**	**85.58**	0.45	0	PCR artifact
P7977-310528	FU4-4	**899**	**2573**	**20495**	**70**	24037	**3.74**	**10.70**	**85.26**	0.29	0	
P7977-310556	FU4-2	**756**	**2738**	**22840**	**94**	26428	**2.86**	**10.36**	**86.42**	0.36	0	PCR artifact
P7977-310563	FU4-1	**819**	**2268**	**24844**	**106**	28037	**2.92**	**8.09**	**88.61**	0.38	5	PCR artifact
P7977-310633	FU4-2	**705**	**2121**	**20802**	**105**	23733	**2.97**	**8.94**	**87.65**	0.44	0	PCR artifact
P7977-310678	FU4-1	**550**	**1843**	**17953**	**60**	20406	**2.70**	**9.03**	**87.98**	0.29	0	PCR artifact

Yellow, samples with PCR artifacts; **Red,** samples with coverage too low to make a call; **VISIT** nomenclature: **FU**, follow up; BL, **Baseline**; a **dash** followed by a number means that sample was re-sequenced; a **dot** followed by a number means that number of days after the original sample for that timepoint was sequenced (i.e., FU4.6 means the first sample was taken at follow up week 4 and a second sample was taken 6 days after the first sample). **GAT 3**+, the number of sequence reads that covered codon 341 and also extended beyond the end of the short PCR product (representing reads that were not amplified with the nested-PCR primers resulting in artifacts).

Eleven subjects had detectable E341D at various timepoints and with **GAT** and **GAC** frequencies that were inconsistent with the PCR artifact (Table 12, subjects highlighted in red). In all but two cases, the coverage for

these samples was too low (<500 sequence reads) to make a reliable call. These probably represent low level cross contamination of samples that occurred during multiplexing or sequence error. For two subjects, P7977-1231-310005 and P7977-1231-310625, there was evidence of E341D emergence. However, the sample from P7977-1231-310005 is not likely to contain a real treatment-emergent substitution for reasons stated previously. However, for subject P7977-1231-310625, E341D was encoded by GAC and present at ~8% frequency at FU4. This substitution is likely to be a true treatment–emergent substitution given that it was not present at baseline and was detected after relapse at FU4. Of note, this substitution and codon was not detected at or above 1% frequency at FU12.

Conclusions reached by Clinical Virology in reassign the emergence of E341D

Clinical virology reanalyzed all of the NGS sequences for all subjects infected with HCV GT3a who had samples that contained E341D at any frequency to determine if the E341D substitution was consistent with new data presented by the sponsor. A total of 46 samples from 35 subjects contained E341D at a frequency greater than 1%, including all 14 GT3a samples re-analyzed by the sponsor. The discrepancy in the number of subjects and samples is likely due to the different analysis approaches. DAVP analyzed all samples that had any E341D signal without using any filtering criteria or cutoffs.

Clinical virology agrees with the sponsor that E341D is an artifact introduced by nested-PCR amplification of the short NS5B sequence fragment. Only one subject infected with HCV GT3a, P7977-1231-310625 had treatment-emergent E341D at a frequency of ~8% at FU4.

Comment: Please provide a strategy for reducing or managing artifacts that result from nested-PCR errors in future NGS runs.

SPONSOR COMMENTS AT ADVISORY COMMITTEE MEETING

An Antiviral Drugs Advisory Committee was conducted for sofosbuvir on October 25, 2013. During this meeting, there was discussion about the potential for resistance developing to sofosbuvir. The sponsor agreed with the Division of Antiviral Products that L159F and V321A did emerge on treatment. However, they did not call these resistance-associated substitutions because these did not show an effect on the EC_{50} value of sofosbuvir in the replicon systems in cell culture.

There are four lines of evidence for L159F being associated with resistance: 1) it emerged in 6 GT3a relapsers in two phase 3 trials, 2) it was present at baseline in 4 GT1b subjects all of whom failed in the liver transplant study and it emerged in two other subjects (GT1a and GT2b) who experienced breakthrough in that trial, 3) L159 is in the active site and is a contact residue (Bressanelli et al., 2002), and 4) it's reported to be associated with resistance among other HCV polymerase inhibitors (Tong X et al., 2013). Of note, the Division takes the stance that a cell culture based phenotypic assay cannot rule out emergent substitutions that are associated with resistance in clinical trials.

The advisory committee members raised several questions about resistance and in fact, asked the sponsor to explain how these substitutions could emerge on treatment and not be associated with resistance. The sponsor replied that they did not know and indicated that they would continue to monitor for resistance, paying particular attention to sites L159F and V321A. Clinical virology provided evidence to the advisory committee meeting that in some cases, F159 expressing virus is rapidly displaced by L159 expressing viruses, indicating that HCV viruses bearing L159F are less fit. However, L159F was present at baseline in some subjects who achieved SVR and was present at high frequency over long periods of time during the followup period for some relapsers, indicating that not all HCV viruses bearing L159F are of low fitness.

Comment: Please perform a bioinformatics study comparing the NS5B genes of HCV viruses bearing the L159F substitution at baseline in subjects who achieved SVR12 to the NS5B genes that developed the L159F substitution on treatment (at all timepoints) with sofosbuvir to identify additional genetic factors that may be important for resistance development to sofosbuvir.

Potential Impact of C316N on the Efficacy of Sofosbuvir in Subjects Infected with HCV GT1b

In the sofosbuvir phase 3 trials, subjects infected with HCV GT1 had overall SVR12 rates of 89% (261/292), with GT1a infected subjects experiencing an overall SVR12 rate of 92% (206/225) and GT1b infected subjects exhibiting an overall SVR12 rate of 82% (54/66). However, in previous direct-acting antiviral drug development programs it has been commonly observed that HCV GT1b infected subjects have better response rates than HCV GT1a infected subjects. To determine if there were viral genetic factors that contributed to this difference, Clinical Virology performed sequence and structural bioinformatics analyses to determine if amino acid differences between these two genotypes in the NS5B polymerase could account for this difference in efficacy.

Structural Analysis of the polymerases (NS5Bs) of HCV GT1a with C316 and HCV GT1b with N316

A structural comparison was generated using PyMol (Schrödinger, LLC) to model the X-ray crystal structures of NS5B for GT1a with the C316 residue and GT1b with the N316 residue using structures with the following accession numbers: 3HKW (GT1a - H77) and 3HHK (GT1b - Con1)(Figure 8). The two structures were loaded into PyMol and superimposed upon one another to perform this analysis.

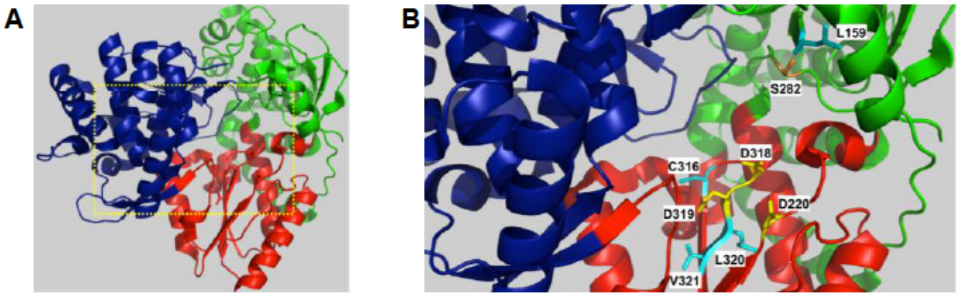

Figure 8. The X-ray crystal structure of HCV GT1a polymerase (generated by DAVP from 3HKW). **A.** Chain A of the HCV GT1a NS5B polymerase shown in the left-handed notation, colored by palm (red), fingers (green), and thumb (blue) domains. The yellow rectangle shows the area that was zoomed in to create panel B. **B.** A Zoomed view of the polymerase active site, showing the catalytic triad (yellow), sites identified as potentially related to resistance in phase 3 trials (cyan), and sites identified as resistance-associated by cell culture (orange).

The active site resides (D220, D318, D319) were highlighted and all residues within 8 angstroms of this catalytic triad were analyzed (Figure 8). There was only one difference noted and that was at position 316, where C316 was present in the GT1a structure and N316 was present in the GT1b structure (Figure 9). Importantly, C316 is highly conserved in GT1a (99.89%) but polymorphic in GT1b (81.83%) based on calculations provided by the sponsor.

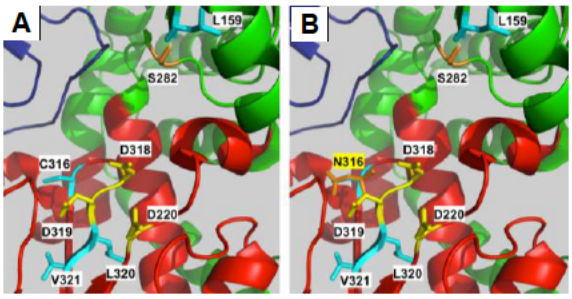

Figure 9. Comparison of the X-ray crystal structures of the HCV GT1a and GT1b polymerases (generated by DAVP from 3HKW and 3HHK, respectively). **A.** The active site of the HCV GT1a NS5B polymerase (Chain A, zoomed). **B.** The active site of the HCV GT1b NS5B polymerase (Chain A, zoomed). The structures are shown in the left-handed notation, colored by palm (red), fingers (green), and thumb (blue) domains. The catalytic triad (yellow), sites identified as potentially related to resistance in phase 3 trials (cyan), and sites identified as resistance-associated by cell culture (orange) are highlighted. The difference at position 316 is highlighted in orange in panel B.

Of note, N316 found in the HCV GT1b polymerase structure is a larger and more bulky amino acid with two additional functional groups in its side chain. Given the increased bulk present with this amino acid, it is possible that N316 or additional substitutions that add bulk (i.e., histadine, phenylalanine), could interfere with the ability of sofosbuvir to interact with to the active site and get incorporated into the nascent viral RNA strand to inhibit the HCV polymerase function (Figure 10). The large amino acid is predicted to interfere with sofosbuvir's ability to enter the active by blocking the space required to accommodate the additional 2'Me and 2'F groups of sofosbuvir.

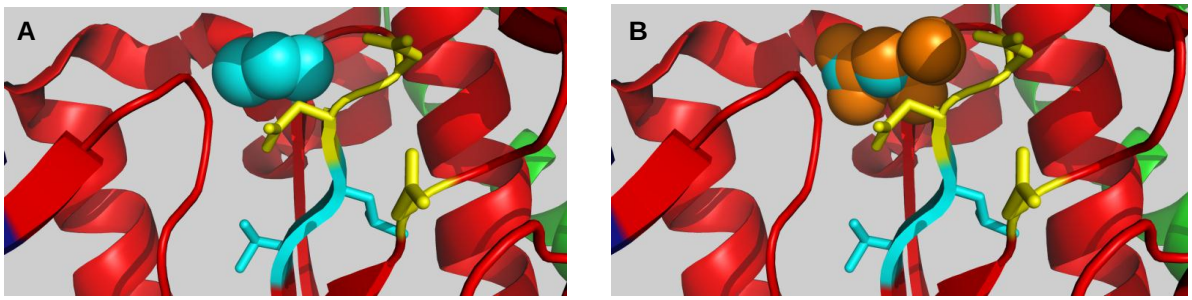

Figure 10. Comparison of the X-ray crystal structures of the HCV GT1a and GT1b polymerases at position 316 (generated by DAVP from 3HKW and 3HHK, respectively). **A.** The active site of the HCV GT1a NS5B polymerase (Chain A, zoomed) with C316 shown in cyan spheres. **B.** The active site of the HCV GT1b NS5B polymerase (Chain A, zoomed) with N316 shown in orange spheres. The structures are shown in the left-handed notation, colored by palm (red), fingers (green), and thumb (blue) domains. The catalytic triad (D220, D318 and D319, yellow) and some sites identified as potentially related to resistance in phase 3 trials (V321A and L320, cyan sticks) are shown.

Structural bioinformatics analyses showed that the only difference within 8 angstroms of the catalytic triad between the HCV GT1a and GT1b NS5B polymerases occurred at position 316. Although there is no crystal structure showing any HCV polymerase in complex with sofosbuvir, nucleoside analogs are known to directly interact with the active site to facilitate incorporation into the nascent RNA template leading to chain termination. Given that C316 is the only polymorphic amino acid position in the active site between the polymerases of GT1a and 1b indicates that substitutions at C316, particularly substitutions that add bulkier amino acids, may interfere with the ability of sofosbuvir to access the active site.

Sequence analysis of the polymerases (NS5Bs) of HCV GT1a with C316 and HCV GT1b with N316

To further explore the potential impact of substitutions at C316, resistance data from Phase 3 trial GS-US-334-0110 and from the pre-transplant study P7977-2025 were analyzed to determine the impact of substitutions at this position.

In study GS-US-334-0110, one GT1a subject, 0530-6412 had C316F emerge with sofosbuvir treatment, and this substitution was detected at Week 4 follow up at which time this subject was determined to be a relapser. All of the subjects in GS-US-334-0110 infected with HCV GT1a had virus with C316 in the polymerase at baseline.

Among subjects in study GS-US-334-0110 infected with HCV GT1b, no subjects developed substitutions at position C316 over the course of treatment; however, six GT1b subjects had C316N (n=4) or C316H (n=2) at baseline and in both of these subgroups the SVR12 rate was 50% (Table 13). Emergence of a C316N substitution is unlikely as 2 mutations in the 316 codon are required. Of note, among GT1b subjects' viruses that had C316 in NS5B at baseline, the SVR12 rate was ~83% (52/63).

In the liver transplant study, P7977-2025, C316N was present at baseline in 6 subjects infected with HCV GT1b, all of whom where treatment failures. By contrast, subjects infected with GT1b with C316, the SVR12

rate was 42% (5/12). C316N was not present at baseline in the virus of any subjects infected with GT1a. No substitutions at position C316 emerged in subjects infected with GT1 in the pre-transplant liver study.

Table 13. Efficacy assessment based on amino acid at position 316 of the polymerase.

EFFICFL	USUBJID	C	H	N	SVR12 rate	Subjects
N	GS-US-334-0110-4308-6656	0	1	0	**50%**	**2**
Y	GS-US-334-0110-3060-6450	0	1	0		
N	GS-US-334-0110-0532-6602	0	0	1	**50%**	**4**
N	GS-US-334-0110-5586-6473	0	0	1		
Y	GS-US-334-0110-0532-6672	0	0	1		
Y	GS-US-334-0110-7864-6536	0	0	1		
N	GS-US-334-0108-0057-1480	1	0	0	**83%**	**63**
N	GS-US-334-0108-0451-1507	1	0	0		
N	GS-US-334-0110-0407-6418	1	0	0		
N	GS-US-334-0110-0407-6696	1	0	0		
N	GS-US-334-0110-0530-6409	1	0	0		
N	GS-US-334-0110-3060-6417	1	0	0		
N	GS-US-334-0110-3060-6569	1	0	0		
N	GS-US-334-0110-4007-6448	1	0	0		
N	GS-US-334-0110-4308-6454	1	0	0		
N	GS-US-334-0110-4322-6580	1	0	0		
N	GS-US-334-0110-4421-6716	1	0	0		
Y	GS-US-334-0108-0530-1405	1	0	0		
Y	GS-US-334-0110-0057-6438	1	0	0		
Y	GS-US-334-0110-0057-6457	1	0	0		
Y	GS-US-334-0110-0331-6560	1	0	0		
Y	GS-US-334-0110-0331-6574	1	0	0		
Y	GS-US-334-0110-0380-6588	1	0	0		
Y	GS-US-334-0110-0407-6480	1	0	0		
Y	GS-US-334-0110-0446-6533	1	0	0		
Y	GS-US-334-0110-0451-6616	1	0	0		
Y	GS-US-334-0110-0519-6568	1	0	0		
Y	GS-US-334-0110-0519-6609	1	0	0		
Y	GS-US-334-0110-0532-6587	1	0	0		
Y	GS-US-334-0110-0532-6725	1	0	0		
Y	GS-US-334-0110-0535-6552	1	0	0		
Y	GS-US-334-0110-0535-6591	1	0	0		
Y	GS-US-334-0110-0642-6524	1	0	0		
Y	GS-US-334-0110-0642-6528	1	0	0		
Y	GS-US-334-0110-1536-6571	1	0	0		
Y	GS-US-334-0110-1668-6493	1	0	0		
Y	GS-US-334-0110-2130-6650	1	0	0		
Y	GS-US-334-0110-2130-6652	1	0	0		
Y	GS-US-334-0110-2130-6668	1	0	0		
Y	GS-US-334-0110-2487-6497	1	0	0		
Y	GS-US-334-0110-2493-6464	1	0	0		
Y	GS-US-334-0110-2493-6467	1	0	0		
Y	GS-US-334-0110-2689-6416	1	0	0		
Y	GS-US-334-0110-2689-6534	1	0	0		
Y	GS-US-334-0110-2728-6721	1	0	0		
Y	GS-US-334-0110-2760-6561	1	0	0		
Y	GS-US-334-0110-2760-6645	1	0	0		
Y	GS-US-334-0110-3060-6635	1	0	0		
Y	GS-US-334-0110-3996-6626	1	0	0		
Y	GS-US-334-0110-4007-6434	1	0	0		
Y	GS-US-334-0110-4007-6484	1	0	0		
Y	GS-US-334-0110-4238-6455	1	0	0		
Y	GS-US-334-0110-4238-6712	1	0	0		
Y	GS-US-334-0110-4262-6429	1	0	0		
Y	GS-US-334-0110-4323-6726	1	0	0		
Y	GS-US-334-0110-4421-6686	1	0	0		
Y	GS-US-334-0110-4434-6634	1	0	0		
Y	GS-US-334-0110-5369-6515	1	0	0		
Y	GS-US-334-0110-5369-6553	1	0	0		
Y	GS-US-334-0110-5498-6443	1	0	0		
Y	GS-US-334-0110-5498-6459	1	0	0		
Y	GS-US-334-0110-5498-6503	1	0	0		
Y	GS-US-334-0110-5518-6426	1	0	0		
Y	GS-US-334-0110-5518-6525	1	0	0		
Y	GS-US-334-0110-5518-6586	1	0	0		
Y	GS-US-334-0110-5518-6625	1	0	0		
Y	GS-US-334-0110-5665-6445	1	0	0		
Y	GS-US-334-0110-5852-6699	1	0	0		
Y	GS-US-334-0110-5852-6717	1	0	0		

Conclusions based on analysis of the NS5Bs of HCV GT1a with C316 and HCV GT1b with N316

- C316N is the only amino acid difference between the HCV GT1a and GT1b polymerases in the active site
- Substitutions that add bulk to this position are predicted to interfere with sofosbuvir's ability to enter the active site and inhibit HCV RNA replication
- C316 is highly conserved in GT1a (99.89%) but not in GT1b (81.83%), indicating that amino acid substitutions at position 316 that reduce efficacy would occur more frequently in HCV GT1b
- C316N/H/F has been associated with sofosbuvir failure in 10 subjects infected with HCV GT1b in studies P7977-2025 and GS-US-334-0110.
- Subjects infected with HCV GT1b with C316N/H at baseline had SVR12 rates of 0% (0/6) and 50% (3/6) in studies P7977-2025 and GS-US-334-0110, respectively, compared to 85% for GT1b with C316.
- One subject infected with HCV GT1b who relapsed after treatment with sofosbuvir had emergent C316F.
- Substitutions at position C316, which is a polymorphic site in the GT1b NS5B polymerase, may contribute to reduced efficacy among subjects infected with this genotype of HCV.

CONCLUSIONS

- In clinical trial P7977-2025, the L159F polymorphism at baseline was associated with SOF failure in subjects infected with HCV GT1b, and this substitution emerged at low frequency in two subjects who experienced virologic breakthrough (1 GT1a and 1 GT2b) and in 1 subject who was infected with GT1a who experienced relapse. No subject who achieved pTVR12 had an L159F polymorphism at baseline. In addition, in Phase 3 studies L159F was treatment-emergent in 6 subjects infected with HCV GT3a who relapsed.

- C316N was present at baseline in 6 subjects infected with GT1b who failed in trial P7977-2025 and no subject who achieved pTVR12 had a C316N polymorphism at baseline.

- In the Phase 3 trials, six GT1b subjects had C316N (n=4) or C316H (n=2) at baseline and in both of these subgroups the SVR12 rate was 50%. In addition, C316F emerged in one subject infected with HCV GT1a who was a relapser. Of note, C316N and C316H substitutions are unlikely in GT1a subjects as each of these requires 2 mutations to occur. In total, C316N/H/F has been associated with sofosbuvir failure in 10 subjects infected with HCV GT1b in studies P7977-2025 and GS-US-334-0110 (NEUTRINO).

- D61G emerged in three on-treatment failures in P7977-2025, and all three of these had variable substitutions at position 62. Substitutions D61G and 62 should be analyzed for phenotypic susceptibility to sofosbuvir.

- E440G emerged in 3 subjects infected with HCV GT1b and this substitution should be studied in a phenotypic assay to determine if it reduces HCV GT1b susceptibility to sofosbuvir.

- E341D, which was identified as a treatment-emergent substitution of interest, has been shown to be a PCR artifact introduced into samples during the nest-PCR amplification of the short NSB5 fragment. This substitution emerged in one subject.

- Substitutions at position C316, which is a polymorphic site in the HCV GT1b NS5B polymerase, may contribute to reduced efficacy among subjects infected with HCV GT1b.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.

NDA#: 204671 **SDN** 016, 027, 030, 031, 040, 047 **DATES REVIEWED:** 9/16/13, 10/04/13, 10/10/13, 10/21/13

RECOMMENDATIONS

1. Determine the phenotypic susceptibility of SOF against HCV GT1b replicons with E440G and against HCV GT1a replicons with D61G (by itself and with N62H, G62D/N, and S62N).

2. Table 2 from page 7 of the Efficacy Virology Report submitted on 10/10/2013 contains errors (not all STUDY IDs and SUBJIDs are correct). Please send a corrected version of this table and provide a second table that indicates all subjects and samples by HCV genotype for which the short NS5B fragment was generated using the nested-primer sets.

3. Please provide a strategy for reducing or managing artifacts that result from nested-PCR errors in future NGS runs.

4. Please perform a bioinformatics study comparing the NS5B genes of HCV viruses bearing the L159F substitution at baseline in subjects who achieved SVR12 to the NS5B genes that developed the L159F substitution on treatment with sofosbuvir to identify additional genetic factors that may be important for resistance development to sofosbuvir.

ADMINISTRATIVE

Reviewer's Signature(s)

Eric F. Donaldson
[Eric F. Donaldson, Ph.D.]
Clinical Virology Reviewer, HFD-530

CONCURRENCE

HFD-530/Virol TL/J O'Rear _____Date _____

cc:
HFD-530/IND
HFD-530/Division File
HFD-530/RPM/Onaga

Reference ID: 3409507

APPENDICES

Appendix 1: Listing of all emerging substitutions detected by Gilead versus by DAVP in P7977-2025

HCV_GT	NS5B AAPOS	USUBJID	SUBS10+-	DETECT	PVD75	QbVD	GIL
1a	57	P7977-2025-0773-7712	L57V	2	X	X	
1a	61	P7977-2025-0451-7732	D61G	2	X	X	
1a	61	P7977-2025-0773-7712	D61G	1		X	
1a	61	P7977-2025-1249-7720	D61G	1	X		
1a	62	P7977-2025-0773-7712	G62D/N	1		X	
1a	62	P7977-2025-0773-7712	G62S	1		X	
1a	62	P7977-2025-0451-7732	N62H	1	X		
1a	62	P7977-2025-0773-7712	S62D	1		X	
1a	62	P7977-2025-0773-7712	S62N	1	X		
1a	62	P7977-2025-1249-7720	S62N	2	X	X	
1a	84	P7977-2025-1249-7720	T84S	1			X
1a	117	P7977-2025-0773-7712	T117A	1			X
1a	189	P7977-2025-1249-7720	N189S	2	X		X
1a	252	P7977-2025-0773-7712	A252V	1	X		
1a	273	P7977-2025-0773-7712	N273S	1	X		
1a	377	P7977-2025-0451-7732	T377A	1			X
1a	421	P7977-2025-0451-7732	A421V	1	X		
1a	461	P7977-2025-1249-7720	L461P	1			X
1a	510	P7977-2025-0773-7712	K510R	1			X
1a	517	P7977-2025-0773-7712	K517R	1			X
1a	544	P7977-2025-1249-7720	R544Q	2	X	X	
1a	574	P7977-2025-0773-7712	F574L	1	X		
1b	42	P7977-2025-0585-7751	S42F	2		X	X
1b	76	P7977-2025-0585-7751	S76P	2		X	X
1b	120	P7977-2025-0585-7751	H120R	1			X
1b	187	P7977-2025-0585-7751	M187L	2		X	X
1b	188	P7977-2025-0585-7751	G188S	2		X	X
1b	300	P7977-2025-0585-7751	S300T	2	X		X
1b	304	P7977-2025-0585-7751	R304Q	2		X	X
1b	355	P7977-2025-0585-7751	Q355H	2	X		X
1b	514	P7977-2025-0585-7751	Q514R	2	X		X
1b	537	P7977-2025-0585-7751	T537A	2		X	X
1b	549	P7977-2025-0585-7751	S549G	1			X
1b	556	P7977-2025-0585-7751	S556A	3	X	X	X
1b	571	P7977-2025-0585-7751	W571R	2		X	X
2b	15	P7977-2025-0773-7734	S15G	1			X
2b	43	P7977-2025-0773-7734	K43R	1			X
2b	47	P7977-2025-0773-7734	G47Q	1		X	
2b	71	P7977-2025-0773-7734	V71I	1			X
2b	80	P7977-2025-0773-7734	A80T	1			X
2b	120	P7977-2025-0773-7734	R120K	1			X
2b	131	P7977-2025-0773-7734	Q131H	1			X
2b	179	P7977-2025-0773-7734	T179A	1			X
2b	312	P7977-2025-0773-7734	I312T	1			X
2b	462	P7977-2025-0773-7734	I462V	2		X	X
2b	471	P7977-2025-0773-7734	A471T	1			X
2b	520	P7977-2025-0773-7734	T520V	1			X
2b	554	P7977-2025-0773-7734	G554A	1			X

Appendix 2: Codon frequencies for HCV GT3a NS3/4A amino acid position 341

The following four tables present all of the samples from all subjects infected with GT3a who relapsed during treatment in clinical trials P7977-1231 and GS-US-334-0107. Subjects that had E341D detected at or above a frequency of 1% were analyzed in greater detail (see Table 12).

Here is the list of abbreviations for these tables: **Yellow,** samples with PCR artifacts; **Red,** samples with coverage too low to make a call; **VISIT** nomenclature: **FU**, follow up; BL, **Baseline**; a **dash** followed by a number means that the sample was re-sequenced; a **dot** followed by a number means that a second sample was collected under that timepoint heading at the number of days after the original sample for that timepoint was sequenced (i.e., FU4.6 means the first sample was taken at Follow Up Week 4 and a second sample was taken 6 days after the first sample). **GAT/GAG 3**+, the number of sequence reads with the indicated codon that covered amino acid 341 and also extended beyond the end of the short NS5B PCR product (representing reads that were not amplified with the PCR primers resulting in artifacts).

Due to the size of the tables, they will start on the next page.

SUBJID	VISIT	Amino Acid 341 codons				Total	Codon Frequencies				Extended Reads				Resistance	Substitution
		GAG [E]	GAA [E]	GAT [D]	GAC [D]	Reads	GAG [E]	GAA [E]	GAT [D]	GAC [D]	GAT +3	Diff	GAG +3	Diff	Explanation	E341D real?
P7977-310002	BL-1	250	2	0	0	252	99.21	0.79	0.00	0.00	0	0	9	241	E341D substitution likely due to primer issue	No
P7977-310002	BL-2	218	192	1475	7	1892	11.52	10.15	77.96	0.37	9	1466	1	217		
P7977-310002	FU12	20	0	9	0	29	68.97	0.00	31.03	0.00	0	9	0	20		
P7977-310002	FU24	38	0	0	0	38	100.00	0.00	0.00	0.00	0	0	7	31		
P7977-310002	FU8	8	0	0	0	8	100.00	0.00	0.00	0.00	0	0	2	6		
P7977-310005	BL-1	3355	8	0	1	3364	99.73	0.24	0.00	0.03	0	0	1672	1683	E341D emerges at 1.86% frequency in FU12 at 0.01% at FU4	No
P7977-310005	BL-2	2482	1	1	0	2484	99.92	0.04	0.04	0.00	0	1	1276	1206		
P7977-310005	FU12	1050	2	20	1	1073	97.86	0.19	1.86	0.09	0	20	3	1047		
P7977-310005	FU20	383	0	0	0	383	100.00	0.00	0.00	0.00	0	0	13	370		
P7977-310005	FU24	4883	4	4	1	4892	99.82	0.08	0.08	0.02	0	4	44	4839		
P7977-310005	FU4	8639	67	1	3	8710	99.18	0.77	0.01	0.03	0	1	56	8583		
P7977-310013	BL-1	4915	1	0	0	4916	99.98	0.02	0.00	0.00	0	0	4318	597	E341D substitution likely due to primer issue	No
P7977-310013	BL-2	8391	13	1	0	8405	99.83	0.15	0.01	0.00	0	1	7639	752		
P7977-310013	FU12-1	326	0	0	0	326	100.00	0.00	0.00	0.00	0	0	2	324		
P7977-310013	FU12-2	11	0	0	0	11	100.00	0.00	0.00	0.00	0	0	3	8		
P7977-310013	FU12-3	816	2404	25957	101	29278	2.79	8.21	88.66	0.34	0	25957	0	816		
P7977-310013	FU12.4-1	10	0	0	0	10	100.00	0.00	0.00	0.00	0	0	2	8		
P7977-310013	FU12.4-2	764	2201	21431	93	24489	3.12	8.99	87.51	0.38	0	21431	0	764		
P7977-310013	FU16	8151	16	1	0	8168	99.79	0.20	0.01	0.00	0	1	20	8131		
P7977-310013	FU24	8406	9	2	1	8418	99.86	0.11	0.02	0.01	0	2	33	8373		
P7977-310021	BL	9352	12	3	0	9367	99.84	0.13	0.03	0.00	0	3	90	9262	No E341D emergence	No
P7977-310021	FU12	11434	6	10	2	11452	99.84	0.05	0.09	0.02	0	10	54	11380		
P7977-310021	FU24	6122	3	3	1	6129	99.89	0.05	0.05	0.02	0	3	29	6093		
P7977-310021	FU4	9670	16	2	2	9690	99.79	0.17	0.02	0.02	0	2	25	9645		
P7977-310036	BL	2175	3	0	0	2178	99.86	0.14	0.00	0.00	0	0	23	2152	No E341D emergence	No
P7977-310036	FU12	10643	8	6	1	10658	99.86	0.08	0.06	0.01	0	6	36	10607		
P7977-310036	FU24	8272	5	3	2	8282	99.88	0.06	0.04	0.02	0	3	26	8246		
P7977-310036	FU4	9063	8	1	0	9072	99.90	0.09	0.01	0.00	0	1	29	9034		
P7977-310036	FU8	8242	6	1	1	8250	99.90	0.07	0.01	0.01	0	1	25	8217		
P7977-310061	BL	5681	5	1	2	5689	99.86	0.09	0.02	0.04	0	1	5321	360	E341D substitution likely due to primer issue	No
P7977-310061	FU12	9	0	0	0	9	100.00	0.00	0.00	0.00	0	0	3	6		
P7977-310061	FU16	822	3455	29062	105	33444	2.46	10.33	86.90	0.31	0	29062	2	820		
P7977-310061	FU4	15	0	0	0	15	100.00	0.00	0.00	0.00	0	0	7	8		
P7977-310111	BL	304	0	0	0	304	100.00	0.00	0.00	0.00	0	0	3	301	E341D emergence detected but very low coverage. Potential cross contamination	Not likely
P7977-310111	FU12	73	1	18	0	92	79.35	1.09	19.57	0.00	0	18	1	72		
P7977-310111	FU24	37	0	0	0	37	100.00	0.00	0.00	0.00	0	0	1	36		
P7977-310111	FU4	25	0	0	0	25	100.00	0.00	0.00	0.00	0	0	2	23		
P7977-310130	BL	10960	0	1	1	10962	99.98	0.00	0.01	0.01	0	1	81	10879	No E341D emergence	No
P7977-310130	FU12	5677	13	4	1	5695	99.68	0.23	0.07	0.02	0	4	33	5644		
P7977-310130	FU16	11323	12	8	0	11343	99.82	0.11	0.07	0.00	0	8	77	11246		
P7977-310130	FU24	6835	7	5	0	6847	99.82	0.10	0.07	0.00	0	5	45	6790		
P7977-310130	FU4	9607	33	2	1	9643	99.63	0.34	0.02	0.01	0	2	351	9256		
P7977-310130	FU8	7929	10	2	0	7941	99.85	0.13	0.03	0.00	0	2	38	7891		
P7977-310139	BL	69	0	0	0	69	100.00	0.00	0.00	0.00	1	-1	6362	-6293	No E341D emergence	No
P7977-310139	FU12	40	0	0	0	40	100.00	0.00	0.00	0.00	1	-1	6643	-6603		
P7977-310139	FU24	41	0	0	0	41	100.00	0.00	0.00	0.00	3	-3	5659	-5618		
P7977-310139	FU8	47	0	0	0	47	100.00	0.00	0.00	0.00	0	0	8407	-8360		
P7977-310148	BL	11073	1	2	2	11078	99.95	0.01	0.02	0.02	0	2	76	10997	No E341D emergence	No
P7977-310148	FU12	11067	9	8	0	11084	99.85	0.08	0.07	0.00	0	8	32	11035		
P7977-310148	FU24	6004	4	2	2	6012	99.87	0.07	0.03	0.03	0	2	30	5974		
P7977-310148	FU4	6844	6	0	2	6852	99.88	0.09	0.00	0.03	0	0	24	6820		
P7977-310167	BL	6846	3	4	0	6853	99.90	0.04	0.06	0.00	4	0	6332	514	E341D substitution likely due to primer issue	No
P7977-310167	FU12	680	1813	22267	76	24836	2.74	7.30	89.66	0.31	0	22267	0	680		
P7977-310167	FU24	7305	2	3	1	7311	99.92	0.03	0.04	0.01	3	0	6998	307		
P7977-310167	FU4-1	6790	3	0	0	6793	99.96	0.04	0.00	0.00	0	0	6497	293		
P7977-310167	FU4-2	6725	4	0	0	6729	99.94	0.06	0.00	0.00	0	0	6440	285		
P7977-310179	BL	9281	9	4	1	9295	99.85	0.10	0.04	0.01	3	1	8403	878	No E341D emergence	No
P7977-310179	FU12	11886	7	12	0	11905	99.84	0.06	0.10	0.00	1	11	11498	388		
P7977-310179	FU16	6705	4	2	1	6712	99.90	0.06	0.03	0.01	2	0	6409	296		
P7977-310179	FU8	8078	4	0	2	8084	99.93	0.05	0.00	0.02	0	0	7764	314		
P7977-310195	BL	5046	11	0	1	5058	99.76	0.22	0.00	0.02	0	0	1737	3309	E341D substitution likely due to primer issue	No
P7977-310195	FU12.5-1	8837	2	0	0	8839	99.98	0.02	0.00	0.00	0	0	31	8806		
P7977-310195	FU12.5-2	652	2181	16956	62	19851	3.28	10.99	85.42	0.31	0	16956	0	652		
P7977-310195	FU12	6	0	0	0	6	100.00	0.00	0.00	0.00	0	0	3	3		
P7977-310195	FU24	6239	4	2	4	6249	99.84	0.06	0.03	0.06	0	2	28	6211		
P7977-310199	BL	46	2	0	0	48	95.83	4.17	0.00	0.00	0	0	39	7	E341D emergence detected but very low coverage. Potential cross contamination	Not likely
P7977-310199	FU12	78	0	8	0	86	90.70	0.00	9.30	0.00	0	8	25	53		
P7977-310199	FU24	50	0	0	0	50	100.00	0.00	0.00	0.00	0	0	32	18		
P7977-310199	FU4	63	0	0	0	63	100.00	0.00	0.00	0.00	0	0	37	26		
P7977-310228	BL	7245	4	1	1	7251	99.92	0.06	0.01	0.01	0	1	48	7197	No E341D emergence	No
P7977-310228	FU12	11322	11	15	1	11349	99.76	0.10	0.13	0.01	0	15	39	11283		
P7977-310228	FU24	6292	2	0	1	6295	99.95	0.03	0.00	0.02	0	0	21	6271		
P7977-310228	FU4	6974	8	2	0	6984	99.86	0.11	0.03	0.00	0	2	16	6958		
P7977-310252	FU24	6215	3	2	3	6223	99.87	0.05	0.03	0.05	0	2	43	6172	E341D substitution likely due to primer issue	No
P7977-310252	FU4.7	1363	2507	19284	81	23235	5.87	10.79	83.00	0.35	3	19281	2	1361		
P7977-310252	FU4	319	2	0	0	321	99.38	0.62	0.00	0.00	0	0	1	318		
P7977-310257	BL	7442	6	4	0	7452	99.87	0.08	0.05	0.00	3	1	6872	570	E341D substitution likely due to primer issue	No
P7977-310257	FU12	8945	13	0	4	8962	99.81	0.15	0.00	0.04	0	0	8536	409		
P7977-310257	FU4-1	15	0	0	0	15	100.00	0.00	0.00	0.00	0	0	5	10		
P7977-310257	FU4-2	837	2341	21858	100	25136	3.33	9.31	86.96	0.40	0	21858	1	836		
P7977-310257	FU8.22	8820	10	2	1	8833	99.85	0.11	0.02	0.01	1	1	8488	332		
P7977-310257	FU8	7526	5	0	1	7532	99.92	0.07	0.00	0.01	0	0	7162	364		
P7977-310284	BL	5299	1	0	1	5301	99.96	0.02	0.00	0.02	0	0	57	5242	No E341D emergence	No
P7977-310284	FU8	7820	12	1	2	7835	99.81	0.15	0.01	0.03	0	1	28	7792		
P7977-310286	BL	122	0	0	0	122	100.00	0.00	0.00	0.00	1	-1	3659	-3537	E341D emergence detected but very low coverage. Potential cross contamination	Not likely
P7977-310286	FU12	101	1	9	0	111	90.99	0.90	8.11	0.00	3	6	5496	-5395		
P7977-310286	FU16	76	0	0	0	76	100.00	0.00	0.00	0.00	0	0	2801	-2725		
P7977-310286	FU24	47	0	0	0	47	100.00	0.00	0.00	0.00	0	0	355	-308		
P7977-310286	FU4.3	35	0	1	1	37	94.59	0.00	2.70	2.70	0	1	1205	-1170		
P7977-310286	FU4	42	0	0	1	43	97.67	0.00	0.00	2.33	1	-1	2002	-1960		
P7977-310307	BL	6933	1	0	0	6934	99.99	0.01	0.00	0.00	0	0	58	6875	E341D substitution likely due to primer issue	No
P7977-310307	FU12	7152	7	2	1	7162	99.86	0.10	0.03	0.01	0	2	31	7121		
P7977-310307	FU24	6907	8	0	0	6915	99.88	0.12	0.00	0.00	0	0	16	6891		
P7977-310307	FU4-1	4	0	0	0	4	100.00	0.00	0.00	0.00	0	0	1	3		
P7977-310307	FU4-2	693	2666	24166	149	27674	2.50	9.63	87.32	0.54	3	24163	6	687		
P7977-310307	FU8	1664	0	0	0	1664	100.00	0.00	0.00	0.00	0	0	6	1658		

SUBJID	VISIT	Amino Acid 341 codons				Total	Codon Frequencies				Extended Reads				Resistance	Substitution
		GAG [E]	GAA [E]	GAT [D]	GAC [D]	Reads	GAG [E]	GAA [E]	GAT [D]	GAC [D]	GAT +3	Diff	GAG +3	Diff	Explanation	E341D real?
P7977-310310	BL	7956	4	2	1	7963	99.91	0.05	0.03	0.01	0	2	96	7860	No E341D emergence	No
P7977-310310	FU12	10253	14	8	1	10276	99.78	0.14	0.08	0.01	0	8	51	10202		
P7977-310310	FU24	6618	0	0	1	6619	99.98	0.00	0.00	0.02	0	0	33	6585		
P7977-310310	FU4.8	7702	11	4	2	7719	99.78	0.14	0.05	0.03	0	4	39	7663		
P7977-310310	FU4	164	1	0	0	165	99.39	0.61	0.00	0.00	0	0	4	160		
P7977-310335	BL	1694	23	1	0	1718	98.60	1.34	0.06	0.00	0	1	107	1587	No E341D emergence	No
P7977-310335	FU4	44	0	0	0	44	100.00	0.00	0.00	0.00	0	0	54	-10		
P7977-310343	BL	2689	2	0	2	2693	99.85	0.07	0.00	0.07	0	0	2533	156	No E341D emergence	No
P7977-310343	FU12	9067	2	4	3	9076	99.90	0.02	0.04	0.03	1	3	8200	867		
P7977-310343	FU24	8197	4	4	1	8206	99.89	0.05	0.05	0.01	4	0	7619	578		
P7977-310345	BL	1063	2	3	1	1069	99.44	0.19	0.28	0.09	0	3	21	1042	No E341D emergence	No
P7977-310345	FU4	6829	6	0	0	6835	99.91	0.09	0.00	0.00	0	0	48	6781		
P7977-310345	FU8	7251	8	3	3	7265	99.81	0.11	0.04	0.04	0	3	61	7190		
P7977-310349	BL	5902	11	0	0	5913	99.81	0.19	0.00	0.00	0	0	61	5841	No E341D emergence	No
P7977-310349	FU12	7153	10	0	1	7164	99.85	0.14	0.00	0.01	0	0	32	7121		
P7977-310349	FU4	8406	8	1	0	8415	99.89	0.10	0.01	0.00	0	1	47	8359		
P7977-310357	BL	6019	10	1	2	6032	99.78	0.17	0.02	0.03	1	0	1512	4507	No E341D emergence	No
P7977-310357	FU12	9545	29	0	5	9579	99.65	0.30	0.00	0.05	0	0	1850	7695		
P7977-310369	BL	6876	6	1	1	6884	99.88	0.09	0.01	0.01	0	1	179	6697	E341D substitution ikely due to primer issue	No
P7977-310369	FU12	7969	9	0	0	7978	99.89	0.11	0.00	0.00	0	0	38	7931		
P7977-310369	FU8-1	820	2369	17962	78	21229	3.86	11.16	84.61	0.37	0	17962	2	818		
P7977-310369	FU8-2	8	0	0	0	8	100.00	0.00	0.00	0.00	0	0	1	7		
P7977-310371	FU8-1	1181	2147	21464	94	24886	4.75	8.63	86.25	0.38	2	21462	0	1181	E341D substitution ikely due to primer issue	No
P7977-310371	FU8-2	1281	1	0	0	1282	99.92	0.08	0.00	0.00	0	0	8	1273		
P7977-310373	BL	1855	2	0	0	1857	99.89	0.11	0.00	0.00	0	0	1421	434	No E341D emergence	No
P7977-310373	FU12	7589	3	0	1	7593	99.95	0.04	0.00	0.01	0	0	7196	393		
P7977-310373	FU8.7	7669	2	3	1	7675	99.92	0.03	0.04	0.01	3	0	7323	346		
P7977-310373	FU8	651	0	0	0	651	100.00	0.00	0.00	0.00	0	0	641	10		
P7977-310382	BL	1933	0	1	0	1934	99.95	0.00	0.05	0.00	0	1	10	1923	E341D substitution ikely due to primer issue	No
P7977-310382	FU12	12920	12	9	0	12941	99.84	0.09	0.07	0.00	0	9	66	12854		
P7977-310382	FU24	7688	4	5	2	7699	99.86	0.05	0.06	0.03	0	5	33	7655		
P7977-310382	FU4.13-1	8396	5	1	0	8402	99.93	0.06	0.01	0.00	0	1	27	8369		
P7977-310382	FU4.13-2	7923	0	2	1	7926	99.96	0.00	0.03	0.01	0	2	23	7900		
P7977-310382	FU4-1	4	0	0	0	4	100.00	0.00	0.00	0.00	0	0	1	3		
P7977-310382	FU4-2	349	896	7786	19	9050	3.86	9.90	86.03	0.21	0	7786	52	297		
P7977-310385	BL	5190	5	1	0	5196	99.88	0.10	0.02	0.00	0	1	4983	207	E341D substitution ikely due to primer issue	No
P7977-310385	FU12	9432	3	3	0	9438	99.94	0.03	0.03	0.00	1	2	9101	331		
P7977-310385	FU24	8297	7	14	1	8319	99.74	0.08	0.17	0.01	10	4	8109	188		
P7977-310385	FU4-1	672	1809	23164	73	25718	2.61	7.03	90.07	0.28	0	23164	1	671		
P7977-310385	FU4-2	4	0	0	0	4	100.00	0.00	0.00	0.00	0	0	2	2		
P7977-310385	FU4.7	10241	8	12	1	10262	99.80	0.08	0.12	0.01	11	1	9924	317		
P7977-310400	BL	7203	5	3	4	7215	99.83	0.07	0.04	0.06	0	3	28	7175	No E341D emergence	No
P7977-310400	FU12	8052	4	1	0	8057	99.94	0.05	0.01	0.00	0	1	34	8018		
P7977-310400	FU8	5668	4	2	2	5676	99.86	0.07	0.04	0.04	0	2	15	5653		
P7977-310401	BL	11195	15	1	1	11212	99.85	0.13	0.01	0.01	0	1	538	10657	No E341D emergence	No
P7977-310401	FU12	6696	5	3	0	6704	99.88	0.07	0.04	0.00	2	1	1457	5239		
P7977-310401	FU4	11006	8	0	2	11016	99.91	0.07	0.00	0.02	0	0	338	10668		
P7977-310402	BL	8146	6	0	0	8152	99.93	0.07	0.00	0.00	0	0	50	8096	No E341D emergence	No
P7977-310402	FU12	7763	6	3	1	7773	99.87	0.08	0.04	0.01	0	3	28	7735		
P7977-310402	FU16.2	6173	3	0	2	6178	99.92	0.05	0.00	0.03	0	0	34	6139		
P7977-310402	FU16	5896	5	1	2	5904	99.86	0.08	0.02	0.03	0	1	26	5870		
P7977-310402	FU8	6706	6	3	3	6718	99.82	0.09	0.04	0.04	0	3	29	6677		
P7977-310405	BL	9549	8	1	0	9558	99.91	0.08	0.01	0.00	0	1	114	9435	No E341D emergence	No
P7977-310405	FU16	9833	7	0	0	9840	99.93	0.07	0.00	0.00	0	0	32	9801		
P7977-310409	BL	13527	7	0	0	13534	99.95	0.05	0.00	0.00	0	0	122	13405	No E341D emergence	No
P7977-310409	FU12	6605	4	2	1	6612	99.89	0.06	0.03	0.02	0	2	43	6562		
P7977-310409	FU4	8808	7	0	0	8815	99.92	0.08	0.00	0.00	0	0	192	8616		
P7977-310411	BL	11737	0	2	1	11740	99.97	0.00	0.02	0.01	0	2	70	11667	No E341D emergence	No
P7977-310411	FU4.8	4049	0	1	0	4050	99.98	0.00	0.02	0.00	0	1	38	4011		
P7977-310411	FU4	240	0	0	0	240	100.00	0.00	0.00	0.00	0	0	3	237		
P7977-310423	BL	4620	0	0	0	4620	100.00	0.00	0.00	0.00	0	0	35	4585	No E341D emergence	No
P7977-310423	FU12	7539	6	2	2	7549	99.87	0.08	0.03	0.03	0	2	29	7510		
P7977-310423	FU4.12	6691	6	0	0	6697	99.91	0.09	0.00	0.00	0	0	29	6662		
P7977-310423	FU4	426	0	1	0	427	99.77	0.00	0.23	0.00	0	1	0	426		
P7977-310428	BL	2971	0	0	0	2971	100.00	0.00	0.00	0.00	0	0	16	2955	No E341D emergence	No
P7977-310428	FU12	278	0	0	0	278	100.00	0.00	0.00	0.00	0	0	16	262		
P7977-310428	FU4	2110	2	0	0	2112	99.91	0.09	0.00	0.00	0	0	8	2102		
P7977-310429	BL-1	836	2	0	0	838	99.76	0.24	0.00	0.00	0	0	3	833	No E341D emergence	No
P7977-310429	BL-2	34	0	0	0	34	100.00	0.00	0.00	0.00	0	0	2	32		
P7977-310429	FU12	6133	8	0	1	6142	99.85	0.13	0.00	0.02	0	0	26	6107		
P7977-310429	FU4.12	5501	6	0	0	5507	99.89	0.11	0.00	0.00	0	0	24	5477		
P7977-310429	FU4	227	0	0	0	227	100.00	0.00	0.00	0.00	0	0	1	226		
P7977-310436	BL	4102	7	0	1	4110	99.81	0.17	0.00	0.02	0	0	2118	1984	E341D substitution ikely due to primer issue	No
P7977-310436	FU12	7622	2	1	1	7626	99.95	0.03	0.01	0.01	0	1	7310	312		
P7977-310436	FU4.12	11142	10	1	1	11154	99.89	0.09	0.01	0.01	2	-1	10467	675		
P7977-310436	FU4-1	7	0	0	0	7	100.00	0.00	0.00	0.00	0	0	4	3		
P7977-310436	FU4-2	93	310	2733	10	3146	2.96	9.85	86.87	0.32	0	2733	0	93		
P7977-310448	BL	2588	1	0	0	2589	99.96	0.04	0.00	0.00	0	0	21	2567	No E341D emergence	No
P7977-310448	FU4	6490	8	0	2	6500	99.85	0.12	0.00	0.03	0	0	35	6455		
P7977-310452	BL	9329	9	0	0	9338	99.90	0.10	0.00	0.00	0	0	88	9241	No E341D emergence	No
P7977-310452	FU12	9474	5	0	0	9479	99.95	0.05	0.00	0.00	0	0	48	9426		
P7977-310452	FU4	9534	7	1	0	9542	99.92	0.07	0.01	0.00	0	1	36	9498		
P7977-310470	BL	47	0	0	0	47	100.00	0.00	0.00	0.00	0	0	21	26	No E341D emergence	No
P7977-310470	FU12	21	0	0	0	21	100.00	0.00	0.00	0.00	0	0	15	6		
P7977-310470	FU4	20	0	0	0	20	100.00	0.00	0.00	0.00	0	0	11	9		
P7977-310472	BL	340	0	0	0	340	100.00	0.00	0.00	0.00	0	0	4	336	E341D substitution ikely due to primer issue	No
P7977-310472	FU12	1862	2	0	0	1864	99.89	0.11	0.00	0.00	0	0	9	1853		
P7977-310472	FU4-1	65	0	0	0	65	100.00	0.00	0.00	0.00	0	0	3	62		
P7977-310472	FU4-2	1768	4747	33159	138	39812	4.44	11.92	83.29	0.35	0	33159	2	1766		
P7977-310488	BL	2334	2	0	0	2336	99.91	0.09	0.00	0.00	0	0	43	2291	E341D substitution ikely due to primer issue	No
P7977-310488	FU12	7848	2	2	1	7853	99.94	0.03	0.03	0.01	0	2	30	7818		
P7977-310488	FU8-1	1878	2669	20576	84	25207	7.45	10.59	81.63	0.33	10	20566	0	1878		
P7977-310488	FU8-2	162	0	9	0	171	94.74	0.00	5.26	0.00	0	9	3	159		
P7977-310495	BL	9326	7	0	1	9334	99.91	0.07	0.00	0.01	0	0	41	9285	No E341D emergence	No
P7977-310495	FU12	8795	8	2	0	8805	99.89	0.09	0.02	0.00	0	2	29	8766		
P7977-310495	FU4	8033	5	0	0	8038	99.94	0.06	0.00	0.00	0	0	30	8003		

SUBJID	VISIT	Amino Acid 341 codons				Total	Codon Frequencies				Extended Reads				Resistance Explanation	Substitution E341D real?
		GAG [E]	GAA [E]	GAT [D]	GAC [D]	Reads	GAG [E]	GAA [E]	GAT [D]	GAC [D]	GAT +3	Diff	GAG +3	Diff		
P7977-310504	BL	6685	9	0	1	6695	99.85	0.13	0.00	0.01	0	0	6429	256	No E341D emergence	No
P7977-310504	FU12	722	2	5	1	730	98.90	0.27	0.68	0.14	0	5	672	50		
P7977-310504	FU16	121	0	0	0	121	100.00	0.00	0.00	0.00	0	0	115	6		
P7977-310506	BL	9892	5	2	2	9901	99.91	0.05	0.02	0.02	3	-1	9286	606	No E341D emergence	No
P7977-310506	FU12	6684	8	1	1	6694	99.85	0.12	0.01	0.01	0	1	6275	409		
P7977-310506	FU4	5300	6	1	0	5307	99.87	0.11	0.02	0.00	0	1	5064	236		
P7977-310507	BL	5929	4	4	0	5937	99.87	0.07	0.07	0.00	0	4	78	5851	No E341D emergence	No
P7977-310507	FU12-1	409	4	0	0	413	99.03	0.97	0.00	0.00	0	0	13	396		
P7977-310507	FU12-2	290	2	0	0	292	99.32	0.68	0.00	0.00	0	0	5	285		
P7977-310507	FU8	897	1	2	0	900	99.67	0.11	0.22	0.00	0	2	4	893		
P7977-310518	BL	181	0	0	0	181	100.00	0.00	0.00	0.00	0	0	25	156	No E341D emergence	No
P7977-310518	FU16	72	0	0	0	72	100.00	0.00	0.00	0.00	0	0	32	40		
P7977-310518	FU4	49	0	0	0	49	100.00	0.00	0.00	0.00	0	0	29	20		
P7977-310521	BL-1	648	1	0	0	649	99.85	0.15	0.00	0.00	0	0	13	635	No E341D emergence	No
P7977-310521	BL-2	36	1	0	0	37	97.30	2.70	0.00	0.00	0	0	3	33		
P7977-310521	FU12	7222	4	2	2	7230	99.89	0.06	0.03	0.03	0	2	23	7199		
P7977-310521	FU4	9986	2	4	1	9993	99.93	0.02	0.04	0.01	0	4	29	9957		
P7977-310527	FU8	1078	2390	16616	51	20135	5.35	11.87	82.52	0.25	0	16616	0	1078	E341D substitution likely due to primer issue	No
P7977-310528	BL	6088	3	3	1	6095	99.89	0.05	0.05	0.02	5	-2	5688	400	E341D substitution likely due to primer issue	No
P7977-310528	FU12	7264	9	1	2	7276	99.84	0.12	0.01	0.03	1	0	6861	403		
P7977-310528	FU4-1	14	0	0	0	14	100.00	0.00	0.00	0.00	0	0	2	12		
P7977-310528	FU4-2	22	0	0	0	22	100.00	0.00	0.00	0.00	0	0	1	21		
P7977-310528	FU4-3	877	2707	21957	116	25657	3.42	10.55	85.58	0.45	0	21957	1	876		
P7977-310528	FU4-4	899	2573	20495	70	24037	3.74	10.70	85.26	0.29	0	20495	1	898		
P7977-310530	BL	7273	12	2	0	7287	99.81	0.16	0.03	0.00	0	2	90	7183	No E341D emergence	No
P7977-310530	FU12	9338	5	1	2	9346	99.91	0.05	0.01	0.02	1	0	33	9305		
P7977-310530	FU8	840	0	0	0	840	100.00	0.00	0.00	0.00	0	0	3	837		
P7977-310534	BL	8982	5	1	0	8988	99.93	0.06	0.01	0.00	0	1	96	8886	No E341D emergence	No
P7977-310534	FU4	9819	8	0	2	9829	99.90	0.08	0.00	0.02	0	0	39	9780		
P7977-310534	FU8	9060	3	6	1	9070	99.89	0.03	0.07	0.01	0	6	53	9007		
P7977-310541	FU12	4787	4	1	0	4792	99.90	0.08	0.02	0.00	0	1	173	4614	No E341D emergence	No
P7977-310541	FU4	8081	7	3	2	8093	99.85	0.09	0.04	0.02	0	3	70	8011		
P7977-310549	BL	6584	9	2	3	6598	99.79	0.14	0.03	0.05	0	2	58	6526	No E341D emergence	No
P7977-310549	FU12	7725	3	1	1	7730	99.94	0.04	0.01	0.01	0	1	29	7696		
P7977-310549	FU4	6736	11	0	1	6748	99.82	0.16	0.00	0.01	0	0	20	6716		
P7977-310556	BL	4056	12	0	0	4068	99.71	0.29	0.00	0.00	0	0	71	3985	E341D substitution likely due to primer issue	No
P7977-310556	FU12	7263	3	2	0	7268	99.93	0.04	0.03	0.00	0	2	26	7237		
P7977-310556	FU4-1	13	0	0	0	13	100.00	0.00	0.00	0.00	0	0	5	8		
P7977-310556	FU4-2	756	2738	22840	94	26428	2.86	10.36	86.42	0.36	0	22840	5	751		
P7977-310560	BL-1	4797	5	0	0	4802	99.90	0.10	0.00	0.00	0	0	42	4755	No E341D emergence	No
P7977-310560	BL-2	30	0	0	0	30	100.00	0.00	0.00	0.00	0	0	1	29		
P7977-310560	FU12	9548	5	1	2	9556	99.92	0.05	0.01	0.02	1	0	34	9514		
P7977-310560	FU4	8136	3	2	4	8145	99.89	0.04	0.02	0.05	0	2	24	8112		
P7977-310563	BL	5925	2	4	0	5931	99.90	0.03	0.07	0.00	0	4	201	5724	E341D substitution likely due to primer issue	No
P7977-310563	FU12	7817	5	1	3	7826	99.88	0.06	0.01	0.04	0	1	32	7785		
P7977-310563	FU4-1	819	2268	24844	106	28037	2.92	8.09	88.61	0.38	5	24839	1	818		
P7977-310563	FU4-2	3	0	0	0	3	100.00	0.00	0.00	0.00	0	0	1	2		
P7977-310563	FU8	4099	1	8	0	4108	99.78	0.02	0.19	0.00	0	8	15	4084		
P7977-310566	BL	7047	0	1	1	7049	99.97	0.00	0.01	0.01	0	1	36	7011	No E341D emergence	No
P7977-310566	FU12	7697	7	0	1	7705	99.90	0.09	0.00	0.01	0	0	36	7661		
P7977-310566	FU4	6463	6	0	0	6469	99.91	0.09	0.00	0.00	0	0	25	6438		
P7977-310578	BL	7809	10	1	1	7821	99.85	0.13	0.01	0.01	0	1	3	7806	No E341D emergence	No
P7977-310578	FU4	8045	3	0	0	8048	99.96	0.04	0.00	0.00	0	0	0	8045		
P7977-310578	FU8	9220	1	13	4	9238	99.81	0.01	0.14	0.04	0	13	3	9217		
P7977-310601	BL	8277	13	2	0	8292	99.82	0.16	0.02	0.00	2	0	7691	586	No E341D emergence	No
P7977-310601	FU12	9006	6	0	2	9014	99.91	0.07	0.00	0.02	0	0	8415	591		
P7977-310601	FU8	6964	4	1	0	6969	99.93	0.06	0.01	0.00	1	0	6617	347		
P7977-310604	BL	7295	14	5	6	7320	99.66	0.19	0.07	0.08	0	5	185	7110	No E341D emergence	No
P7977-310604	FU12	8322	12	2	2	8338	99.81	0.14	0.02	0.02	0	2	68	8254		
P7977-310604	FU4	10775	5	1	0	10781	99.94	0.05	0.01	0.00	0	1	53	10722		
P7977-310605	BL	7540	7	2	0	7549	99.88	0.09	0.03	0.00	1	1	4842	2698	No E341D emergence	No
P7977-310605	FU12	9006	9	0	2	9017	99.88	0.10	0.00	0.02	0	0	7887	1119		
P7977-310605	FU4	11020	6	3	1	11030	99.91	0.05	0.03	0.01	3	0	10341	679		
P7977-310606	BL	12421	11	2	1	12435	99.89	0.09	0.02	0.01	0	2	125	12296	No E341D emergence	No
P7977-310606	FU12	8281	13	0	0	8294	99.84	0.16	0.00	0.00	0	0	38	8243		
P7977-310606	FU4	8643	118	4	0	8765	98.61	1.35	0.05	0.00	0	4	23	8620		
P7977-310610	BL	8997	5	0	1	9003	99.93	0.06	0.00	0.01	0	0	120	8877	No E341D emergence	No
P7977-310610	FU12	7009	8	3	1	7021	99.83	0.11	0.04	0.01	0	3	26	6983		
P7977-310610	FU4	2486	1	0	1	2488	99.92	0.04	0.00	0.04	0	0	6	2480		
P7977-310620	BL	9847	13	1	0	9861	99.86	0.13	0.01	0.00	0	1	230	9617	No E341D emergence	No
P7977-310620	FU12	7726	3	0	1	7730	99.95	0.04	0.00	0.01	0	0	32	7694		
P7977-310620	FU4.7	499	0	1	0	500	99.80	0.00	0.20	0.00	0	1	32	467		
P7977-310620	FU4	182	1	0	0	183	99.45	0.55	0.00	0.00	0	0	2	180		
P7977-310625	BL	8596	14	2	3	8615	99.78	0.16	0.02	0.03	0	2	121	8475	E341D emerged in FU4 (8.3%). However, none of the reads bearing	Not likely
P7977-310625	FU12	5834	3	4	6	5847	99.78	0.05	0.07	0.10	0	4	34	5800		
P7977-310625	FU4	685	0	0	62	747	91.70	0.00	0.00	8.30	0	0	0	685		
P7977-310632	BL-1	2851	0	0	0	2851	100.00	0.00	0.00	0.00	0	0	2654	197	No E341D emergence	No
P7977-310632	BL-2	249	0	0	0	249	100.00	0.00	0.00	0.00	0	0	227	22		
P7977-310632	FU4	111	0	0	0	111	100.00	0.00	0.00	0.00	0	0	103	8		
P7977-310632	FU8	6879	4	0	0	6883	99.94	0.06	0.00	0.00	0	0	6566	313		
P7977-310633	BL	10402	10	3	0	10415	99.88	0.10	0.03	0.00	0	3	342	10060	E341D substitution likely due to primer issue	No
P7977-310633	FU12	7129	8	2	1	7140	99.85	0.11	0.03	0.01	0	2	30	7099		
P7977-310633	FU4-1	13	1	0	0	14	92.86	7.14	0.00	0.00	0	0	3	10		
P7977-310633	FU4-2	705	2121	20802	105	23733	2.97	8.94	87.65	0.44	0	20802	0	705		
P7977-310637	BL	4578	8	1	0	4587	99.80	0.17	0.02	0.00	0	1	31	4547	No E341D emergence	No
P7977-310637	FU12	10	0	0	0	10	100.00	0.00	0.00	0.00	0	0	5	5		
P7977-310637	FU16	467	0	0	0	467	100.00	0.00	0.00	0.00	0	0	0	467		
P7977-310678	BL	5949	6	2	3	5960	99.82	0.10	0.03	0.05	0	2	66	5883	E341D substitution likely due to primer issue	No
P7977-310678	FU12	163	0	0	0	163	100.00	0.00	0.00	0.00	0	0	157	6		
P7977-310678	FU4-1	550	1843	17953	60	20406	2.70	9.03	87.98	0.29	0	17953	1	549		
P7977-310678	FU4-2	2	0	0	0	2	100.00	0.00	0.00	0.00	0	0	1	1		
P7977-310678	FU8	4980	2	6	0	4988	99.84	0.04	0.12	0.00	5	1	4749	231		
GS-US-334-0107-7201	BL	8555	8	17	2	8582	99.69	0.09	0.20	0.02	1	16	8018	537	No E341D emergence	No
GS-US-334-0107-7201	FU4	577	0	0	0	577	100.00	0.00	0.00	0.00	0	0	529	48		
GS-US-334-0107-7207	BL	6140	5	2	0	6147	99.89	0.08	0.03	0.00	1	1	5650	490	No E341D emergence	No
GS-US-334-0107-7207	FU4-1	8	0	0	0	8	100.00	0.00	0.00	0.00	0	0	5	3		
GS-US-334-0107-7207	FU4-2	2536	2	0	0	2538	99.92	0.08	0.00	0.00	0	0	2460	76		

SUBJID	VISIT	Amino Acid 341 codons				Total	Codon Frequencies				Extended Reads				Resistance	Substitution
		GAG [E]	GAA [E]	GAT [D]	GAC [D]	Reads	GAG [E]	GAA [E]	GAT [D]	GAC [D]	GAT +3	Diff	GAG +3	Diff	Explanation	E341D real?
GS-US-334-0107-7217	BL	7897	15	9	0	7921	99.70	0.19	0.11	0.00	1	8	156	7741	No E341D emergence	No
GS-US-334-0107-7217	FU12.15	6853	2	0	1	6856	99.96	0.03	0.00	0.01	0	0	25	6828		
GS-US-334-0107-7217	FU12	11570	11	0	1	11582	99.90	0.09	0.00	0.01	0	0	45	11525		
GS-US-334-0107-7225	BL	6914	6	6	1	6927	99.81	0.09	0.09	0.01	0	6	101	6813	No E341D emergence	No
GS-US-334-0107-7225	FU4	4031	3	0	0	4034	99.93	0.07	0.00	0.00	0	0	11	4020		
GS-US-334-0107-7226	BL-1	641	1	0	0	642	99.84	0.16	0.00	0.00	0	0	235	406	No E341D emergence	No
GS-US-334-0107-7226	BL-2	8780	7	14	0	8801	99.76	0.08	0.16	0.00	1	13	2377	6403		
GS-US-334-0107-7226	FU4	1298	2	1	1	1302	99.69	0.15	0.08	0.08	0	1	5	1293		
GS-US-334-0107-7230	BL	9716	6	10	4	9736	99.79	0.06	0.10	0.04	0	10	68	9648	No E341D emergence	No
GS-US-334-0107-7230	FU4	4069	2	2	1	4074	99.88	0.05	0.05	0.02	0	2	18	4051		
GS-US-334-0107-7234	BL	35	3	3	0	41	85.37	7.32	7.32	0.00	0	3	18	17	E341D emergence detected but very low coverage. Potential cross contamination	No
GS-US-334-0107-7234	FU4-1	2	0	0	0	2	100.00	0.00	0.00	0.00	0	0	2	0		
GS-US-334-0107-7234	FU4-2	1	0	0	0	1	100.00	0.00	0.00	0.00	0	0	0	1		
GS-US-334-0107-7234	FU4.7	5	0	4	1	10	50.00	0.00	40.00	10.00	0	4	2	3		
GS-US-334-0107-7238	BL	25	1	3	0	29	86.21	3.45	10.34	0.00	0	3	2	23	E341D emergence detected but very low	No
GS-US-334-0107-7238	FU4	1	0	0	0	1	100.00	0.00	0.00	0.00	0	0	2	-1		
GS-US-334-0107-7260	BL-1	3958	4	2	0	3964	99.85	0.10	0.05	0.00	0	2	60	3898	No E341D emergence	No
GS-US-334-0107-7260	BL-2	6427	3	1	2	6433	99.91	0.05	0.02	0.03	0	1	122	6305		
GS-US-334-0107-7260	FU12	9159	3	8	0	9170	99.88	0.03	0.09	0.00	0	8	39	9120		
GS-US-334-0107-7265	BL	9404	17	10	0	9431	99.71	0.18	0.11	0.00	0	10	82	9322	No E341D emergence	No
GS-US-334-0107-7265	FU12.15	7144	5	3	1	7153	99.87	0.07	0.04	0.01	0	3	24	7120		
GS-US-334-0107-7265	FU12	5055	3	3	1	5062	99.86	0.06	0.06	0.02	0	3	12	5043		
GS-US-334-0107-7269	BL-1	1634	1	0	0	1635	99.94	0.06	0.00	0.00	0	0	1510	124	E341D emergence detected but in FU4.11 with low coverage. Potential cross	No
GS-US-334-0107-7269	BL-2	7431	11	9	3	7454	99.69	0.15	0.12	0.04	6	3	7044	387		
GS-US-334-0107-7269	FU4.11	97	1	5	0	103	94.17	0.97	4.85	0.00	0	5	87	10		
GS-US-334-0107-7269	FU4	589	0	0	0	589	100.00	0.00	0.00	0.00	0	0	535	54		
GS-US-334-0107-7271	BL	5483	3	9	4	5499	99.71	0.05	0.16	0.07	5	4	4916	567	No E341D emergence	No
GS-US-334-0107-7271	FU4	1345	2	0	1	1348	99.78	0.15	0.00	0.07	0	0	1224	121		
GS-US-334-0107-7276	BL	7843	5	7	2	7857	99.82	0.06	0.09	0.03	1	6	7406	437	No E341D emergence	No
GS-US-334-0107-7276	FU4	4914	4	0	3	4921	99.86	0.08	0.00	0.06	0	0	4519	395		
GS-US-334-0107-7278	BL	6682	7	7	5	6701	99.72	0.10	0.10	0.07	0	7	67	6615	No E341D emergence	No
GS-US-334-0107-7278	FU4-1	346	1	0	0	347	99.71	0.29	0.00	0.00	0	0	1	345		
GS-US-334-0107-7278	FU4-2	513	0	0	0	513	100.00	0.00	0.00	0.00	0	0	3	510		
GS-US-334-0107-7281	BL	8125	3	5	4	8137	99.85	0.04	0.06	0.05	0	5	405	7720	No E341D emergence	No
GS-US-334-0107-7281	FU4	7693	2	1	4	7700	99.91	0.03	0.01	0.05	0	1	540	7153		
GS-US-334-0107-7294	BL-1	3727	5	0	0	3732	99.87	0.13	0.00	0.00	0	0	27	3700	E341D substitution likely due to primer issue	No
GS-US-334-0107-7294	BL-2	21	2	2	0	25	84.00	8.00	8.00	0.00	0	2	3	18		
GS-US-334-0107-7294	FU4-1	34	0	0	0	34	100.00	0.00	0.00	0.00	0	0	0	34		
GS-US-334-0107-7294	FU4-2	2171	2645	28213	106	33135	6.55	7.98	85.15	0.32	16	28197	10	2161		
GS-US-334-0107-7294	FU4.9	59	1	4	0	64	92.19	1.56	6.25	0.00	0	4	1	58		
GS-US-334-0107-7295	FU4.14	10	17	99	0	126	7.94	13.49	78.57	0.00	13	86	5	5	E341D emergence detected at FU4 and Fu4.14. Although this is low coverage, it could be	Yes
GS-US-334-0107-7295	FU4-1	3	0	0	0	3	100.00	0.00	0.00	0.00	0	0	0	3		
GS-US-334-0107-7295	FU4-2	1	0	0	0	1	100.00	0.00	0.00	0.00	0	0	0	1		
GS-US-334-0107-7295	FU4-3	17	33	127	0	177	9.60	18.64	71.75	0.00	31	96	16	1		
GS-US-334-0107-7306	BL	7272	5	6	1	7284	99.84	0.07	0.08	0.01	2	4	6858	414	No E341D emergence	No
GS-US-334-0107-7306	FU4	5111	3	0	0	5114	99.94	0.06	0.00	0.00	0	0	4828	283		
GS-US-334-0107-7307	FU4.12	5659	1	1	0	5661	99.96	0.02	0.02	0.00	0	1	17	5642	E341D substitution likely due to primer issue	No
GS-US-334-0107-7307	FU4-1	10	0	0	0	10	100.00	0.00	0.00	0.00	0	0	1	9		
GS-US-334-0107-7307	FU4-2	8	0	0	0	8	100.00	0.00	0.00	0.00	0	0	2	6		
GS-US-334-0107-7307	FU4-3	4	0	0	0	4	100.00	0.00	0.00	0.00	0	0	1	3		
GS-US-334-0107-7307	FU4-4	1810	3039	22672	87	27608	6.56	11.01	82.12	0.32	17	22655	3	1807		
GS-US-334-0107-7315	BL	8982	9	33	2	9026	99.51	0.10	0.37	0.02	0	33	60	8922	No E341D emergence	No
GS-US-334-0107-7315	FU12	9733	93	3	2	9831	99.00	0.95	0.03	0.02	0	3	43	9690		
GS-US-334-0107-7324	BL	11017	11	16	2	11046	99.74	0.10	0.14	0.02	0	16	78	10939	No E341D emergence	No
GS-US-334-0107-7324	FU12.9	8005	0	0	1	8006	99.99	0.00	0.00	0.01	0	0	19	7986		
GS-US-334-0107-7324	FU12	6448	2	3	2	6455	99.89	0.03	0.05	0.03	0	3	13	6435		
GS-US-334-0107-7326	BL	9118	13	12	3	9146	99.69	0.14	0.13	0.03	0	12	5651	3467	No E341D emergence	No
GS-US-334-0107-7326	FU12.10	10775	15	1	2	10793	99.83	0.14	0.01	0.02	1	0	9978	797		
GS-US-334-0107-7326	FU12	9006	4	2	1	9013	99.92	0.04	0.02	0.01	1	1	8403	603		
GS-US-334-0107-7328	BL	5893	3	2	0	5898	99.92	0.05	0.03	0.00	0	2	2625	3268	No E341D emergence	No
GS-US-334-0107-7328	FU4	2739	3	0	1	2743	99.85	0.11	0.00	0.04	1	-1	2457	282		
GS-US-334-0107-7333	BL	8689	7	4	2	8702	99.85	0.08	0.05	0.02	0	4	117	8572	No E341D emergence	No
GS-US-334-0107-7333	FU4-1	581	0	0	0	581	100.00	0.00	0.00	0.00	0	0	1	580		
GS-US-334-0107-7333	FU4-2	10	0	0	0	10	100.00	0.00	0.00	0.00	0	0	2	8		
GS-US-334-0107-7333	FU4-3	507	2	0	0	509	99.61	0.39	0.00	0.00	0	0	1	506		
GS-US-334-0107-7344	BL	3923	3	0	0	3926	99.92	0.08	0.00	0.00	0	0	36	3887	No E341D emergence	No
GS-US-334-0107-7344	FU4.2	8282	8	1	0	8291	99.89	0.10	0.01	0.00	0	1	29	8253		
GS-US-334-0107-7344	FU4	12273	5	2	0	12280	99.94	0.04	0.02	0.00	0	2	42	12231		
GS-US-334-0107-7345	BL	2521	4	1	1	2527	99.76	0.16	0.04	0.04	0	1	2130	391	No E341D emergence	No
GS-US-334-0107-7345	FU4	3760	1	1	0	3762	99.95	0.03	0.03	0.00	1	0	3532	228		
GS-US-334-0107-7350	BL	470	2	1	0	473	99.37	0.42	0.21	0.00	1	0	105	365	No E341D emergence	No
GS-US-334-0107-7350	FU4	42	0	0	0	42	100.00	0.00	0.00	0.00	0	0	24	18		
GS-US-334-0107-7418	BL	6557	7	2	2	6568	99.83	0.11	0.03	0.03	2	0	6235	322	No E341D emergence	No
GS-US-334-0107-7418	FU4.14	9390	8	1	0	9399	99.90	0.09	0.01	0.00	1	0	8978	412		
GS-US-334-0107-7418	FU4	8575	10	13	0	8598	99.73	0.12	0.15	0.00	0	13	8127	448		
GS-US-334-0107-7439	BL	7413	2	0	0	7415	99.97	0.03	0.00	0.00	0	0	35	7378	No E341D emergence	No
GS-US-334-0107-7439	FU4	5442	0	2	0	5444	99.96	0.00	0.04	0.00	0	2	23	5419		
GS-US-334-0107-7445	BL-1	6551	3	1	0	6555	99.94	0.05	0.02	0.00	0	1	44	6507	E341D emergence detected but in FU4 with low coverage. Potential cross contamination	No
GS-US-334-0107-7445	BL-2	72	0	0	0	72	100.00	0.00	0.00	0.00	0	0	0	72		
GS-US-334-0107-7445	FU4.10	7190	8	1	0	7199	99.87	0.11	0.01	0.00	0	1	15	7175		
GS-US-334-0107-7445	FU4	60	0	1	0	61	98.36	0.00	1.64	0.00	0	1	0	60		
GS-US-334-0107-7449	FU4.1	12244	13	6	1	12264	99.84	0.11	0.05	0.01	0	6	52	12192	No E341D emergence	No
GS-US-334-0107-7449	FU4.9	7638	4	2	3	7647	99.88	0.05	0.03	0.04	0	2	21	7617		
GS-US-334-0107-7473	BL-1	124	0	0	0	124	100.00	0.00	0.00	0.00	0	0	124	0	No E341D emergence	No
GS-US-334-0107-7473	BL-2	61	0	0	0	61	100.00	0.00	0.00	0.00	0	0	50	11		
GS-US-334-0107-7473	FU12	12	0	0	0	12	100.00	0.00	0.00	0.00	0	0	6	6		
GS-US-334-0107-7474	BL	9644	1	13	2	9660	99.83	0.01	0.13	0.02	0	13	124	9520	No E341D emergence	No
GS-US-334-0107-7476	BL-1	9158	9	0	0	9167	99.90	0.10	0.00	0.00	0	0	8698	460	E341D detected at Baseline at low coverage	No
GS-US-334-0107-7476	BL-2	0	1	3	0	4	0.00	25.00	75.00	0.00	0	3	0	0		
GS-US-334-0107-7476	FU4.7	9578	42	9	5	9634	99.42	0.44	0.09	0.05	1	8	9161	417		
GS-US-334-0107-7476	FU4	6532	11	0	0	6543	99.83	0.17	0.00	0.00	0	0	6239	293		
GS-US-334-0107-7477	BL-1	289	1	0	0	290	99.66	0.34	0.00	0.00	0	0	14	275	E341D substitution likely due to primer issue	No
GS-US-334-0107-7477	BL-2	8123	5	7	4	8139	99.80	0.06	0.09	0.05	2	5	474	7649		
GS-US-334-0107-7477	FU4.18	207	2	19	0	228	90.79	0.88	8.33	0.00	0	19	5	202		
GS-US-334-0107-7477	FU4-1	117	0	0	0	117	100.00	0.00	0.00	0.00	0	0	0	117		
GS-US-334-0107-7477	FU4-2	57	0	0	0	57	100.00	0.00	0.00	0.00	0	0	0	57		
GS-US-334-0107-7477	FU4-3	44	0	0	0	44	100.00	0.00	0.00	0.00	0	0	0	44		
GS-US-334-0107-7477	FU4-4	4275	5641	35364	139	45419	9.41	12.42	77.86	0.31	31	35333	12	4263		

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/s/

ERIC F DONALDSON
11/19/2013

JULIAN J O REAR
11/19/2013

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA: 204671 **SDN**: 000 **DATE REVIEWED:** 09/04/2013
Virology Reviewer: Lisa K. Naeger, Ph.D.

NDA#: 204,671 **Serial #:** 000
Reviewer's Name(s): Lisa K. Naeger, Ph.D.

Sponsor's Name and Address:
Gilead Sciences, Inc
333 Lakeside Drive
Foster City, CA 94404

Initial Submission Dates:

Correspondence Date:	04/06/2013
CDER Receipt Date:	04/08/2013
Assigned Date:	04/08/2013
Review Complete Date:	09/06/2013
PDUFA Date:	12/08/2013

Amendments:

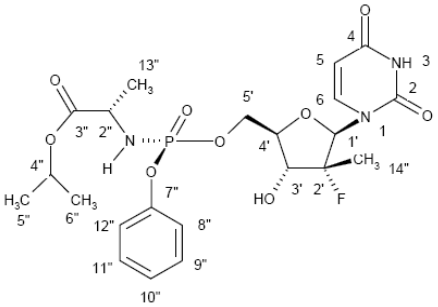
SDN	Date Submitted	Date Received	Date Assigned
003	05/01/13	05/01/13	05/09/13
006	06/21/13	06/24/13	07/26/13
012	07/12/13	07/12/13	07/30/13
016	07/29/13	07/30/13	08/14/13
017	08/09/13	08/12/13	08/21/13

Related/Supporting Documents: IND106739

Product Name(s)
Proprietary: To be determined
Non-Proprietary/USAN: Sofosbuvir
Code Name/Number: GS-7977, PSI-7977 (Pharmasset)

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

Molecular Weight: 529.46
Molecular Formula: $C_{22}H_{29}FN_3O_9P$
Structural Formula:



Reference ID: 3369468

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA: 204671 **SDN**: 000 **DATE REVIEWED:** 09/04/2013
Virology Reviewer: Lisa K. Naeger, Ph.D.

Sofosbuvir (SOF)

Dosage Form(s): tablet
Route(s) of Administration: Oral
Indication(s): NS5B nucleotide analog polymerase inhibitor indicated in combination with ribavirin (RBV) for the treatment of chronic genotypes 2 and 3 HCV infection and with pegylated interferon + RBV for the treatment of chronic genotype 1 HCV infection in adults.

Dispensed: Rx

Abbreviations: BL, baseline; DAA, direct acting antiviral; EC_{50}, effective concentration at 50%; FC, fold-change; FDA, Food and Drug Administration; GT, genotype; HCV, hepatitis C virus; HSA, human serum albumin; IC_{50}, inhibitory concentration at 50%; IFN, recombinant human interferon α; mt, mitochondria; NGS, next generation sequencing; NRTIs, nucleoside reverse transcriptase inhibitors; PBL, peripheral blood lymphocytes; PDVF, protocol defined virologic failure; PEG, pegylated human interferon; PR, protease; P/R, pegylated interferon/ribavirin; RBV, ribavirin; SDM, site-directed mutants; SOF, sofosbuvir; WT, wild-type; SVR, sustained virologic response

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Reference ID: 3369468

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA: 204671 **SDN**: 000 **DATE REVIEWED:** 09/04/2013
Virology Reviewer: Lisa K. Naeger, Ph.D.

EXECUTIVE SUMMARY

This NDA for sofosbuvir (SOF) is approvable with respect to virology for the treatment of HCV genotypes 2 and 3 in combination with ribavirin (RBV) or genotype 1 in combination with pegylated recombinant human interferon α + RBV. Sofosbuvir is a nucleotide prodrug of 2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate that is converted by cellular enzymes to the active uridine triphosphate form (GS-461203) within the hepatocyte and inhibits HCV replication.

Although other members of this class of drugs have been linked to cardiac toxicity possibly related to mitochondrial toxicity, SOF showed no clear evidence of mitochondrial toxicity. SOF showed no decrease in mtDNA following a 10- to 14-day treatment of multiple cell lines. SOF and its metabolites showed no inhibition of mitochondrial biogenesis (mitochondrial DNA replication, RNA transcription and protein expression) with $mtCC_{50}$ values >100 μM in PC3 and HepG2 cells. In biochemical assays, the active triphosphate form of SOF did not inhibit RNA polymerase II at concentrations up to 200 μM, mitochondrial RNA polymerase at concentrations up to 500 μM, or DNA polymerases α, β, or γ at concentrations up to 200 μM. In addition, the active metabolite of SOF is a poor substrate for mitochondrial RNA polymerase with an incorporation rate into RNA of <0.5% relative to UTP in a biochemical assay.

SOF had EC_{50} values ranging from 14-110 nM in stable full-length replicon cells of genotype 1a, 1b, 2a, 3a and 4a; chimeric GT1b Con-1 replicons carrying NS5B coding sequences from genotypes 2b, 5a, or 6a; and NS5B amplified from individual patient plasma samples and cloned into the replicon-based shuttle vectors. Interestingly, the EC_{50} values were slightly higher for GT1b and GT3a replicons compared to the other genotypes/subtypes replicons which may provide some supportive evidence for why subjects infected with GT1a and GT2 HCV had higher overall clinical efficacy compared to subjects infected with GT1b and GT3a HCV.

SOF showed no antiviral activity at the highest concentration tested (100 μM) against other non-HCV viruses with an RNA genome: human rhinovirus (HRV) types 10 and 14, respiratory syncytial virus (RSV), influenza A virus, and HIV. SOF showed no antagonism when combined with other classes of direct acting antivirals for HCV, i.e. nonnucleoside palm inhibitor (GS-9190), nonnucleoside thumb-1 inhibitor (GS-9669), NS5A inhibitors (GS-5885, GS-5816), NS3/4A protease inhibitors (boceprevir, telaprevir, GS-9451), RBV, IFNα or with HIV-1 NRTIs (ABC, AZT, ddI, d4T, FTC, TDF, 3TC).

The NS5B S282T subsitution was selected in GT1b, 2a, 3a and 4a subgenomic replicons when the replicon cells were treated with SOF concentrations of 10 to 30 times the wild-type EC_{50} value. Detection of the S282T substitution was associated with a 4- to 24- fold decrease in susceptibility to SOF for all four genotypes. The S282T substitution was not cross-resistant with the NS5A inhibitor, GS-5885, or RBV. SOF remained active against NS5B nucleoside mutants (except S282T) and nonnucleoside inhibitor mutants, NS5A resistance mutants, and NS3/4A protease inhibitor resistance-associated mutants tested indicating that these mutants are not cross-resistant to SOF.

There were four Phase 3 trials of SOF, three trials in subjects infected with GT2 or 3 HCV and one trial in subjects infected with GT1 HCV. The VERSANT HCV genotype

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assay, Version LiPA 2.0 was used for screening genotype/subtype for entry into the Phase 3 studies. Subsequently, nucleotide sequence and phylogenetic analysis of the HCV NS5B region was used to confirm genotype/subtype. In some circumstances, NS5B sequencing indicated a different genotype and/or subtype for a subject. Overall, concordance between the LiPa screening assay and NS5B sequencing was ≥94%. However, some subjects were found to have been misclassified by the screening assay when the NS5B sequencing analysis was performed. Most misclassifications were of the same genotype but different subtype. However, 9 subjects were misclassified as GT2 infection by the screening assay when they were later determined to be GT1 by the sequencing assay. Of these 9 subjects who received a suboptimal SOF+RBV regimen when they should have received SOF+P/R, 3 (33%) achieved SVR12 and all 3 were IL28B CC genotype. The sponsor used the genotype/subtype determined by NS5B sequencing and classified these 9 subjects as GT1 infection for efficacy and virology analyses. However for intent-to-treat (ITT) analyses, these subjects should be classified as GT2 infection as determined by the screening assay. In the “real world” clinical settings, NS5B sequencing may not be performed, so physicians may not have this more accurate genotyping/subtyping. In the near term, genotyping/subtyping will usually be determined in the clinic by assays similar to the screening assay. Therefore, the efficacy should reflect what can be expected in the clinic with a screening assay.

For subjects with GT2 infection, the most common HCV subtype when assessed by NS5B nucleotide sequence analysis was subtype 2b in 79% of subjects followed by GT2a in 19.5% of subjects. There were no obvious differences in response rates to SOF+RBV across the GT2 subtypes. For subjects with GT3 infection, 99% of subjects were infected with subtype 3a by NS5B sequencing and only 6 subjects had another subtype. Therefore, no conclusions could be made about the response rates for other GT3 subtypes. In Study 110, 77% of the subjects were infected with GT1a and 23% were infected with GT1b. There were only 28 subjects with GT4 HCV infection most of which were GT4a, 6 with GT6 infection (a, e, j or q) and 1 with GT5a infection. SVR12 rates were 10% higher for subjects infected with GT1a compared to GT1b (92% vs. 82%). Response rates of subjects with GT4a infection were 96% (25/26) and the one subject each infected with 4d and 4k also achieved SVR12. The one subject infected with GT5a and the 6 subjects infected with GT6 (3 with GT6a, and 1 each with GTe, j, q) all achieved SVR12.

Response rates were examined for each genotype by various baseline factors including prior response rates, IL28B genotype, and cirrhosis status. The data from these baseline factor subgroups support a longer 16-week duration of SOF+RBV for all subjects with GT3 HCV infection and for treatment-experienced subjects with GT2 HCV infection.

For **genotype 3** infection, SVR12 rates were much lower for subjects with prior pegylated interferon α/ribavirin (P/R) experience than treatment-naïve subjects following 12 weeks of SOF+RBV. A longer duration of 16 weeks SOF+RBV improved SVR12 rates for all prior P/R failures by approximately 30%. For treatment-naïve and treatment-experienced subjects infected with GT3 HCV, SVR12 rates were lower for IL28B genotypes TT compared to CC and CT. In treatment-experienced subjects in Study 108, response rates were about 30% better with the longer 16-week duration for all IL28B genotypes compared to the 12-week duration. For both treatment-naïve and treatment-experienced cirrhotic subjects with GT3 infection, SVR12 rates were 20-40% lower than

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non-cirrhotics following 12 week SOF+RBV treatment. The 16-week duration of SOF+RBV improved SVR12 rates by 3 times for cirrhotic treatment-experienced subjects to 61% similar to 63% for non-cirrhotics.

For treatment-naïve subjects infected with **genotype 2** HCV, SVR12 rates were 70% after 12 weeks SOF+RBV for subjects who were prior P/R null or partial responders. A longer duration of 16 weeks SOF+RBV improved SVR12 rates for prior P/R nulls and partial responders to 88%. For treatment-experienced cirrhotic GT2 infected subjects, SVR12 rates were 60% with 12 weeks of treatment. The 16-week duration of SOF+RBV improved SVR12 rates for cirrhotic treatment-experienced subjects to 78%. These data support a longer duration of 16 weeks SOF+RBV for treatment-experienced subjects with GT2 infection. Specifically, treatment-experienced nulls and partial responders and treatment-experienced cirrhotic subjects had better SVR rates following 16 weeks of treatment compared to 12 weeks of treatment.

In Study 110 for both GT1a and 1b HCV infected subjects who received SOF with P/R for 12 weeks, response rates were higher for IL28B CC than CT/TT. GT1a and GT1b subjects with cirrhosis had lower response rates (84% and 67%, respectively) compared to non-cirrhotics (93% and 84%, respectively).

An analysis of early response predictors (SVR12 rates by response (<25 IU/mL target not detected (TND), (<25 IU/mL target detected), (< and > 1,000 IU/mL) at Week 1, Week 2 and Week 4) showed that there are no clear recommendations that can be made to increase SVR12 rates based on early responses at Week 1, 2 or 4 for GT1, 2, or 3 infection.

There were no significant differences between baseline sequences of subjects who achieved SVR12 and those who relapsed. Notably, S282T was not present in any of the baseline samples.

For the analysis of SOF resistant substitution emergence, next generation nucleotide sequencing (NGS) data were submitted for 4 Phase 3 clinical trials. Of the 982 subjects in SOF+RBV or SOF/PEG/RBV groups of the Phase 3 Studies P7977-1231, GS-US-334-0107, GS-US-334-0108 and GSUS-334-110, a total of 224 subjects had NGS data with 676 files. The FDA virology analyses of the NGS data used two algorithms (i.e., PVD75 and QbVD) for the bioinformatics analysis and interpretation of the NGS data. The FDA used a cutoff of 10% for emergence of substitutions (i.e., substitutions were not present at baseline but were present at a frequency of at least 10% at failure). The FDA analysis focused on emergent substitutions identified in 2 or more subjects and identified by at least 2 of the algorithms used (i.e., Gilead, PVD75 and QbVD**)**.

No S282T substitutions were identified at baseline or at time of relapse in any of the subjects who relapsed from the Phase 3 trials in either the Gilead or FDA analyses. Multiple other substitutions in NS5B were identified by two algorithms in ≥2 subjects who relapsed in the phase 3 studies. However, many of these substitutions were at polymorphic sites and did not show a detectable reduction in phenotypic susceptibility to SOF or RBV when tested in replicons by Gilead. Substitutions L159F, V321A or E341D emerged in multiple GT3a-infected subjects across the phase 3 studies and were detected with 2 bioinformatic algorithms. The phenotypic susceptibility of subject

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isolates with L159F and V321A was tested by Gilead and the results showed no shift in SOF susceptibility. Gilead did not analyze the phenotypic susceptibility of substitution E341D.

One subject in Study P7977-1231 experienced DAA breakthrough at Week 8. This subject was infected with GT3a genotype/subtype, had IL28B CT genotype and a baseline viral load of 7 $\log_{10}$ IU/mL. This subject had evidence of poor compliance with low drug levels. There was only population sequence available from the Week 8 sample for this subject. The substitutions T84S, A150T and E202D emerged in NS5B on treatment. The positions T84 and E202 are 95% and 98% conserved, respectively. We will request that Gilead analyze substitutions T84S, E202D and E341D for phenotypic susceptibility.

The S282T substitution was detected by NGS analysis in one GT2b infected subject (Subject 1030-5068 from Phase 2 Study P7977-0523 [ELECTRON]) who received SOF monotherapy for 24 weeks and relapsed at Week 4 post-treatment. The sample from this genotype 2b subject with S282T detected displayed a mean 13.5-fold reduced susceptibility to SOF. Overall, S282T mutant replicons from genotypes 1-6 displayed a decreased replication capacity in cell culture. For this subject, the S282T substitution was no longer detectable at Week 12 post-treatment by NGS analysis with an assay cut off of 1%. Given the S282T emerged in this relapse subject in the Phase 2 study, is selected in cell culture replicons and confers decreased susceptibility to SOF, the S282T should be included in the resistance section of the label even though it was not detected in the Phase 3 studies.

1. RECOMMENDATIONS

1.1. Recommendation and Conclusion on Approvability

This NDA for sofosbuvir (SOF) is approvable with respect to virology for the treatment of HCV in combination with ribavirin (RBV) for Genotype 2 and 3 or pegylated interferon + RBV for Genotype 1.

1.2. Recommendation on Phase IV (Post-Marketing) Commitments, Agreements, and/or Risk Management Steps, if Approvable.

Substitutions T84S+A150T+E202D emerged in one subject who experience on-treatment failure. Substitution E341D was a treatment emergent substitution that developed in 13 subjects infected with GT3a HCV in two clinical trials (P7977-1231, n=10; GS-US-334-0107, n=3) and it was only found in subjects who relapsed. The L159F substitution emerged in 6 subjects with GT3a and is a previously identified NS5B substitution which with L320F was been shown to confer low-level resistance to HCV nucleotide inhibitors (Tong X et al., 2012). The L159F and E341D emerged together in one GT3a subject. Therefore, we are requesting they be characterized in combination in phenotypic assays to determine if they contribute to decreased SOF susceptibility.

1. Please assess the phenotypic susceptibility of substitutions T84S, E202D, T84S+A150T+E202D, and E341D.
2. Please also assess the phenotypic susceptibility of the combination of substitutions L159F+E341D.

2. SUMMARY OF OND VIROLOGY ASSESSMENTS

2.1 Non-Clinical Virology

Sofosbuvir 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. SOF had EC_{50} values ranging from 14-110 nM in stable full-length replicon cells of genotype 1a, 1b, 2a, 3a and 4a; and chimeric GT1b Con-1 replicons carrying NS5B coding sequences from genotypes 2b, 5a, or 6a. SOF antiviral activity was assessed against NS5B amplified from individual subject plasma samples and cloned into the replicon-based shuttle vectors. The mean EC_{50} value against all GT NS5B patient isolates was 78.4 nM. Mean EC_{50} values were 67.8 nM, 105.3 nM, 35.0 nM, and 84.6 nM against GT1a (n= 67), GT1b (n= 29), GT2 (n= 15), and GT3a (n= 106) subject isolates, respectively. SOF showed no antiviral activity against human rhinovirus (HRV) types 10 and 14, respiratory syncytial virus (RSV), influenza A virus, and HIV, at the highest concentration tested (100 μM). No significant effect on the activity of GS-7977 was observed in the presence of human serum or the addition of human serum albumin.

GS-7977 (SOF) and its metabolites had CC_{50} values >50 μM in the human cell lines tested (pancreatic cancer cells, prostate cancer cells, T cell lines, replicon hepatic carcinoma cell lines, erythroid and myeloid bone marrow progenitor cells). Mitochondrial toxicity was assessed. SOF showed no decrease in mtDNA following a 10- to 14-day treatment of multiple cell lines. SOF and its metabolites showed no inhibition of mitochondrial biogenesis (mitochondrial DNA replication, RNA transcription and protein expression) with $mtCC_{50}$ values >100 μM in PC3 and HepG2 cells. In biochemical reactions, the active triphosphate form of SOF did not inhibit RNA polymerase II at concentrations up to 200 μM, mitochondrial RNA polymerase at concentrations up to 500 μM, or DNA polymerases α, β, or γ at concentrations up to 200 μM. In addition, the active metabolite of SOF is a poor substrate for mitochondrial RNA polymerase with an incorporation rate into RNA of <0.5% relative to UTP in a biochemical reaction.

SOF showed no antagonism when combined with other classes of direct acting antivirals for HCV, i.e. nonnucleoside palm inhibitor (GS-9190), nonnucleoside thumb-1 inhibitor (GS-9669), NS5A inhibitors (GS-5885, GS-5816), NS3/4A protease inhibitors (boceprevir, telaprevir, GS-9451), with RBV, with IFN or with HIV-1 NRTIs (ABC, AZT, ddI, d4T, FTC, TDF, 3TC.

Full-length genotype 1b, 2a, 3a, and 4a replicons and chimeric replicons encoding the genotype 2b, 5a, or 6a NS5B sequence in a genotype 1b backbone were used to select for resistance to SOF via passaging under escalating concentrations of SOF. In GT1b, 2a, 3a and 4a subgenomic replicons, S282T was observed when the replicon cells were treated with SOF concentrations of 10 to 30 times the wild-type EC_{50} value. Detection of the S282T substitution was associated with a decrease in susceptibility to SOF with the presence of S282T at >99% correlating with at least 4- to 24- fold decreases in susceptibility to SOF for all four genotypes. Several substitutions in addition to S282T were observed in GT1b, GT2a and GT4a replicons. The S549N substitution was observed in GT1b and GT2a replicons with S282T, but alone was associated with a <1-fold decrease in SOF susceptibility. The M289L substitution was observed with S282T

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in GT2a, 5 and 6 replicons. Multiple substitutions including V67A, E237G, R304K, were observed in the GT4a replicon during the resistance selection.

Replicons encoding S282T alone demonstrated 4- to 11-fold increases in SOF EC_{50} values. In the GT2a replicon, the M289L substitution when present as a single substitution showed a 2-fold shift in SOF EC_{50} value, while as the double mutant M289L+S282T showed a 3.3-fold increase in EC_{50} fold shift. R498K, observed in GT2b passages during SOF selection, did not exhibit any phenotypic shift when present as a single substitution. When present as a double mutant (R498K+S282T), the fold shift in SOF was similar to S282T alone.

NS5B S282T substitution was not cross-resistant with the NS5A inhibitor GS-5885 or RBV. SOF remained active against NS5B nucleoside mutants (except S282T), nonnucleoside inhibitor mutants, NS5A resistance mutants, and NS3/4A protease inhibitor resistance-associated mutants tested.

2.2 Clinical Virology

Response by Genotype/Subtype

There were 4 Phase 3 trials of SOF, 3 trials in subjects infected with GT2 or 3 HCV using SOF in combination with RBV and 1 trial in subjects infected with GT1 HCV using SOF in combination with P/R.

For subjects infected with GT2 (n=348), subtype 2b was the most common subtype seen in 79% of subjects (n=274), 19.5% of subjects were infected with GT2a (n=68), and only 6 subjects were infected with GT 2c, 2i, 2j, or 2r as determined by NS5B nucleotide sequence analysis. There were no obvious differences in response rates to SOF+RBV across these subtypes. For subjects infected with GT3 (n=621), 99% of subjects had subtype 3a infection by NS5B sequencing and only 6 subjects had other subtype. Therefore, no conclusions could be made about the response rates for other subtypes of GT3. In Study 110, 77% of the subjects were infected with GT1a and 23% with GT1b. There were only 28 subjects infected with GT4 HCV most of which were GT4a, 6 with GT6 (a, e, j or q) and 1 with GT5a. All subjects with genotype 1, 1b, 4, 5, and 6 infections were from the U.S., because Study 110 was conducted only in the U.S. In the studies 1231, 107 and 108, 92% and 59% of subjects with GT2 and GT3 infection, respectively, were from the U.S.

The VERSANT HCV genotype assay, Version LiPA 2.0 was used for screening genotype/subtype for entry into the Phase 3 studies. Subsequently, nucleotide sequence and phylogenetic analysis of the HCV NS5B region was used to confirm subtype. In some circumstances, NS5B sequencing would indicate a different genotype and/or subtype for a subject. The sponsor used the genotype/subtype determined by NS5B sequencing for their analyses. Overall, concordance between the LiPa screening assay and NS5B sequencing was ≥94%. However, some subjects were found to have been misclassified by the screening assay when the NS5B sequencing analysis was performed.

Most misclassifications were of the same genotype but different subtype. However, 9 subjects' viruses were misclassified as GT2 by the screening assay when they were

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later determined to be GT1 by the sequencing assay. Of these 9 subjects who received a suboptimal SOF+RBV regimen when they should have received SOF+P/R, 3 (33%) achieved SVR12 and all 3 were IL28B CC genotype. The sponsor classified these subjects as GT1 for efficacy and virology analyses. However for ITT analyses, this reviewer believes these subjects should be classified as GT2 as determined by the screening assay. In the "real world" clinical settings, NS5B sequencing will not be performed, so physicians would not be able to get this more accurate genotyping/ subtyping. Genotyping/subtyping will be determined in the clinic by assays similar to the screening assay. Therefore, the efficacy should reflect what can be expected in the clinic with a screening assay.

Response Rates by Baseline Factors

Response rates were examined for each genotype by various baseline factors including prior response rates, IL28B genotype, and cirrhosis status. The data from all these baseline factor subgroups support a longer 16-week duration of SOF+RBV for GT3 HCV infection. For genotype 3 infection, SVR12 rates were much lower for subjects with prior P/R experience than treatment-naïve subjects following 12 weeks of SOF+RBV. SVR12 rates were lower for prior nulls and partial responders compared to prior P/R breakthroughs and relapsers. A longer duration of 16 weeks SOF+RBV improved SVR12 rates for all prior P/R failures by approximately 30%.

For treatment-naïve subjects with GT3 HCV infection in Studies 107 and 1231, SVR12 rates were lower for IL28B genotypes TT (33-42%) compared to CC and CT (57-65%). In treatment-experienced subjects in Study 108, response rates were about 30% better with the longer 16-week duration for IL28B genotype CC and CT compared to the 12-week duration.

For both treatment-naïve and treatment-experienced cirrhotic subjects with GT3 infection, SVR12 rates were 20-40% lower than non-cirrhotics following 12 week SOF+RBV treatment. The 16-week duration of SOF+RBV improved SVR12 rates by 3 times for cirrhotic treatment-experienced subjects to 61% similar to the 63% rate for non-cirrhotics.

For treatment-naïve subjects with GT2 HCV infection, SVR12 rates were 93-95% following 12 week SOF+RBV treatment. SVR12 rates were also high for subjects with prior P/R breakthrough or relapse following both 12 and 16 weeks of SOF+RBV (86% for 12 weeks; 89% for 16 weeks). Response rates after 12 weeks SOF+RBV for subjects who were prior P/R null or partial responders were 70%. A longer duration of 16 weeks SOF+RBV improved SVR12 rates for prior P/R nulls and partial responders to 88%.

Subgroups were too small to make definitive conclusions regarding differences in response by IL28B genotype. However, in treatment-experienced GT2-infected subjects in Study 108, response rates were in general better with the longer 16-week duration for IL28B genotype subgroups compared to the 12-week duration although again subgroups were small.

For treatment-naïve cirrhotic GT2 infected subjects, SVR12 rates were >90% following 12 week SOF+RBV treatment. For treatment-experienced cirrhotic GT2 infected subjects, SVR12 rates were lower (60%) with 12 weeks of treatment. The 16-week

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duration of SOF+RBV improved SVR12 rates for cirrhotic treatment-experienced subjects to 78%.

These data support a longer duration of 16 weeks SOF+RBV for treatment-experienced subjects infected with GT2. Specifically, treatment-experienced nulls and partial responders and treatment-experienced cirrhotic subjects had better SVR rates following 16 weeks of treatment compared to 12 weeks of treatment.

In Study 110, where all GT1 infected subjects were treatment-naïve, SVR12 rates were high. Interestingly, response rates were higher for GT1a compared to GT1b (92% vs. 82%) infections. For both GT1a and 1b HCV infected subjects, response rates were higher for IL28B CC than CT/TT. GT1a and GT1b infected subjects with cirrhosis had lower response rates compared to non-cirrhotics.

Early Predictors of Response

In an attempt to determine if there were early response predictors of response, SVR12 rates by response (<25 IU/mL target not detected (TND), (<25 IU/mL target detected), (< and > 1,000 IU/mL) at Week 1, Week 2 and Week 4 were analyzed. Overall, these data indicate there are no clear recommendations that can be made to increase SVR12 rates based on early responses at Week 1 or 4 for GT1, 2, or 3 infections. However, clearly the data show that a longer duration of 16 weeks SOF+RBV increases SVR12 rates for GT3 HCV infection.

Baseline Genotypic Predictors of Response

There were no significant differences between baseline sequences of subjects who achieved SVR12 and those who relapsed. Notably, S282T was not present in any of the baseline samples. In addition, no statistical difference in treatment outcome was associated with the presence of baseline substitutions T390I or F415Y (observed in virologic failures of RBV-containing regimens; Young et al., 2003; Ward et al., 2008; Bartels et al., 2011) or substitutions N142T, M289I or M289L (selected in cell culture by nucleoside analog polymerase inhibitors) in subjects treated with a SOF-containing regimen.

Analysis of Resistance Substitution Emergence On-Treatment

For the analysis of SOF resistant substitution emergence, next generation nucleotide sequencing (NGS) data were submitted for 4 Phase 3 clinical trials. Of the 982 subjects in SOF+RBV or SOF/PEG/RBV groups of the Phase 3 Studies P7977-1231, GS-US-334-0107, GS-US-334-0108 and GSUS-334-110, a total of 224 subjects had NGS data with 676 files. The FDA virology analyses of the NGS data used two algorithms (i.e., PVD75 and QbVD) for the bioinformatics analysis and interpretation of the NGS data. The FDA used a cutoff of 10% for emergence of substitutions (i.e., substitutions were not present at baseline but were present at a frequency of at least 10% at failure). The FDA analysis focused on emergent substitutions identified by at least 2 of the algorithms used (i.e., Gilead, PVD75 and QbVD**)** and identified in 2 or more subjects.

No S282T substitutions were identified at baseline or at time of relapse in any of the subjects from any of the Phase 3 trials in either the Gilead or FDA analyses. Multiple other substitutions in NS5B were identified by two algorithms in ≥2 subjects who relapsed in the phase 3 studies. However, many of these substitutions were at

polymorphic sites with substitutions occurring in both directions and did not show a detectable reduction in phenotypic susceptibility to SOF or RBV when tested in replicons by Gilead. Substitutions L159F, V321A or E341D emerged in multiple subjects across the phase 3 studies and were detected with 2 bioinformatic algorithms. The phenotypic susceptibility of subject isolates with L159F and V321A was tested by Gilead and the results showed no shift in SOF susceptibility. (b) (4) (b) (4) We will request the phenotypic susceptibility data for substitution E341D. The L159F and E341D also emerged together in one GT3a subject from study P7977-1231; therefore, we will request the SOF phenotypic susceptibility data for this combination.

One subject in Study P7977-1231 experienced DAA breakthrough at Week 8. This subject had GT3a genotype/subtype, IL28B CT genotype and a baseline viral load of 7 $\log_{10}$ IU/mL. However, this subject had evidence of noncompliance with low drug levels. There was only population nucleotide sequence available from the Week 8 sample for this subject. The substitutions T84S, A150T and E202D emerged in NS5B on treatment. The positions T84 and E202 are 95% and 98% conserved, respectively. We will request the phenotypic susceptibility data for substitutions T84S, E202D, and the combination T84S+A150T+E202D.

The S282T substitution was detected by NGS in one GT2b subject (Subject 1030-5068 from Phase 2 Study P7977-0523 [ELECTRON]) who received SOF monotherapy for 24 weeks and relapsed at Week 4 post-treatment. The sample from this genotype 2b subject with S282T detected displayed a mean 13.5-fold (range 8.99-19.77) reduced susceptibility to SOF. For this subject, the S282T substitution was no longer detectable at Week 12 post-treatment by NGS with an assay cut off of 1%. A significant reduction in replication capacity (1.5 - 1.8% of wild-type) was observed in the replicon clones containing NS5B from the phase 2 subject with the S282T substitution detected. Overall, S282T mutant replicons from genotypes 1 - 6 displayed a decreased replication capacity in cell culture ranging from 1% to 11% as compared to corresponding wild-type replicons. These data are consistent with the short persistence (≤8 weeks) of S282T in the single subject with this mutant detected following SOF treatment. Given the S282T emerged in the relapse subject in the Phase 2 study, is selected in cell culture replicons and confers decreased susceptibility to SOF, the S282T should be included in the resistance section of the label even though it was not detected in the Phase 3 studies.

3. ADMINISTRATIVE

3.1. Reviewer's Signature(s)

Lisa K. Naeger
[Lisa K. Naeger, Ph.D.]
Sr. Virologist, HFD-530

3.2. Concurrence

HFD-530/Micro TL _____ Date _____

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4. VIROLOGY REVIEW

4.1 Important Milestones in Product Development

GS-7977 was originally developed by Pharmasset as PSI-7977 until it was bought by Gilead Sciences.

At the EOP2 meeting in May 2012, we requested Gilead study a longer duration than 12 weeks, but the sponsor only agreed to study 16 weeks in treatment-experienced subjects in Study 108.

We also informed them at this meeting that the adequacy of supporting data for HCV GT4, 5, and 6 indications would be a review issue. A minimum of 10-15 subjects for each of the most common HCV genotype 4, 5, and 6 subtypes in ths U.S. was recommended. We informed Gilead that sufficient subjects across GT4, 5, and 6 as well as sufficient representation of common subtypes within these gentoypes was of particular concern given the importance of subtype in response to DAA-based treatments.

FDA Response to Gilead May, 2012:
A sample size of ≤20 subjects is unlikely to provide sufficient information to characterize the efficacy of the regimen broadly for genotypes 4, 5, and 6. There may be variability in the efficacy of GS-7977 against multiple subtypes of HCV genotype 4, 5, and 6 that are genetically diverse.

While we support your efforts to characterize the efficacy of anti-HCV treatment regimens in HCV genotype 4, 5, and 6 populations, and recognize the feasibility challenges, an indication for HCV genotypes 4, 5 and 6 for the GS-7977/P/R regimen will be a review issue. We recommend you enroll a minimum of 10-15 subjects for each of the most common HCV genotypes 4, 5 and 6 subtypes in the U.S. Sufficient subjects across genotype 4, 5, and 6, as well as sufficient representation of common subtypes within these genotypes is of particular concern given the importance of subtype in response to DAA-based treatments. In addition, the proposed GS-7977 + P/R regimen (12-weeks) was not evaluated for genotypes 4 and 6 in Phase 2. The total number of subjects enrolled across genotypes should be sufficient to determine if the treatment duration is appropriate for all subjects regardless of baseline predictive factors as discussed above in the response to Question 1.

In parallel with clinical studies of HCV genotype 4-, 5-, and 6-infected subjects, conduct detailed nonclinical virology studies (e.g., using the HCV replicon system) to characterize the anti-HCV activity and durability of GS-7977 for isolates representative of the most common HCV genotype 4, 5 and 6 subtypes in the U.S.
Analyses should characterize the following, at minimum:

- *Antiviral activity*
- *Resistance pathways*
- *Numbers of amino acid substitutions needed to confer significant reductions in GS-7977 susceptibility*
- *Fold-reductions in HCV susceptibility conferred by individual and combinations of resistance-associated substitutions*

- *Analyses should include HCV genotype 1a and 1b isolates for comparison.*

For resistance analysis data from SOF Phase 3 trials, we agreed to accept population nucleotide sequence analysis for baseline samples of all subjects and NGS nucleotide sequence analysis of isolates from treatment failures at failure timepoints. We requested that Gilead analyze substitutions that emerged in 2 or more subjects for SOF susceptibility.

We had several teleconferences with Gilead regarding submission of next generation sequencing (NGS) data. Clinical Virology Reviewer Eric Donaldson, Ph.D., provided a Draft document for "Submitting Next Generation Sequencing Data to the Division of Antiviral Products Experimental Design and Data Submission" and requested comments and suggestions from Gilead. In December 2012, we had discussions by teleconference and agreed upon the cut-off of 15%, data formatting and submission of fastq files, and the summary tabulated data formats.

4.2 Methodology

Population Sequencing
Baseline population nucleotide sequencing of the full-length HCV NS5B coding region was performed by (b) (4) using RT-PCR and standard Sanger population sequencing. The obtained NS5B sequence was also used to confirm the results of genotyping/subtyping by the INNO-LiPA assay performed at screening. For post-baseline resistance analyses of subjects with virologic failure, the HCV NS5B gene was amplified from subject samples if the serum/plasma level of HCV RNA was ≥1,000 IU/mL.

Deep Nucleotide Sequence Analysis (NGS)
For virologic failures, NS5B RT-PCR amplicons at baseline and post-baseline timepoints were subjected by the sponsor to deep nucleotide sequence analysis using the Illumina MiSeq deep sequencing platform. A high level summary of the predominant substitutions for each study was summarized for each individual study.

Population or consensus sequencing results were analyzed for all other amino acid changes observed in >2 resistance analysis subjects. Consensus sequences of post-baseline samples were generated with inclusion of mixed amino acid calls present between 15 and 85%. Cases where the baseline amino acid differed from reference and the post-baseline substitution was a change to the reference amino acid were considered as a post-baseline substitution and the data are reported. Substitutions observed at any amino acid position in post-baseline sample that differ from baseline at frequencies ≥1% were reported. The substitutions were classified by the sponsor into the following categories. "Developed" substitutions were defined as those not detected at baseline and or present at levels below the 1% cutoff and are detected above background at post-baseline. "Enriched" substitutions are those that were present at baseline above the 1% cutoff and have increased more than 2-fold from baseline at post-baseline sampling. "Lost" substitutions are defined as those present above the 1% cutoff at baseline and are no longer detectable by the same criteria at the post-baseline sampling. "Depleted" substitutions are defined as being detected above 1% at baseline

and at sampling, but have decreased in frequency by more than 2-fold at post-baseline. Net is calculated as (#Developed + #Enriched - #Lost - #Depleted). This Net value was used to calculate the percent of subjects who developed the substitutions at failure.

Phenotypic Analysis

Phenotypic analyses of select samples using a chimeric replicon encoding the NS5B region derived from subject plasma/serum were performed by Gilead Sciences, Inc., Janssen Diagnostic (Beerse, Belgium), (b) (4)

Baseline Sequence Data Analysis

Baseline sequences were analyzed for the presence of previously identified nucleoside analog polymerase inhibitors (NI), non-nucleoside analog polymerase inhibitors (NNI) or RBV associated substitutions (Table 1; Report PC-334-2024). The NS5B region of all subjects with baseline sequences available were analyzed at baseline for pre-existing substitutions conferring resistance to NIs, NNIs or RBV using population sequencing. The treatment outcome of subjects with the NI, NNI or RBV resistance-associated substitutions detected at baseline was evaluated for each individual studies.

Table 1. List of Specific Resistance-Associated Substitutions to Be Analyzed

NS5B Substitution[a]	NNI/NI Substitution	Inhibitors Affected
S96T	NI	R1479 {15906}
N142T	NI	R1479{15906}
S282T	NI	SOF, RG7128{15906, 9254, 20393, 21144}
M289L	NI	SOF {20547} PC-334-2010
M289I	NI	PC-334-2010
L320F	NI Site	PSI-6130, Mericitabine {15906, 23664}
L159F	NI Site	Mericitabine {23664}
C316Y	NNI Site IV	Benzothiadiazine, HCV-796, TGV, ABT-333{16599, 19444, 23666}
C316N	NNI Site IV	Benzothiadiazine, TGV {17830}
T390I	RBV	Ribavirin{21377}
M414T	NNI Site III	Benzothiadiazine, ABT-333, ANA598 {23668, 23666}
M414L	NNI Site III	Benzothiadiazine, ANA598 {23668}
F415Y	RBV	Ribavirin {21377, 23665}
L419M	NNI Site II	Thiophene, GS-9669, VX-222 (PC-257-2028, PC-334-2017)
L419S	NNI Site II	Thiophene, GS-9669, VX-222 (PC-257-2028, PC-334-2017)
R422K	NNI Site II	Thiophene, GS-9669, VX-222 (PC-257-2028, PC-334-2017)
M423T	NNI Site II	Thiophene, GS-9669, VX-222 (PC-257-2028, PC-334-2017)
M423I	NNI Site II	Thiophene, GS-9669, VX-222 (PC-257-2028, PC-334-2017)
M423V	NNI Site II	Thiophene, GS-9669, VX-222 (PC-257-2028, PC-334-2017)
M423A	NNI Site II	Thiophene, Filibuvir {20461}
Y448H	NNI Site III/IV	Benzothiadiazine (ABT-333), TGV{19444, 11477, 23666}
I482L	NNI Site II	Thiophene, GS-9669, VX-222 (PC-257-2028, PC-334-2017)
A486V	NNI Site II	Thiophene, GS-9669, VX-222 (PC-257-2028, PC-334-2017)
A486I	NNI Site II	Thiophene, GS-9669, VX-222 (PC-257-2028, PC-334-2017)
A486T	NNI Site II	Thiophene, GS-9669, VX-222 (PC-257-2028, PC-334-2017)
V494A	NNI Site II	Thiophene, GS-9669, VX-222 ((PC-257-2028, PC-334-2017)
P495L	NNI Site I	Indole, BI207127 {23667}
P495S	NNI Site I	Indole, BI207127 {23667}
G554D	NNI Site III	Benzothiadiazine ANA598 {23668}
S556G	NNI Site III	Benzothiadiazine (ABT-333){23666}
D559G	NNI Site III	Benzothiadiazine ANA598 {23668}

a The reference amino acids (amino acid before position, S282) shown in the table above are derived from either GT1a, GT1b or GT2a subtypes. The specific substitutions (amino acid after position, 282T) are potentially associated with resistance across genotypes/subtypes. To obtain the appropriate reference amino acid sequence genotypes/subtypes,

Phylogenetic Analysis
A phylogenetic analysis was performed using an alignment of the full-length HCV NS5B sequences derived from baseline viral HCV samples of the subjects who participated in an active treatment regimen in the 3 Phase 3 Studies: P7977-1231, GS-US-334-0107, and GS-US-334-0108. Multiple sequence alignment was performed using Multiple Sequence Comparison by Log-Expectation. Trees were constructed using the maximum likelihood method, as implemented within the software package PhyML-aLRT. The analyses of nucleotide sequences from the HCV NS5B region are provided as phylogenetic trees for each of the active treatment groups in the 3 Phase 3 studies

4.3 Prior FDA Virological Reviews

IND-106739 reviews through SDN081 were done by Takashi Komatu, Ph.D. and then after SDN081 by Lisa K. Naeger, Ph.D., Sr. Virology Reviewer.

4.4 State of Antivirals Used for the indication (s) Sought*:*

Globally, 170 million people are estimated to be infected with HCV, which induces liver necrosis and inflammation and increases the risk of progressive liver failure and liver cancer (WHO, 2010). The prevalence of chronic HCV infection in the United States (3.9 million infected) is approximately 4 times that of HBV or HIV-1 infection. An estimated 75% of chronically HCV-infected individuals remain undiagnosed compared with individuals infected with HBV (65%) or HIV-1 (21%). HCV accounts for about 15% of acute viral hepatitis, 60-70% of chronic hepatitis and up to 50% of cirrhosis, end-stage liver disease and liver cancer.

HCV is a small, enveloped, single-stranded RNA virus of the Flaviviridae family. The virus contains a single, 10 kb, positive-sense RNA genome which encodes both structural proteins necessary for virus particle formation and nonstructural proteins necessary for replication. Viral RNA encodes a single, long open reading frame producing a polyprotein. Structural proteins are cleaved by cellular proteases and the nonstructural proteins are cleaved by the viral encoded NS2 and NS3/4A proteases.

There are 6 major HCV genotypes with different geographic distributions. Genotypes 1a and 1b are most common in the U.S. representing about 75% of the infected population with genotype 1a predominating (Zein, N et. al., 1996). Genotypes 2 and 3 are present in only 10-20% of U.S. patients with subtypes 2a, 2b and 3a most common in the U.S. (Pawlotsky JM, et. al., 1995; Clement CG et al, 2010, Nizar N Zein, 2000) and genotype 4 is found in about 7% of U.S. patients. In the NHANESIII study done in the U.S., 57% were classified as 1a, 17% as 1b, 3.5% as 2a, 11% as 2b, 7% as 3a, 0.9% as 4, 3% as type 6. [Alter MJ et al., 1999]. In another study, the HCV genotypes identified included 1a (n = 142; 52%), 1b (n = 73; 26%), 2a (n = 8; 3%), 2b (n = 27; 10%), 3a (n = 17; 6%), 4 (n = 3; 1%), and 6 (n = 5; 2%) [Nainan OV et al., 2006].

Unlike HIV-1, which currently requires long-term therapy to maintain viral suppression, clearance of HCV is possible with therapy, because of a presumed lack of an archival form of the HCV RNA genome. A durable sustained virologic response (SVR) has been consistently observed in long-term studies following HCV treatment. However, the current treatments for chronic HCV infection are limited in efficacy and have significant

toxicities. The current standard-of-care for adults with genotype 1 chronic hepatitis C (CHC) virus infection is treatment with an NS3/4A inhibitor, boceprevir or telaprevir, in combination with pegylated recombinant human interferon α (Peg-IFN) plus ribavirin (RBV) and results in sustained clearance of HCV RNA in 60-70% of patients. Subjects with chronic genotype 1 HCV infection usually require a full 48 weeks of therapy to maximize the likelihood of achieving SVR, although subjects who achieve a rapid virologic response (RVR) after 4 weeks of treatment may benefit from a shorter duration of treatment.

The current standard-of-care for adults with genotype 2 and 3 chronic hepatitis C (CHC) virus infection is 24 weeks of pegylated recombinant human interferon α (Peg-IFN) combined with ribavirin (RBV). This combination is more effective for genotypes 2 and 3 than genotype 1. The efficacy (sustained virologic response) for genotype 2 and 3 is 60-80%. Currently, the standard-of-care for treatment of genotype 4, 5, or 6 chronic HCV infection is P/R with weight-based RBV for 48 weeks (Wartell-Bladou C, et. al., 2012).

There are limitations in P/R treatment for some groups of patients. Patients with high viral loads, cirrhosis, homozygous or heterozygous “T” allele in the polymorphic IL28B gene, and African-Americans have lower rates of SVR with P/R treatment. In addition, P/R treatment is not tolerable in all chronic HCV HCV patients, because of the significant adverse events associated with interferon and ribavirin. Furthermore, subjects who have previously failed to respond to P/R therapy have poorer treatment response to retreatment. Therefore, there is an unmet need for effective and safe treatment options in certain patient populations and shorter treatment options for most subjects with HCV.

Direct-acting antivirals (DAAs) specifically target the HCV proteins involved in the HCV life cycle. Boceprevir and telaprevir and are currently marketed DAAs targeting the NS3/4A protease. Variants resistant to DAAs likely pre-exist in all patients, because of the high HCV replication rate and error rate. HCV has a high rate of replication (up to 1 $x10^{12}$ virions produced each day) (Neumann et al., 1998), which is error-prone because the HCV RNA-dependent RNA polymerase lacks a proofreading function. The error rate of the polymerase is approximately 10^{-4}/base/generation for a single mutation, which results in one mutation being introduced into every genome that is copied. New variants are constantly being generated, and it has been estimated that every possible point mutation along the HCV genome occurs at least once and probably many times each day (Kieffer et al., 2010). Treatment with a DAA monotherapy can rapidly select for resistant variants in some patients, as has been demonstrated by the selection of variants with resistance to a number of DAAs, including NS3/4A protease inhibitors and polymerase inhibitors (Sarrazin and Zeuzem, 2010). Therefore, DAAs are being studied in chronic HCV patients in combination with Peg/RBV, RBV or other DAAs to reduce the selection of resistant variants, improve SVR rates and potentially shorten treatment duration. The DAA, SOF, was studied with RBV for 12 and 16 weeks for the treatment of GT2 and 3 and with P/R for 12 weeks for the treatment of GT1.

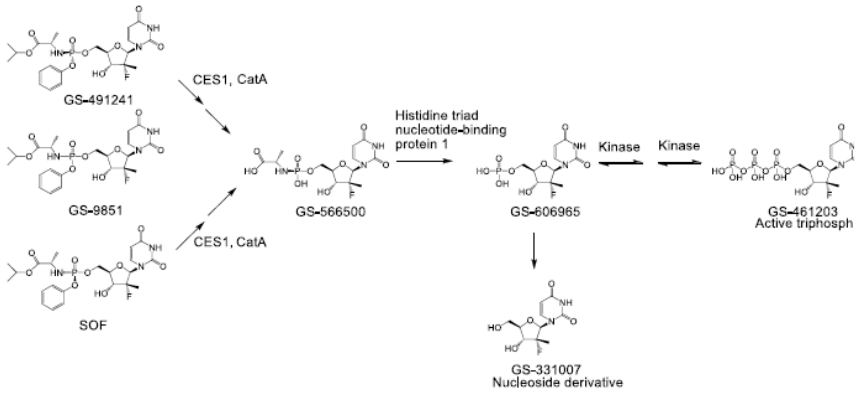
4.5 NON-CLINICAL VIROLOGY

MECHANISM OF ACTION

Sofosbuvir 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. The active triphosphate is incorporated into genomic RNA by the NS5B RNA polymerase inhibiting production of transcripts for production of viral proteins and replication of the viral genome. In a biochemical assay, GS-461203 inhibited recombinant NS5B from GTs 1b, 2a, 3a, and 4a with IC_{50} values of 3.3, 0.36, 1.4 and 2.7 µM.

Studies were performed using the prodrugs GS-9851 or SOF, which have the same metabolites and active moiety (Figure 1). GS-9851 comprises 2 diastereomers, SOF and GS-491241. The ratio of diastereomers in GS-9851 is approximately 1:1, and there is no evidence of interconversion in cell culture or in vivo. Both SOF and GS-491241 are metabolized in the same manner and produce the same metabolites (Figure 1). The diastereomeric mixture (GS-9851) was used in early development in both nonclinical and clinical studies, but the single diastereomer SOF was eventually chosen for development and registration.

Figure 1. Intracellular Metabolic Pathway of GS-9851, GS-491241, and Sofosbuvir (GS-7977)



Cytotoxicity

The cytotoxicity of SOF, GS-9851, and intracellular metabolites was determined in multiple cell lines (Table 2; Report PC-PSI-7977-09-0004). Sofosbuvir demonstrated CC_{50} values of 95.9 µM in Huh-7 and 90.6 µM in HepG2 hepatoma cells in an 8-day assay. The CC_{50} values were greater than the highest concentration of SOF tested in BxPC3 (pancreatic cancer cell line) and CEM cells (peripheral blood T lymphoblast) (>200 µM), in MT4 cells (> 94 µM) and in PC-3 cells (prostate cancer cells) (>89 µM) (Reports PC-PSI-7977-09-0004 and PC-334-2005). In the replicon cell lines tested in a 72 hour assay, GS-7977 (SOF) had CC_{50} values >50 µM following 3 days against GT1a, 1b, 2a, 3a and 4a replicons (Table 3)(PC-334-2005). For GS-9851 and all metabolites tested, CC_{50} values were higher than the maximum concentrations tested in each cell line for 8 days (100-1,000 µM) (PC-PSI-7851-08-0002, PC-PSI-7851-08-0027, PC-PSI-7851-09-0002, and PC-PSI-7977-09-0004). Similarly, the CC_{50} values for GS-9851

were >50 µM (the highest concentration tested) in human erythroid and myeloid bone marrow progenitor cells following 14-16 days (PC-PSI-7851-08-0022).

Table 2. Cytotoxicity of Sofosbuvir, GS-9851, GS-491241, and Intracellular Metabolites in Cell Culture

	CC_{50} (µM)[a]					
	Cell Line					
Compound	BxPC3	CEM	Huh-7	HepG2	MT4	PC-3
SOF	>200	>200	95.9 ± 9.8	90.6 ± 12.9	>94	>89
GS-9851	>100	>100	>100	>100	n.d.b	n.d.b
GS-491241	>200	>200	>200	>200	n.d.b	n.d b
GS-566500	>100	>100	>100	>100	n.d.b	n.d.b
GS-331007	>1000	>1000	>1000	>1000	n.d.b	n.d.b

SOF = sofosbuvir (GS-7977)

a CC_{50} = concentration at which 50% maximum cytotoxicity was achieved

b "n.d" denotes compound CC50 not determined in these cell lines

Table 3. Cytotoxicity of GS-7977 in Stable Replicon Cell Lines

	CC_{50} (µM) in Replicon Genotype (Strain)				
Compound	1a (H77)	1b (Con-1)	2a (JFH-1)	3a (S52)	4a (ED43)
GS-7977	>82	>85	>68	>89	>89

Mitochondrial Toxicity

The effect of SOF on mitochondrial DNA (mtDNA) replication was assessed in HepG2 cells. Analysis of mtDNA levels using quantitative PCR has been a standard method to monitor the potential mitochondrial toxicity of nucleoside analogs. SOF showed no decrease in mtDNA following a 10-day treatment of HepG2 cells at 20 µM, 2 µM and 0.2 µM. 20 µM is approximately 200-fold over the highest HCV replicon EC_{50} value (GT1-6 range = 14-110 nM) (Report PC-334-2012). In contrast, the positive control ddC resulted in a significant decrease in mtDNA levels at 0.2, 2.0 and 20 µM. In addition, mtDNA was not reduced in CEM cells by 14-days of treatment with concentrations of GS-7977 up to 100 µM, the highest concentration tested. Furthermore, there were no decreases in mtDNA levels observed with 100 µM GS-9851 in CEM, HepG2, and BxPC3 cells.

The effect of SOF and GS-9851 on mitochondrial biogenesis was tested in the human prostate carcinoma cell line PC-3 and hepatic carcinoma HepG2 cell line using a [redacted] (b) (4) This assay measures the ratio between a mitochondrially-encoded protein [complex IV or cytochrome c oxidase 1 (COX-1)] and a nuclear-encoded protein [complex II or succinate dehydrogenase (SDH-A)]. The assay directly measures protein expression levels, which can reflect inhibition

of any or all of the following steps in mitochondrial biogenesis: mitochondrial DNA replication, mitochondrial RNA transcription, and mitochondrial protein expression. A panel of nucleoside analogs and nucleoside monophosphate analog prodrugs was tested to allow a relative comparison, including the clinically tested nucleoside analog polymerase inhibitors: BMS-986094, IDX184, NM107 (parent nucleoside of NM283), R1479 (parent nucleoside of R1626), and PSI-6130 (parent nucleoside of RG7128).

In PC-3 cells, SOF, GS-9851, and PSI-6130 showed no inhibition of mitochondrial biogenesis with $mtCC_{50}$ values >100 µM after 5-day treatment (Table 4) (Report PC-334-2015). GS-9851 and PSI-6130 were also tested for effects on mitochondrial biogenesis in HepG2 cells and similarly showed no inhibition. A known inhibitor of mitochondrial DNA synthesis, ddC, showed inhibition of mitochondrial biogenesis in PC-3 cells ($mtCC_{50}$ = 0.14 µM) and HepG2 cells ($mtCC_{50}$ = 59 µM). BMS-986094 and R1479 showed inhibition of mitochondrial biogenesis in PC-3 cells with $mtCC_{50}$ values of 0.8 µM and 10 µM, respectively. An accurate determination of the $mtCC_{50}$ value for BMS-986094 was complicated by the observation of general cellular toxicity at low drug concentrations. The assay signal was below the level of detection in PC-3 cells treated at concentrations greater than 1 µM BMS-986094 due to the low number of cells remaining in the assay plate after 5 days of treatment. Nevertheless, a dose-dependent and selective depletion of COX-1 over SDH-A was observed when cells were treated with BMS-986094 below 1 µM. IDX184, a prodrug sharing the same active metabolite with BMS-986094, showed inhibition of mitochondrial biogenesis in PC-3 cells but at higher drug concentrations ($mtCC_{50}$ = 100 µM).

Table 4. Effect of SOF and other Nucleoside Analog Polymerase Inibitors on Mitochondrial Biogenesis after 5-Day Treatment in PC-3 and HepG2 cells

Catagory	Compounds	PC-3 Mitochondrial Biogenesis CC_{50} (µM)[a]	HepG2 Mitochondrial Biogenesis CC_{50} (µM)[a]	Development Stage/Major Clinical Toxicity[b]
Uridine	SOF	>100	n.d.	Phase III/None reported
	GS-9851	>100	>100	Not a clinical candidate--racemic mixture of SOF and GS-491241
Guanosine	BMS-986094	0.82[c]	n.d.	Stopped in Phase II/Cardiac and kidney toxicity
	IDX184	100	n.d.	Partial clinical hold in Phase II/None reported
Cytidine	PSI-6130 (nuc from RG-7128)	>100	>100	Phase II/ None reported
	R1479	10	77	Stopped in Phase II/Hematologic toxicity including leukopenia
	NM107 (nuc from NM283)	91	>100	Stopped in Phase II/Gastrointestinal toxicity
	ddC	0.14	59	Marketed (HIV)/Peripheral neuropathy, Sensorineural deafness, cardiomyopathy
Positive control	Chloramphenicol	2.5	5.6	Marketed (antibiotic)/Bone marrow suppression, anemia

a Results are the mean +/- standard deviation of at least n = 3 independent studies done in duplicate.
b Information on clinical toxicity was collected from various publicly available sources including publications, conference presentations, and investor meetings as of the time this report is prepared.
c Observed in the presence of general cytotoxicity of the compound

GS-461203 (the active triphosphate form of SOF) was tested for the inhibition of human RNA polymerase II, mitochondrial RNA polymerase (mtRNAP), and DNA polymerases α, β, and γ in biochemical assays. It was also tested for the incorporation into RNA by the human mitochondrial RNA polymerase. Results showed that GS-461203 did not inhibit RNA polymerase II at concentrations up to 200 μM, mtRNAP at concentrations up to 500 μM or DNA polymerases α, β, or γ at concentrations up to 200 μM (Table 5) (Report PC-334-2013). The mtRNAP inhibition assay identified all of the 3'-deoxy forms of the natural ribonucleotides as inhibitors of mtRNP with IC_{50} values ranging from 1.4-4.7 μM. However, GS-461203 showed no inhibition of human mtRNAP at the highest concentration tested (500 μM). In addition, GS-461203 is a poor substrate for mtRNAP with an incorporation rate into RNA of < 0.5% relative to UTP.

Table 5. Inhibition of RNA Polymerase Pol II, Mitochondria RNA Polymerase and DNA Polymerase α, β, and γ

Compounds	IC_{50} (μM)[a]				
	DNA Pol α[b]	DNA Pol β[b]	DNA Pol γ[b]	RNA Pol II[c]	mtRNAP
Positive Controls (IC_{50})	Aphidicolin (7.3)	3'deoxy TTP (1.4)	3'deoxy TTP (0.74)	α-amanitin (0.0024)	3'deoxy GTP[d] (1.9)
GS-461203	>200	>200	>200	>200	>500

a IC_{50} values were reported as arithmetic means of two to three independent experiments. All nucleoside/tide analogs were tested using their corresponding 5'-triphosphate active form.

b The natural dNTP concentrations were kept at 2 μM.

c The natural NTP concentrations were kept at their corresponding K_m values: 25 μM for ATP and 5 μM for GTP, CTP, and UTP.

d IC_{50} values for 3'deoxy ATP, 3'-deoxy GTP, 3'deoxy CTP, and 3'deoxy UTP were 4.6, 1.9, 1.4, and 4.7 μM, respectively.

Antiviral Activity in Cell Culture

Pharmasset Sciences Inc. first described the antiviral activity of GS-7977 against genotype 1a, 1b, 2a full-length replicons and against genotype 1b Con-1 chimeric replicons encoding 2a, 2b and 3a NS5B patient isolates using a luciferase readout following 3 days incubation (Table 6) (Report PC-334-2005). GS-7977 had EC_{50} values ranging from 15-110 nM against the pan-genotypic stable replicons tested.

Table 6. Antiviral Activity of GS-7977 Tested at Pharmasset Sciences, Inc.

Replicon	GS-7977 EC_{50} (nM)	GS-7977 CC_{50} (μM)
1a H77	50	>20
1b Con-1	110	>20
2a JFH-1	50	>20
1b/2a J6 NS5B	15	>20
1b/2b NS5B patient isolate	50	>20
1b/3a NS5B patient isolate	40	>20

GS-7977 was further assessed in stable full-length replicon cells of genotype 1a, 1b, 2a, 3a and 4a and chimeric GT1b Con-1 replicons carrying NS5B coding sequences from

genotype 2b, 5a, or 6a. Antiviral activity was observed across all stable replicons tested with EC_{50} values ranging from 14-110 nM (Table 7; Report PC-334-2005).

Table 7. Antiviral Activity of GS-7977, GS-5885, and GS-9669 against Stable Replicon Cell Lines

	EC_{50} in nM[b]		
Genotype	GS-7977	GS-5885	GS-9669
1a H77	40	0.031	11.1[c]
1b Con-1	110	0.004	2.7[c]
2a JFH-1	50	21	7103[c]
2b[a]	15	865	16980
3a S52	50	35	ND[d]
4a ED43	40	0.11	>423
5a[a]	15	0.15	10.31
6a[a]	14	0.12	1850

a The chimeric GT1b replicons carrying NS5B genes GT2b, 5a or 6a consensus sequences were used for testing GS-7977 and GS-9669 while the chimeric replicons carrying NS5A genes from these three genotypes were used for testing GS-5885.

b EC_{50}s are the average of at least two independent experiments

c Data from PC-257-2019

d Not determined

In addition, GS-7977 was tested against full-length GT1a, 1b and 2a replicons and NS5B GT3a, 4a, 5a, or 6a chimeric replicons in transient replication assays (Table 8; Report PC-334-2005). GS-7977 exhibited similar antiviral activity to the stable replicon cell lines against all transient replicons tested. Interestingly, given the clinical results with more favorable SVR12 rates for GT2 than GT3, the EC_{50} values for SOF in GT3a replicons was higher (50 nM) compared to GT2b (15-20 nM). Most of the GT2 and 3 subjects in the Phase 3 clinical trials had subtypes GT2b and GT3a.

Table 8. Antiviral Activity of GS-7977 against Transient Replicons

	EC_{50} in nM[b]	
Genotype[a]	GS-7977	
1a H77	30.2	
1b Con-1	26.6	
2a JFH-1	241.7	
2b	19.7	
3a	52.4	
4a	75.1	
5a	14.4	
6a	19.6	

GS-7977 inhibited virus derived from H77 (GT1a) and JFH-1 (GT2a) with EC_{50} values of 0.03 μM and 0.02 μM, respectively, and EC_{90} values of 0.14 μM and 0.13 μM, respectively (Table 9; Report PC-334-2005).

Table 9. Antiviral Activity of GS-7977 against GT1a and 1b Virus

HCV inhibitor	HCV EC_{50}	HCV EC_{90}
GT 1a (H77)	0.03 μM	0.14 μM
GT 2a (JFH-1)	0.02 μM	0.13 μM

Similar Susceptibility Results Obtained from Different Laboratories

Since the baseline susceptibilities assays for GT1 were performed by different organizations, the mean EC_{50} values for each laboratory were calculated and compared (Table 10; Report PC-334-2016). The means EC_{50} values for GT1a samples tested by Gilead Sciences, [redacted] (b) (4) were 76.3 nM (n=3), 71.9 nM (n=33) and 62.6 nM (n=31), respectively, using a luciferase readout following 3 days incubation. The means EC_{50} value for GT1b samples tested by Gilead Sciences, [redacted] (b) (4) were 114.9 nM (n=2), 100.8 nM (n=11) and 107.2 nM (n=16). No significant differences between the EC_{50} values from the 3 laboratories were seen for GT1a ($p = 0.26$) or GT1b ($p = 0.78$) using a one way ANOVA test.

Table 10. Comparison of NS5B Baseline Susceptibilities to Sofosbuvir across Three Laboratories

Genotype	Mean EC_{50} ± SD (nM) (number of isolates)	
	Gilead Sciences	(b) (4) [redacted]
GT 1a	76.3 ± 38.5 (n=3)	
GT 1b	114.9 ± 41.2 (n=2)	

Sofosbuvir Activity against a Large Panel of Subject Isolates of GT1a, 1b, 2b and 3

In order to determine the susceptibilities of subject isolates to sofosbuvir, the entire population of the NS5B polymerase gene amplified from individual subject plasma samples was cloned into the replicon-based shuttle vectors. This pooled population of molecules was used in transient replication assays. NS5B from 217 subject serum samples were tested for their susceptibility to sofosbuvir (Report PC-334-2016); 67 of which were from GT1a, 29 from GT1b, 15 from GT2 (14 GT2b and 1 GT2a), and 106 from GT3a. The mean EC_{50} value against all GT isolates was 78.4 nM. Mean EC_{50} values were 67.8 nM, 105.3 nM, 35.0 nM, and 84.6 nM against GT1a, 1b, 2, and 3a, respectively (Table 11; Report PC-334-2016). Among these samples, the EC_{50} values

ranged from 28.9 to 127.6 nM against GT1a isolates, 44.9 to 169.9 nM against GT1b isolates, 13.7 to 80.8 nM against GT2 isolates, and 23.9 to 180.5 nM against GT3a isolates. Minimal variation in susceptibility among GT1a, 1b, 2, and 3a baseline isolates was observed with 3.8, 3.1, 5.9, and 3.7 fold differences between the 95th and 5th percentiles of EC_{50} values, respectively (Table ; Report PC-334-2016).

Table 11. Mean Sofosbuvir EC_{50} Values against Chimeric Replicons Containing NS5B from Subject Isolates

	GT 1a (n=67)	GT 1b (n=29)	GT 2 (n=15)	GT 3a (n=106)
Mean EC_{50} ± SD (nM)	67.8 ± 24.3	105.3 ± 29.2	35.0 ± 18.0	84.6 ± 33.6
Range (Min , Max)	(28.9, 127.6)	(44.9, 169.9)	(13.7, 80.8)	(23.9, 180.5)
5% Percentile EC_{50} (nM)	30.44	53.27	13.75	37.01
95% Percentile EC_{50} (nM)	116.1	163.4	80.78	142.3
Fold Change in the 95th Percentile and the 5th Percentile	3.8	3.1	5.9	3.8

Antiviral Activity against Other Viruses

The antiviral activity of sofosbuvir was tested against human rhinovirus (HRV) types 10 and 14, respiratory syncytial virus (RSV), influenza A virus, and HIV-1. SOF exhibited no antiviral activity against these non-HCV viruses at the highest concentration tested (100 µM) (Table 12; PC-334-2011).

Table 12. Antiviral Activity of Sofosbuvir against Other Viruses

	Positive Control		SOF
Virus	Compound	EC_{50}[a] (µM)	EC_{50}[a] (µM)
HIV-1	AZT	0.023	>100
Human Rhinovirus type 10	Rupintrivir	0.044	>100
Human Rhinovirus type 14	Rupintrivir	0.045	>100
RSV	YM-53403	0.30	>100
Influenza A H3N2/HK/68	Oseltamivir	0.014	>100

a All values based on two or more independent experiments.

Effect of Human Serum and Serum Proteins

The effect of human serum on the activity of GS-7977 was evaluated by monitoring the luciferase activity in NS2-ET cells. Increasing concentrations of GS-7977 (0 to 3 µM)

were added to NS2-ET cells in the presence or absence of 10%, 20%, or 40% human serum. No significant effect on the activity of GS-7977 was observed in the presence of human serum or the addition of human serum albumin (Table 13).

Table 13. EC_{50} and EC_{90} Values of GS-7977 in the Presence of Human Serum Proteins

Human serum proteins	EC_{50} (µM)	SD	EC_{90} (µM)	SD
no human serum proteins	0.045	0.015	0.222	0.064
human serum (10%)	0.045	0.019	0.185	0.065
human serum (20%)	0.039	0.020	0.176	0.040
human serum (40%)	0.032	0.006	0.140	0.043
human serum albumin (5 mg/mL)	0.029	0.007	0.141	0.042
human serum albumin (10 mg/mL)	0.023	0.011	0.182	0.004
human serum albumin (20 mg/mL)	0.022	0.006	0.121	0.037

Antiviral Activity in Combination with Other Antiviral Agents

<u>In Combination with NS5A Inhibitors, Nonnucleoside Analog Polymease Inhibitors and RBV</u>

GS-7977 is likely to be used in combination with other classes of anti-HCV agents for the treatment of chronic HCV infection. The anti-HCV activity and cytotoxicity of GS-7977 combined with other anti-HCV compounds were tested in Huh-7 cells carrying an HCV genotype 1a replicon. There is no significant cytotoxicity observed for all individual compounds up to the highest concentrations tested in the combination assay. The antiviral effects of GS-7977 with the NS5A inhibitors GS-5885 or GS-5816, the nonnucleoside palm inhibitor GS-9190, the nonnucleoside thumb-1 inhibitor GS-9669, the protease inhibitor GS-9451, or ribavirin (RBV) were evaluated with an HCV genotype 1a replicon in the Huh-7 cell line. For each individual drug, the EC_{50} value was selected as the midpoint for the concentration range tested. The results were analyzed using MacSynergy II, which provides surface plots displaying significant deviations from additivity. At the 95% confidence interval, the mean synergy and antagonism volumes were between 25 and -25 μM^2% when GS-7977 was combined with GS-5885, GS-5816, GS-9190, GS-9669, or GS-9451 indicative of additive interaction with GS-7977 (Table 14; Report PC-334-2004). Furthermore, GS-7977 shows a synergy volume in the range of 25 to 50 μM^2% when combined with RBV, indicating a minor synergistic interaction. In combination studies using direct acting antivirals with GS-7977, cell viability was greater than 93% at the highest concentrations of compound combinations tested while studies analyzing the combined effects of GS-7977 and RBV showed cell viability greater than 85% at the highest combined drug concentrations. These results indicated that GS-7977 has additive antiviral activity when combined with GS-5885, GS-5816, GS-9190, GS-9669, or GS-9451. GS-7977 demonstrated minor synergy in combination with RBV in cell culture.

Table 14. Quantification of Antiviral Antagonism and Drug Interactions for Drug Combinations with GS-7977

Compound Used in Combination with GS-7977	Class	Synergy Volume (nM^2)[a]	Antagonism Volume (nM^2)[a]	Interaction
GS-5885	NS5A Inhibitor	3.3 ± 4.2	-7.7 ± 13.3	Additive
GS-5816	NS5A Inhibitor	0.0 ± 0.0	-18.3 ± 15.9	Additive
GS-9190	NS5B Non-nucleoside	4.7 ± 8.1	-11.7 ± 10.0	Additive
GS-9669	NS5B Non-nucleoside	1.3 ± 2.3	-5.7 ± 9.0	Additive
GS-9451	NS3 Protease Inhibitor	1.0 ± 1.7	-3.0 ± 4.4	Additive
RBV	Nucleoside Analog	35.3 ± 3.2	-2.0 ± 2.0	Minor synergy

a Values represent the mean ± standard deviation of three independent experiments performed in four replicates

The antiviral effects of GS-7977 in combination with GS-5885 or GS-5816 were evaluated in GT1b, 2a, 3a, and 4a stable replicon cell lines. GS-7977 had additive interactions with GS-5885 and GS-5816 in GT1b, 2a, 3a, and 4a replicons (Report PC-334-2014).

<u>In Combination with IFNα and NS3/4A Protease Inhibitors</u>

In addition, the antiviral activity of sofosbuvir was evaluated in combination with interferon α, boceprevir or telaprevir using the HCV genotype 1a replicon. The results were analyzed using MacSynergy II. At the 95% confidence interval, the mean synergy and antagonism volumes were between 25 and –25 μM^2% when sofosbuvir was combined with interferon or either protease inhibitor, indicative of additive interaction with sofosbuvir (Table 15; Report PC-334-2018). For all combinations cell viability was greater than 94% at the highest concentrations of compound combinations tested.

Table 15. Quantification of Antiviral Antagonism and Drug Interactions for Drug Combinations with GS-7977

Compound Used in Combination with SOF	Class	Synergy Volume (nM^2)[a]	Antagonism Volume (nM^2)[a]	Interaction
Interferon	Immune Modulator	12.0 ± 14.9	-1.1 ± 1.0	Additive
Boceprevir	NS3 Protease Inhibitor	1.7 ± 2.9	-18.3 ± 15.9	Additive
Telaprevir	NS3 Protease Inhibitor	4.7 ± 8.1	-11.7 ± 10.0	Additive

a Values represent the mean ± standard deviation of three independent experiments performed in four replicates

<u>In Combination with HIV-1 NRTIs</u>

To examine the effects of NRTIs on SOF anti-HCV activity, GT1a replicon cells were incubated with serially-diluted SOF in the presence of multiple concentrations of ABC, AZT, ddI, d4T, FTC, TDF, or 3TC. As the replicon CC_{50} values for all NRTIs tested except AZT were >44.4 μM, 44.4 μM was used as the highest concentration tested in combination with SOF. For AZT, the highest concentration used was 15 μM, the CC_{50} value of this compound in replicon cells. SOF EC_{50} values were similar regardless of the

presence of any of the NRTIs at any concentration (Tables 16 and 17; Report PC-334-2019). Notably, no trending towards an increase in SOF EC_{50} values was observed with increasing concentrations of any of the NRTIs tested. These data indicate there is no antagonistic effect of these NRTIs on SOF activity in cell culture. The cell viability at the highest concentrations of NRTIs with SOF ranged from 82%- 96%.

Table 16. SOF in Combination with HIV NRTIs in GT1a Replicon Cells

NRTI Concentration	SOF EC_{50} (nM)					
	TVF	d4T	FTC	ddI	3TC	ABC
44 μM	17.6 ± 2.6	20.0 ± 3.9	14.5 ± 1.6	22.2 ± 0.6	15.3 ± 0.9	23.2 ± 1.5
5.55 μM	20.1 ± 1.2	14.0 ± 3.5	11.0 ± 2.5	15.6 ± 4.8	12.8 ± 1.1	13.6 ± 3.1
0.694 μM	16.7 ± 0.8	14.5 ± 3.5	16.3 ± 2.3	13.9 ± 2.8	12.8 ± 2.5	17.0 ± 1.9
0 μM	14.2 ± 4.8	10.0 ± 2.0	17.6 ± 4.4	13.9 ± 4.7	18.2 ± 2.4	14.4 ± 4.9

Table 17. SOF in Combination with AZT in GT1a Replicon Cells

AZT Concentration	SOF EC_{50} (nM)
15 μM	18.3 ± 4.2
1.875 μM	12.9 ± 2.2
0.694 μM	15.4 ± 0.6
0 μM	17.9 ± 1.8

Cell Culture Resistance

Full-length genotype 1b, 2a, 3a, and 4a replicons and chimeric replicons encoding the genotype 2b, 5a, or 6a NS5B sequence in a genotype 1b backbone were used to select for resistance to sofosbuvir via passaging under escalating concentrations of sofosbuvir (0.1 to 10 μM) and at concentration of 0.5-1mg/mL G418. Genotypic and phenotypic data were obtained from the resistant viruses in selection experiments (Table 18) (Report PC-334-2010). In GT1b, 2a, 3a and 4a subgenomic replicons, S282T was observed when the replicon cells were treated with SOF concentrations of 10 to 30 times the wild-type EC_{50} value. Detection of the S282T substitution was associated with a decrease in susceptibility to SOF with the presence of S282T at >99% correlating with at least 4- to 24- fold decreases in sensitivity in SOF for all four genotypes. Although, S282T was the only substitution selected in GT3a, several substitutions in addition to S282T were observed in GT1b, 2a and GT4a replicons. The S549N substitution was observed with S282T and associated with a <1-fold decrease in SOF susceptibility in GT1b and GT2a replicons. The M289L substitution was observed with S282T in GT2a, 5 and 6 replicons. Multiple substitutions including V67A, E237G, R304K, were observed in the GT4a replicon during the resistance selection.

Chimeric GT1b replicons encoding the NS5B region from GT2b, GT5a, or GT6a NS5B were similarly cultured in the presence of increasing concentrations of SOF. Genotypic and phenotypic analysis detected the S282T substitution and increased fold-changes in

SOF EC_{50} values compared to the corresponding wild-type for all replicons. Deep sequencing analysis of early passages of chimeric replicons revealed that the levels of S282T reached 99% by day 81 in GT2b and by day 89 for GT6a chimeric replicons. In GT5a NS5B chimeric replicon cells, S282T levels reached 98% by Day 70. The presence of S282T at these >90% frequencies resulted in higher fold changes (30- to 99-fold) to SOF. Additional substitutions were observed by deep sequencing in GT2b and 6a chimeric replicons. R498K was observed at levels >15% after 39 days of selection in GT2b chimeric replicon cells. In GT6a replicons, the E375D substitution was observed thoughout the selection, while T580I and N237S were observed after 89 days of SOF selection.

Table 18. Genotype and Phenotype of SOF-Selected HCV GT1b, 2a, 3a and 4a Replicon Cells and Chimeric GT2b, 5a and 6a NS5B Replicons Cells

Replicon GT	Days of Selec-tion	SOF Concentration used for Selection (nM)	Fold Change in SOF EC_{50}	Substitutions Identified by NS5B Deep Sequencing	
				S282T (%)	Others (>15%)
GT1b	82	2000	2.01	15.43	T344A, C445F
	98	2000	3.36	53.58	T344A, C445F, S549N
	109	4000	4.08	98.36	T344A, S549N
GT2a	6	200	1.69	<1	I178V
	56	1200	11.0	32.09	T286P, M289L, V421A, S549N
	66	1200	21.5	52.06	K100Q, T286P, M289L, T483M
	79	2000	24.3	98.50	K51R, T286P, M289L
GT3a	6	200	0.70	<1	None
	72	2000	6.69	57.35	None
	94	3000	21.2	99.64	None
GT4a	6	200	1.01	<1	K531R, K544N,
	85	3000	40	50.94	V67A, E237G, R304K, K544N
	108	3000	24.17	99.59	V67A, E237G, R304K, A324V, K544N,C575G
GT2b	25	500	3.5	18.3	None
	39	500	6.6	90.8	R498K
	81	1000	99.5	99.8	R498K
GT5a	25	500	2.4	<1	None
	56	1000	14.3	77.9	None
	70	1000	60.4	98.1	None
GT6a	25	500	1.9	<1	E375D
	64	1000	5.5	53.4	E375D
	89	1000	32.7	99.1	N237S, E375D, T580I

a Concentrations of SOF at which replicons cells were cultured in and when the mutation(s) were identified

Phenotypic Analysis of SOF-Selected Amino Acid Substitutions

Individual substitutions detected in selection experiments were engineered into wild-type replicons by site-directed mutagenesis and phenotyped (Table 19) (Report PC-334-2010). The replicons containing the S282T substitution exhibited low replication capacity while other substitutions replicated at varying levels. Replicons encoding S282T alone demonstrated 4- to 11-fold increases in SOF EC_{50} values. In the GT2a replicon, the M289L substitution when present as a single substitution showed a 2-fold shift in SOF EC_{50} value, while as double mutant M289L+S282T showed a 3.3-fold increase in EC_{50} fold shift. R498K, observed in GT2b passages during SOF selection, did not exhibit any phenotypic shift when present as a single substitution. When present as a double mutant (R498K+S282T), the fold shift in SOF was similar to S282T alone. Likewise, all other individual substitutions tested showed no change in SOF susceptibility indicating that these substitutions did not confer an additional reduction in susceptibility to SOF.

Table 19. Phenotypic Analyses of NS5B Substitutions Observed During SOF Selection

Mutations	Replication Capacity (% relative to WT)	Fold Change in SOF EC_{50}
GT 2a: Wild-type EC_{50} = 55.8 nM		
K51R	94±0.66	0.88
K100Q	106±3.03	1.00
T286P	122±9.07	1.27
M289L	64±3.46	1.94
T483M	85±3.64	0.89
S549N	74±3.49	0.78
S282T	11.2±1.20	2.40
M289L +S282T	22±0.69	3.35
GT 2b: Wild-type EC_{50} = 28.1 nM		
R498K	126±8	1.34
S282T	12±0.52	10.04
R498K+S282T	14±0.15	12.3
GT 3a: Wild-type EC_{50} = 30.9 nM		
S282T	5.2±0.4	4.06
GT 4a: Wild-type EC_{50} = 44.1 nM		
A324V	46±1.18	0.90
S282T	4.27±0.25	5.97
GT 5a: Wild-type EC_{50} = 31.0 nM		
S282T	0.59±0.05	11.14
GT 6a: Wild-type EC_{50} = 39.5 nM		
N237S	6.86±0.47	2.36
T580I	438±10.36	0.62
D375E	300±1.75	1.01
S282T	36±2.12	8.79

a Replication fitness was assessed by comparing the Renilla reporter expression at 4h and 72hr for mutants and wild-type replicons. Replication capacity of the wild-type was set to 100 and replication fitness of the mutants was compared.

The NS5B substitutions selected in each replicon genotype and the phenotypic changes for these substitutions are summarized in Table 20.

Table 20. Summary of Substitutions Selected and Phenotypic Changes

Replicon GT	Substitutions Identified by NS5B Deep Sequencing	Maximum Fold Change in SOF EC_{50}	Phenotype of Site-Directed Mutants
GT1a			
GT1b	S282T With T344A, C445F, S549N	4X	
GT2a	S282T With K51R, K100Q, I178V, T286P, M289L, T483M, S549N	24X	**S282T (2.4)** **M289L (1.9)** **S282T+M289L (3.4)** K51R (0.9) K100Q (1.0) T286P (1.3) T483M (0.9) S549N (0.8)
GT2b	S282T With R498K	99X	**S282T (10)** S282T+R498K (12.3) R498K (1.3)
GT3a	S282T With none	21X	**S282T (4.1)**
GT4a	S282T With V67A, E237G, R304K, A324V, K531R, K544N, C575G	40X	**S282T (6.0)** A324V (0.9)
GT5a	S282T With none	60X	**S282T (11)**
GT6a	S282T With N237S, E375D, T580I	33X	**S282T (8.8)** **N237S (2.4)** D375E (1.0) T580I (0.6)

Biochemical Analysis of Recombinant NS5B Encoding the S282T Substitution

The effect of S282T on the activity of the active triphosphate form of SOF, GS-461203, was assessed in a biochemical assay (Table 21) (Report PC-334-2010). IC_{50} values derived from recombinant NS5B polymerase from genotypes 1b, 2a, 3a, and 4a encoding the S282T substitution were compared to respective wild-type controls. The presence of the S282T substitution resulted in significant changes in GS-461203 activity with fold changes in IC_{50} values ranging from 8.5- to 24-fold.

Table 21. Effect of the S282T on the Inhibition of Recombinant NS5B Enzymatic Activity by GS-461203, the Active Metabolite of Sofosbuvir

Active Triphosphate	Wild-Type IC_{50} (μM)[a]				S282T Fold Change in IC_{50}[b]			
	Genotype 1b	Genotype 2a	Genotype 3a	Genotype 4a	Genotype 1b	Genotype 2a	Genotype 3a	Genotype 4a
GS-461203	3.3	0.36	1.4	2.7	24	22.7	14.7	8.5

a ATP, UTP, and CTP concentrations were fixed at 2.5 μM. ^{33}P-labled GTP at a concentration of 1.25 μM was used to track RNA synthesis. IC_{50} values were reported as an average of at least 3 independent experiments.

b Fold change was calculated as the ratio of mutant IC_{50} to the wild-type IC_{50}. Data represent the average of 2 to 3 independent measurements.

Cross-Resistance

Amino acid variants selected with SOF in the stable replicon passages of GT2a, 2b, 3a, 4a, 5a or 6a were tested to see if they conferred resistance to the NS5A inhibitor GS-5816 and ribavirin (RBV). Passages of selected replicon cells with <1% S282T detected and 98-99% S282T detected were tested (Table 22; Report PC-334-2010). Susceptibility to GS-5816 and RBV did not change for replicons with the S282T substitution. These data indicate that the NS5B S282T substitution does not confer cross-resistance to the NS5A inhibitor GS-5816 or RBV.

Table 22. Cross-Resistance Studies of GS-5816 and RBV against SOF-Selected GT2a, 3a and 4a Replicon Cells and Chimeric GT2b, 5a and 6a NS5B in GT1b Con-1 Replicon Cells

Replicons	Days of selection	S282T (%)	EC_{50} Fold Change[a]	
			GS-5816	RBV
GT 2a	6	<1	0.49	0.86
	79	98.50	0.38	0.23
GT 3a	6	<1	0.38	0.69
	94	99.04	1.12	0.46
GT 4a	6	<1	0.66	0.80
	108	99.59	0.99	0.4
GT 2b	25	18.3	1.3	1.1
	81	99.8	2.3	0.6
GT 5a	25	<1	1.3	1.0
	70	98.1	1.9	0.6
GT 6a	25	<1	0.8	0.9
	89	99.1	1.2	0.8

a EC_{50} fold change was determined by normalizing the EC_{50} value of the SOF-selected cells with that of the no drug control cells

Antiviral Activity of GS-7977 against NS5A and NS5B Mutant Replicons

The NS5B S282T subsitution was introduced into the full-length GT1a, 1b, and 2a replicons and chimeric replicons containing a 2b, 3a, 4a, or 5a NS5B sequence cloned into a GT1b backbone. The susceptibility to GS-7977, the NS5A inhibitor GS-5885, and ribavirin (RBV) was evaluated using a transient replication assay (Table 23; Report PC-334-2006). The S282T substitution displayed reduced susceptibility to GS-7977 across all seven genotypes with EC_{50} values ranging from 117 nM to 368 nM and fold increase in EC_{50} ranging from 2.4 to 18 compared to the wild-type. For the wild-type replicon, the EC_{50} values for RBV were also similar across the 7 genotypes tested with the lowest EC_{50} value being against GT5a. The EC_{50} values for S282T replicons were 3- to 10-fold more sensitive to treatment with RBV than the corresponding wild-type for all seven genotypes. No significant differences were observed in EC_{50} values for GS-5885 between the wild-type and S282T replicons indicating that this substitution does not alter susceptibility to GS-5885.

Table 23. Antiviral Activity of GS-7977 and RBV against Wild-type and S282T Mutant GT1b, 2a, 2b, 3a, 4a, and 5a Replicons

Replicon	GS-7977			RBV			GS-5885		
	EC_{50} nM[a]		Fold	EC_{50} µM[a]		Fold	EC_{50} pM[a]		Fold
	WT	S282T	change[b]	WT	S282T	change[b]	WT	S282T	change[b]
GT1a	30.2	253.5	8.4	26.1	3.8	0.1	12.2	7.6	0.6
GT1b	21.5	189.2	8.8	6.6	1.6	0.2	0.5	0.4	0.8
GT2a	146.8	346.1	2.4	8.3	0.6	0.1	5165	2336	0.5
GT2b	13.3	215.6	16.2	2.6	0.6	0.2	0.5	0.5	0.9
GT3a	33.9	117.1	3.5	6.7	1.0	0.2	0.4	0.2	0.6
GT4a	35.8	217.5	6.1	6.2	0.6	0.1	0.5	0.4	0.8
GT5a[c]	20.3	367.8	18.1	1.8	0.6	0.3	0.3	0.3	1.0

a EC_{50} indicates average for two or more independent experiments.
b Fold change from the corresponding wild type
c For GT5a n=1

Activity of GS-7977 against Other NS5B Mutants

GS-7977 was tested against a panel of genotype 1a and 1b NS5B mutant replicons to examine if GS-7977 demonstrated cross-resistance to mutants selected by other nucleoside and nonnucleoside inhibitors (Table 24 and 25; Report PC-334-2006 and PC-334-2017). GS-7977 remained fully active against all NS5B mutant replicons tested indicating that these mutants are not cross-resistant to GS-7977**.**

Table 24. Activity of GS-7977, GS-9669, VX-222, and Ribavirin against Genotype 1a Replicons Expressing NS5B Non-nucleoside Inhibitor Substitutions

	Fold Change in EC_{50} vs. Wild-type[a,b,c]			
Compound	SOF	GS-9669	VX-222	Ribavirin
WT EC_{50}[a]	142.3 nM	10.1 nM	16.3 nM	36,000 nM
L419M	0.9	87.3	110.5	0.9
L419S	0.5	197.1	134.6	0.2
R422K	0.7	144.7	97.8	0.3
M423I	0.9	10.6	10.5	0.8
M423T	0.8	15.9	28.3	0.6
M423V	0.8	8.5	17.9	0.7
I482L	0.9	26.1	34.3	0.7
A486V	0.9	39.6	48.5	0.7
V494A	0.6	17.4	27.2	0.5
P495L	0.9	1.7	N.T.	1.1

a Values represent the mean of two or more independent experiments and were generated in 96-well assays
b Fold resistance is calculated as the ratio of mutant EC_{50} to wild-type EC_{50}.
c N.T. indicates "not tested".

Table 25. Activity of GS-7977, GS-9669, VX-222, and Ribavirin against Genotype 1b Replicons Expressing Non-nucleoside Inhibitor Substitutions

	Fold Change in EC_{50} vs. Wild-type[a,b,c]			
Compound	SOF	GS-9669	VX-222	Ribavirin
WT EC_{50}[a]	132.5 nM	3.4 nM	7.5 nM	15,500 nM
C316Y	1.2	0.9	N.T.	0.9
M414T	1.0	1.0	N.T.	1.1
L419M	0.9	123.4	128.2	0.9
L419S	0.9	789.8	209.1	0.6
R422K	0.8	814.6	>665	0.9
M423I	0.8	4.6	5.6	0.7
M423T	1.0	19.3	49.8	0.7
M423V	0.8	7.0	14.3	1.0
Y448H	0.8	0.6	N.T.	0.7
I482L	1.0	51.4	101.7	1.1
A486I	0.8	48.7	102.6	0.8
A486T	0.8	31.1	55.9	0.9
A486V	0.8	49.8	77.8	0.9
V494A	1.0	18.1.	32.6	1.1
P495A	1.1	0.9	N.T.	0.9
P495L	0.9	1.7	N.T.	1.1

a Values represent the mean of two or more independent experiments and were generated in 96-well assays
b Fold resistance is calculated as the ratio of mutant EC_{50} to wild-type EC_{50}.
c N.T. indicates "not tested".

Cross-Resistance with NS3/4A Protease Inhibitors

SOF was tested against a panel of genotype 1a and 1b replicons encoding individual NS3 protease inhibitor resistance-associated substitutions. No reduction in susceptibility to SOF was observed for any of the mutants tested (Tables 26 and 27; Report PC-334-2017). These data indicate that SOF does not display cross-resistance to NS3 PI resistance associated substitutions.

Table 26. Antiviral Activity of SOF and NS3 Protease Inhibitors against Genotype 1a Replicons Expressing NS3/4A Protease Inhibitor Resistance-Associated Substitutions

	Fold Change in EC_{50} vs. Wild-type[a,b,c]					
Compound	**SOF**	**GS-9451**	**Boceprevir**	**Telaprevir**	**TMC-435450**	**MK-5172**
WT EC_{50}[a]	**142.3 nM**	**23.1 nM**	**906.4 nM**	**942.6 nM**	**18.8 nM**	**1.3 nM**
V36M	1.0	0.4	N.T.	N.T.	N.T.	N.T.
R155K	2.5	>150	2.7	9.5	30.0	3.6.
R155T	1.4	2.8	>27	>55	17.0	11.5
R155W	0.8	26.0	1.1	1.2	23.0	34.6
D168A	1.9	425.0	1.4	0.9	>50	87.5
D168E	2.6	25.1	0.4	0.5	36.1	8.2
D168G	0.8	115.0	0.7	0.9	8.1	32.6
D168H	2.2	394.0	0.4	0.8	>50	80.0
D168N	1.1	20.5	1.1	0.9	>43	6.6
D168V	2.2	407.0	1.5	1.0	>59	92.6
D168Y	1.7	295.0	1.7	0.9	>50	108.0

a Values represent the mean of two or more independent experiments and were generated in 96-well assays
b Fold resistance is calculated as the ratio of mutant EC_{50} to wild-type EC_{50}.
c N.T. indicates "not tested".

Table 27. Antiviral Activity of SOF and NS3 Protease Inhibitors against Genotype 1b Replicons Expressing NS3/4A Protease Inhibitor Resistance-Associated Substitutions

	Fold Change in EC_{50} vs. Wild-type[a,b,c]					
Compound	SOF	GS-9451	Boceprevir	Telaprevir	TMC-435	MK-5172
WT EC_{50}[a]	132.5 nM	9.2 nM	264.1 nM	414.3 nM	16.6 nM	0.4 nM
V36A	1.5	1.1	N.T.	4.3	2.9	N.T.
V36M	1.0	1.1	2.6	10.1	2.8	0.8
Q41R	1.7	4.4	N.T.	2.5	N.T.	N.T.
F43S	1.3	0.5	N.T.	2.8	N.T.	N.T.
T54A	0.8	0.4	4.6	9.1	2.5	1.1
T54S	N.T.	1.0	6.9	12.6	1.9	1.2
R155C	0.7	0.2	4.5	7.2	0.8	1.2
R155K	1.4	989.0	5.9	16.1	18.8	3.1
R155Q	0.3	17.1	1.2	2.1	1.2	1.4
R155W	0.8	408.4	1.3	2.0	33.7	15.3
A156D	2.6	>519	3.1	>13.0	14.6	15.0
A156G	1.7	25.0	2.3	0.9	21.6	2.5
A156S	1.0	1.9	>11.8	N.T.	0.5	N.T.
A156T	1.2	>685	>68	>542	31.5	>150
A156V	1.0	951.0	54.0	24.4	112.4	>150
D168A	1.8	>679	1.0	0.6	>249	32.4
D168E	1.7	147.9	0.9	1.3	54.2	7.0
D168G	1.4	85.3	1.0	0.9	8.4	10.5
D168H	2.0	>1000	0.9	2.1	191.0	15.1
D168N	1.6.	28.4	1.3	1.2	13.0	3.4
D168V	0.9	>1000	0.7	0.5	>296	10.8
D168Y	0.4	>960	0.8	0.7	>140	10.4

a Values represent the mean of two or more independent experiments and were generated in 96-well assays
b Fold resistance is calculated as the ratio of mutant EC_{50} to wild-type EC_{50}.
c N.T. indicates "not tested".

Activity of GS-7977 and GS-5885 against NS5A Mutants

To determine if NS5A drug resistance substitutions are cross-resistant to GS-7977, a panel of NS5A mutant replicons was assayed for susceptibility to both GS-5885 and GS-7977. All eleven NS5A mutants displayed reduced susceptibility to GS-5885 with increases in EC_{50} values ranging from 10- to 3029-fold. In contrast, no significant shift in the EC_{50} values were observed for GS-7977 and RBV against the NS5A mutants (Table 28; Report PC-334-2006).

Table 28. Activity of GS-7977 or GS-5885 against Genotype 1a Replicons Expressing NS5A Resistance-Associated Substitution

	Fold Shift in EC_{50} (NS5A Mutant EC_{50} / 1a-H77 EC_{50})[a]										
Compound	K24E	K24N	K24R	L31M	M28T	Q30H	Q30R	Q30E	Y93C[b]	Y93H	Y93N
GS-5885	20	74	10	140	25	73	170	997	2531	3029	>984
GS-7977	0.6	0.6	0.9	1.1	0.9	1.0	0.8	1.0	0.5	0.7	0.7
RBV	0.7	0.8	1.4	0.5	0.4	0.7	0.8	0.8	0.7	1.0	1.1

a Fold change from the corresponding wild type calculated from two independent experiments
b Data from n = 1 experiments

These results indicate that GS-5885 NS5A resistance substitutions are not cross-resistance with GS-7977.

Activity of GS-7977 in Genotype 1a Replicons Containing the NS5B F415Y, T390I, L159F or L320F Substitutions: Analysis of Cross-Resistance with RBV and Other Nuscleoside Analog Polymerase Inhibitors

The NS5B substitutions F415Y and T390I were previously identified as being enriched in HCV patients experiencing virologic failure following treatment with ribavirin-containing treatment regimens (Young et al., 2003; Ward et al., 2008; Bartels et al., 2011). In addition, a recent report indicates that the NS5B double substitution L159F+L320F confers low-level resistance to HCV nucleotide inhibitors (Tong X et al., 2012).

SOF and RBV were tested against GT1a replicons encoding NS5B T390I or F415Y substitutions as well as a wild-type 1a replicon control (Table 29; Report PC-334-2020). F415Y and T390I mutant replicons replicated well as ascertained by replication capacity measurements. SOF and RBV maintained activity against these mutants with less than 2-fold shifts in EC_{50} values (less than assay cut off) indicating that these substitutions do not confer significantly reduced susceptibility to SOF and RBV.

SOF was also tested against mutant GT1a and 1b replicons encoding L159F, L320F, or L159F+L320F substitutions and corresponding wild-type GT1a and GT1b replicons. These mutants showed less than 2.5-fold susceptibility shifts in EC_{50} values for SOF (Table 29). In addition, L159F, L320F, and L159F+L320F mutant replicons demonstrated significantly poorer replicative capacities than wild-type.

(b) (4)

Table 29. Activity of SOF and RBV against Replicons Encoding NS5B T390I, F415Y, L159F, L320F, or L159F+L320F Substitutions

GT	Mutation	Replication Capacity (% relative to Wild-type)	Fold-Change EC_{50} vs. Wild-type	
			SOF	RBV
1a	Wild-type (H77)	100		
	T390I	192±11	0.89	1.17
	F415Y	102±2	1.04	1.22
	L159F	10.1±1.27	1.2	nd
	L320F	16.2±1.28	1.8	nd
	L159F+L320F	0.5±0.08	2.3	nd
1b	Wild-type (Con-1)	100		
	L159F	20.9±2.2	1.3	nd
	L320F	22.9±3.3	1.7	nd
	L159F+L320F	3.9±0.77	2.2	nd

"nd" denotes not determined

4.6 Clinical Studies

PHASE III STUDIES

Study P7977-1231 (FISSION) is a Phase 3 randomized active-controlled study in treatment-naïve subjects with chronic GT2 or GT3 HCV receiving SOF+RBV for 12 weeks compared to PEG/RBV for 24 weeks.

Study GS-US-334-0107 (POSITRON) is a Phase 3 randomized double blind placebo-controlled study of GS-7977+RBV for 12 weeks in subjects with chronic GT2 or GT3 HCV infection who are IFN-intolerant, IFN-ineligible or unwilling to take IFN.

Study GS-US-334-0108 (FUSION) is a Phase 3 randomized double-blind study of GS-7977+RBV for 12 or 16 weeks in treatment-experienced subjects with chronic GT2 or GT3 HCV infection.

Study GS-US-334-0110 (NEUTRINO) is a Phase 3 open-label study of GS-7977+PEG +RBV for 12 weeks in treatment-naïve subjects with chronic GT1, 4, 5, or 6 HCV infection.

For all these studies, the primary efficacy endpoint was SVR12, defined as HCV RNA <LLOQ 12 weeks after discontinuation of treatment. The proportion of subjects with genotype 2 or 3 HCV infection who achieved an SVR12 following 12 weeks SOF+RBV treatment in Studies P7977-1231, GS-US-334-0107, and GS-US-334-0108 was 67%, 78% and 50%, respectively (Table 30). The proportion of subjects with genotype 2 or 3 HCV infection who achieved an SVR12 following 16 weeks SOF+RBV in GS-US-334-0108 was 73%.

In Study GS-US-334-0110**,** the proportion of subjects with GT1, 4, 5, or 6 that achieved SVR12 after treatment with SOF+PEG+RBV for 12 weeks was 90% (295/327).

Table 30. P7977-1231, GS-US-334-0107, and GS-US-334-0108: SVR12 in Subjects with Genotype 2 or 3 HCV Infection (Full Analysis Set)

	P7977-1231 (FISSION)		GS-US-334-0107 (POSITRON)	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
SVR12	170/253 (67.2%)	162/243 (66.7%)	161/207 (77.8%)	50/100 (50.0%)	69/95 (72.6%)
95% CI	61.0% to 72.9%	60.4% to 72.6%	71.5% to 83.2%	39.8% to 60.2%	62.5% to 81.3%

In subjects with GT2 HCV infection receiving 12 or 16 weeks SOF+RBV, the proportion who had SVR12 in Studies P7977-1231, GS-US-334-0107, and GS-US-334-0108 ranged from 86% to 97% (Table 31).

Table 31. P7977-1231, GS-US-334-0107, and GS-US-33-0108: SVR12 in Subjects with Genotype 2 HCV Infection (Full Analysis Set)

	P7977-1231 (FISSION)		GS-US-334-0107 (POSITRON)	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 = 70	N = 67	N = 109	N = 36	N = 32
SVR12	68/70 (97.1%)	52/67 (77.6%)	101/109 (92.7%)	31/36 (86.1%)	30/32 (93.8%)
95% CI	90.1% to 99.7%	65.8% to 86.9%	86.0% to 96.8%	70.5% to 95.3%	79.2% to 99.2%

However, the proportion of subjects with GT3 HCV infection who achieved SVR12 was lower ranging from 30% to 63% (Table 32). In Study GS-US-334-0108, SVR12 was achieved in 19 subjects (30%) with GT3 HCV infection in the SOF+RBV 12 Week group compared with 39 subjects (62%) in the SOF+RBV 16 Week group. These data support a longer duration of 16 weeks for GS-7977+RBV in the treatment of GT3 HCV infection.

Table 32. P7977-1231, GS-US-334-0107, and GS-US-334-0108: SVR12 in Subjects with Genotype 3 HCV Infection (Full Analysis Set)

	P7977-1231 (FISSION)		GS-US-334-0107 (POSITRON)	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 = 183	N = 176	N = 98	N = 64	N = 63
SVR12	102/183 (55.7%)	110/176 (62.5%)	60/98 (61.2%)	19/64 (29.7%)	39/63 (61.9%)
95% CI	48.2% to 63.1%	54.9% to 69.7%	50.8% to 70.9%	18.9% to 42.4%	48.8% to 73.9%

The most common reason for virologic failure in the SOF+RBV groups was virologic relapse, with most of the relapses occurring at the posttreatment Week 4 visit. One subject in Study P7977-1231 (1224-310357) in the SOF+RBV group had on-treatment virologic failure at Week 8. This subject had HCV GT3 infection with a baseline HCV RNA of 7.00 $\log_{10}$ IU/mL. At the Week 1 and 4 visits, the subject exhibited plasma concentrations of SOF and GS-331007, the predominant metabolite of SOF, as

expected based on recorded dosing information (Week 1 and 4 GS-331007 plasma concentrations: 257.3 ng/mL and 305.7 ng/mL, respectively, from samples drawn 26 to 29 hours postdose). However, at the Week 8 and EOT visits, GS-331007 plasma concentrations were below the quantification limit (i.e., < 10 ng/mL), which is indicative of non-compliance with study drug at or around these study visits given the established GS-331007 half-life (i.e., median apparent half-life of approximately 12 to 18 h).

4.7 Clinical Virology

PHASE III STUDIES

SVR12 by Genotype/Subtype

The proportion of subjects with SVR12 by genotype and subtype was analyzed. For subjects with GT2 infection, subtype 2b was the most common subtype in 79% of subjects, 19.5% of subjects were infected with GT2a, and only 6 subjects were infected with 2c, 2i, 2j, or 2r as determined by NS5B nucleotide sequence analysis (Table 33). For subjects with GT3 infection, 99% of subjects had subtype 3a by NS5B sequencing and only 6 subjects had other subtypes (Table 33). In the studies 1231, 107 and 108, 92% and 59% of subjects infected with GT2 and GT3, respectively, were from the U.S (Table 34).

Table 33. Proportion of Subjects* Infected with GT2, GT3 and GT1 Subtypes by NS5B Sequencing

	Study 107	Study 108	P7977-1231	Study 110	Total
GT2a	28	12	28		68/348 (19.5%)
GT2b	110	55	109		274 (79%)
GT2c	2				2 (0.6%)
GT2i		1			1 (0.3%)
GT2j	2				2 (0.6%)
GT2r	1				1 (0.3%)
GT3a	133	125	357		615/621 (99%)
GT3b	1	1	1		3
GT3g			1		1
GT3i	1	1			2
GT1a			5	225	230 (77%)
GT1b			4	66	70 (23%)
GT4					28
GT5					1
GT6					6

FDA Analysis*

Table 34. Proportion of GT2- and GT3-Infected Subjects from the U.S.

	GT2	GT3
Study 1231	98%	50%
Study 107	89%	75%
Study 108	88%	67%
Total	92%	59%

For subjects with GT2 infection, there were no obvious differences in response rates to SOF+RBV across subtypes 2b and 2a determined by the screening assay. Although subgroups were too small to make conclusions, SVR12 rates were generally high across all subtypes of GT2 (Table 35). Given that 99% of subjects infected with GT3 had subtype 3a by NS5B sequencing and only 6 subjects were infected other subtypes, no conclusions could be made about the response rates for other subtypes of GT3 (Table 35).

Table 35. Response by Genotype 2 and 3 Subtype (screening subtype assay)

	Study 107		**Study 108**		**P7977-1231**	
	SOF+RBV	**Placebo**	**SOF RBV 12 Weeks**	**SOF RBV 16 Weeks**	**SOF+RBV**	**P/R**
GT2b	94% (76/81)	0/23	89% (25/28)	92% (22/24)	96% (51/53)	78% (40/51)
GT2a					1/1	
GT2a/2c	87% (13/15)	0/5	67% (2/3)	100% (5/5)	100% (9/9)	70% (7/10)
GT2	92% (11/12)	0/6	80% (4/5)	100% (3/3)	100% (8/8)	80% (4/5)
GT3a	60% (58/96)	0/35	28% (17/60)	61% (35/57)	56% (100/180)	63% (104/164)
GT3b			0/1		1/1	1/1
GT3	2/2	0/2	67% (2/3)	67% (4/6)	50% (1/2)	45% (5/11)

In Study 110, 77% of the subjects were infected with GT1a and 23% were infected with GT1b by NS5B nucleotide sequence analysis (Table 33). There were only 28 subjects with GT4 HCV infection most of which were GT4a, 6 with GT6 (a, e, j or q) and 1 with GT5a. All subjects infected with genotype 1, 1b, 4, 5, and 6 were from the U.S., because Study 110 was conducted only in the U.S. SVR12 rates were 10% higher for subjects with GT1a infection compared to GT1b infection (92% vs. 82%, respectively) (Table 36). Response rates of subjects infected with GT4a were 96% and the one subject each with 4d and 4k also achieved SVR12. The one subject with GT5a infection and the 6 subjects with GT6 infection (3 with GT6a, and 1 each with GTe, j, q) all achieved SVR12.

Table 36. Response for Subjects Infected with HCV Genotypes 1, 4, 5 and 6

	Study 110 (SOF+P/R)
GT1a	92% (207/226)
GT1b	82% (55/67)
Screening GT4	93% (14/15)
Screening GT4a/4c/4d	100% (13/13)
Analysis 4a	96% (25/26)
Analysis 4d	1/1
Analysis 4k	1/1
Screening GT5a	1/1
Analysis GT5a	1/1

Screening GT6a/6b	3/3
Screening GT6c-l	3/3
Analysis 6a	3/3
Analysis 6e	1/1
Analysis 6j	1/1
Analysis 6q	1/1

IL28B genotypes:
GT4: (CC, 14%) (CT, 75%) (TT, 11%)
GT5: (CT)
GT6: (CC, 83%) (CT, 17%)

The VERSANT HCV genotype assay, Version LiPA 2.0 was used for screening genotype/subtype for entry into the Phase 3 studies. Subsequently, nucleotide sequence and phylogenetic analysis of the HCV NS5B region was used to confirm subtype. In some circumstances, NS5B sequencing would indicate a different genotype and/or subtype for a subject. The sponsor used genotype determined by sequencing for analyses. Overall, concordance between the LiPa screening assay and NS5B sequencing was ≥94% (Table 37). However, some subjects were found to have been misclassified by the screening assay when the NS5B sequencing analysis was performed. Most misclassifications were of the same genotype but different subtype (Table 38). As stated above, there were no differences in efficacy rates that were determined or could be determined by subtype.

Table 37. Concurrence between Genotype Screening and Analysis

Study	GT3a	GT2b	GT1a	GT1b
107	98% (131/133)	95% (104/110)		
108	94% (117/125)	95% (52/55)		
1231	96% (344/357)	95% (104/109)		
110			98% (225/230)	94% (66/70)

Table 38. Comparison of Screening (InnoLIPA) and Analysis (NS5B nucleotide sequencing) Assays for Determination of Genotype 2 and 3 Subtypes

	Study 107		Study 108		P7977-1231	
Genotype	**Screening**	**Analysis**	**Screening**	**Analysis**	**Screening**	**Analysis**
3	4		9		13	
3a	131	133	117	125	344	357
3b		1 (placebo; failure)	1	1 (12 wk; failure)	2	1 (P/R; success)
3i		1 (success)		1 (12 wk; success)		
3g						1 (P/R; success)
2	18		8		13	
2a		28		12	1	28
2a/2c	20		8		19	

2b	104	110	52	55	104	109
2c		2 (SOF/RBV; successes)				
2i				1 (SOF/RBV success)		
2j		2 (SOF/RBV; 1/2 success)				
2r		1 (SOF/RBV; success)				

Nine subjects were misclassified as GT2 infection by the screening assay when they were later determined to be GT1 infection by the NS5B sequencing assay (Table 39). Of these 9 subjects, 3 (33%) achieved SVR12 and all 3 were IL28B CC genotype. The sponsor classified these subjects as GT1 infection for efficacy and virology analyses. However for ITT analyses, this reviewer believes these subjects should be classified as GT2 as determined by the screening assay. In the "real world" clinical settings, NS5B sequencing will not be performed, so physicians would not be able to get this more accurate genotyping/subtyping. Genotyping/subtyping will be determined in the clinic by assays similar to the screening assay. Therefore, the efficacy should reflect what can be expected in the clinic with a screening assay.

Table 39. Comparison of Screening and Analysis Genotype Assays for GT1 Subtypes

	Study 110			
n	**Screening**	**Analysis**	**SVR12**	**Treatment**
2	1	1	2/2	SOF+P/R
225	1a	1a	92% (206/225)	SOF+P/R
66	1b	1b	82% (54/66)	SOF+P/R
	Study 108 and P7977-1231			
n	**Screening**	**Analysis**	**SVR12**	**Treatment**
1	2	1a	1/1	SOF+RBV 12 Weeks
4	2b	1a	1/4 (one success on 16 WK)	2 SOF+RBV (12 WK) 2 SOF+RBV (16 WK)
1	2a/2c	1b	0/1	SOF+RBV 12 WK
3	2b	1b	1/3 (one success on 12 WK)	2 SOF+RBV (12 WK) 1 SOF+RBV (16 WK)

Response by Baseline Factors

Response rates were examined for each genotype by various baseline factors including prior response rates, IL28B genotype, and cirrhosis status.

Genotype 3

For treatment-naïve subjects with GT3 HCV infection, SVR12 rates were 56 - 61% following 12 weeks of SOF+RBV treatment (Table 40). However, SVR12 rates were much lower for subjects with prior P/R experience following 12 weeks of SOF+RBV. Response rates after 12 weeks SOF+RBV were 31% for subjects with prior P/R breakthrough or relapse and 27% for subjects who were prior P/R null or partial responders (Table 40). A longer duration of 16 weeks SOF+RBV improved SVR12 rates for all prior P/R failures by approximately 30%. Response rates after 16 weeks SOF+RBV were 65% for subjects with prior P/R breakthrough or relapse and 53% for subjects who were prior P/R null or partial responders (Table 40). SVR12 rates were lower for prior nulls and partial responders compared to prior P/R breakthroughs and relapsers.

For treatment-naïve subjects with GT3 HCV infection in Studies 107 and 1231, SVR12 rates were lower for IL28B genotypes TT (33-42%) compared to CC and CT (57-65%) (Table 40). In treatment-experienced subjects in Study 108, response rates were about 30% better with the longer 16-week duration for IL28B genotype CC and CT compared to the 12-week duration. The unexpectedly high SVR12 rate of 63% for IL28B TT subjects following 12 week duration of SOF+RBV likely reflects the small numbers in this subset.

For both treatment-naïve and treatment-experienced cirrhotic subjects with GT3 infection, SVR12 rates were 20-40% lower than non-cirrhotics following 12 weeks of SOF+RBV treatment (Table 40). The 16-week duration of SOF+RBV improved SVR12 rates for cirrhotic treatment-experienced subjects to 61% similar to 63% for non-cirrhotics. The 16-week duration improved SVR12 rates for cirrhotics 3 times better than the 12-week duration (61% vs. 19%). The data from all these baseline factor subgroups support a longer 16-week duration of SOF+RBV for GT3 HCV infection.

Table 40. Response by Baseline Factors: Percentage of Subjects with GT3 Infection Achieving SVR12

	Study 107		**Study 108**		**P7977-1231**	
	SOF+ RBV	**Placebo**	**SOF RBV 12 Weeks**	**SOF RBV 16 Weeks**	**SOF+RBV**	**P/R**
GT3 (n=621)	61% (60/98)	0% (0/37)	30% (19/64)	62% (39/63)	56% (102/183)	63% (110/176)
Prior P/R			30% (19/64)	62% (39/63)		
P/R Breakthrough or Relapser			31% (15/49)	65% (30/46)		
P/R Null or			27%	53%		

Partial Responder			(4/15)	(9/17)		
Naive	61% (60/98)	0% (0/37)			56% (102/183)	63% (110/176)
IL28B CC	65% (34/52)	0/12	39% (9/23)	63% (10/16)	57% (43/75)	75% (54/72)
IL28B CT	62% (21/34)	0/22	15% (5/33)	65% (24/37)	58% (52/89)	61% (46/76)
IL28B TT	42% (5/12)	0/3	63% (5/8)	50% (5/10)	33% (9/27)	41% (7/17)
IL28B CT/TT	57% (26/46)	0/25	24% (10/41)	62% (29/47)	53% (61/116)	57% (53/93)
Cirrhosis Yes	21% (3/14)	0/10	19% (5/26)	61% (14/23)	34% (13/38)	30% (11/37)
No	68% (57/84)	0/27	38% (14/37)	63% (25/40)	61% (89/145)	71% (99/139)

Genotype 2

For treatment-naïve subjects with GT2 HCV infection, SVR12 rates were 93-95% following 12 weeks of SOF+RBV treatment (Table 41). SVR12 rates were also high for subjects with prior P/R breakthrough or relapse following both 12 and 16 weeks of SOF+RBV (86% for 12 weeks; 89% for 16 weeks). Response rates after 12 weeks of SOF+RBV for subjects who were prior P/R null or partial responders were 70% (Table 41). A longer duration of 16 weeks of SOF+RBV improved SVR12 rates for prior P/R nulls and partial responders to 88%.

SVR12 rates were 71-100% for both treatment-naïve and P/R-experienced subjects with GT2 HCV infection regardless of IL28B genotype (Table 41). Subgroups were too small to make definitive conclusions regarding differences in response by IL28B genotype. However, in treatment-experienced GT2-infected subjects in Study 108, response rates were in general better with the longer 16-week duration for IL28B genotype subgroups compared to the 12-week duration although again subgroups were small.

For treatment-naïve cirrhotic GT2 infected subjects, SVR12 rates were >90% following 12 weeks of SOF+RBV treatment (Table 41). For treatment-experienced cirrhotic GT2 infected subjects, SVR12 rates were lower (60%) following 12 weeks of SOF+RBV treatment. The 16-week duration of SOF+RBV improved SVR12 rates for cirrhotic treatment-experienced subjects to 78%.

Table 41. Response by Baseline Factors: Percentage of Subjects Infected with GT2 Achieving SVR12

	Study 107		Study 108*		P7977-1231*	
	SOF+RBV	Placebo	SOF RBV 12 Weeks	SOF RBV 16 Weeks	SOF+RBV	P/R
GT2 (n=357)	93% (101/109)	0/34	82% (32/39)	89% (31/35)	95% (69/73)	78% (52/67)
Prior P/R			82% (32/39)	94% (30/32)		
P/R Breaktrhough or Relapser			86% (25/29)	89% (24/27)		
P/R Null or Partial Responder			70% (7/10)	88% (7/8)		
Naive	93% (101/109)	0/34			95% (69/73)	78% (52/67)
IL28B CC	89% (40/45)	0/17	88% (7/8)	71% (10/14)	97% (32/33)	82% (28/34)
IL28B CT	98% (49/50)	0/14	75% (15/20)	100% (19/19)	94% (30/32)	77% (17/22)
IL28B TT	86% (12/14)	0/3	91% (10/11)	100% (2/2)	88% (7/8)	64% (7/11)
IL28B CT/TT	95% (61/64)	0/17	86% (25/29)	100% (21/21)	93% (37/40)	73% (24/33)
Cirrhosis Yes	94% (16/17)	0/3	60% (6/10)	78% (7/9)	83% (10/12)	62% (8/13)
No	92% (85/92)	0/31	90% (26/29)	92% (24/26)	97% (59/61)	81% (44/54)

***Analysis includes 9 subjects that screened GT2 but were determined GT1a or 1b by NS5B sequencing.**

Genotype 1

In Study 110, where all GT1-infected subjects were treatment-naïve, SVR12 rates were high. Interestingly, response rates were higher for GT1a-infected subjects compared to GT1b-infected subjects (92% vs. 82%, respectively) (Table 42). For both GT1a and 1b, response rates were higher for subjects with IL28B CC than CT/TT. However, different proportions of IL28B genotypes in GT1a and GT1b subsets do not appear to explain the decrease in GT1b response rates compared to GT1a response rates even though the proportion of IL28B CC subjects was higher in the GT1a subset compared to the GT1b subset. The SVR12 rates for subjects with IL28B TT genotype were higher in GT1a subjects compared to GT1b subjects (90% vs. 76%, respectively). Likewise, SVR12 rates for subjects with the CC genotype were also higher for GT1a compared to GT1b (99% vs. 93%, respectively). Subjects with cirrhosis had lower response rates for both GT1a and GT1b compared to non-cirrhotics; however, SVR12 rates were again higher in GT1a than GT1b (84% vs. 67%, respectively) although the subset of GT1b was very small. The median baseline viral load was higher for GT1b-infected subjects, 4,600,000

IU/mL compared to 3,700,000 IU/mL for GT1a-infected subjects. It is unclear if this would be an explanation for differences in response rates for these subtypes.

Table 42. Response by Baseline Factors: Percentage of Subjects Infected with GT1a and GT1b Achieving SVR12

	Study 110 (SOF+P/R)	
GT1a* (n=226)	**SVR12**	
Naive	92% (207/226)	Proportion
IL28B CC	99% (72/73)	32%
IL28B CT	88% (107/122)	54%
IL28B TT	90% (28/31)	14%
IL28B CT/TT	88% (135/153)	
Cirrhosis Yes	84% (36/43)	19%
No	93% (169/181)	81%
GT1b* (n= 67)		
Naive	82% (55/67)	Proportion
IL28B CC	93% (13/14)	21%
IL28B CT	81% (29/36)	54%
IL28B TT	76% (13/17)	25%
IL28B CT/TT	78% (42/54)	
Cirrhosis Yes	67% (6/9)	14%
No	84% (48/57)	86%

Median baseline viral load: GT1a = 3,700,000 IU/mL; GT1b = 4,600,000 IU/mL
***Analysis excludes 9 subjects that screened GT2 but were determined GT1a or 1b by NS5B sequencing (where analyzed by sponsor as GT1a or 1b).**

The EC_{50} values of SOF are 40 nM and 110 nM for GT1a H77 and GT1b Con-1 stable replicons, respectively. However, in transient replicons, EC_{50} values are 30 nM and 27 nM against GT1a H77 and GT1b Con-1, respectively. Interestingly, data of GT1a vs. GT1b response rates for other nucleotides shows a consistent trend that HCV nucleotide analogs may be slightly more effective for HCV genotype 1a vs. 1b even though there are small numbers. Within the mericitabine (RO5024048) + P/R group [24 weeks; if no RVR then 48 weeks], SVR24 rates were 30/50 (60%) and 12/24 (50%) for HCV genotype 1a and 1b subjects, respectively (Table 43). Within the placebo + P/R group, SVR24 rates were 24/68 (35%) and 7/17 (41%) for HCV genotype 1a and 1b subjects, respectively.

Table 43. SVR24 Rates of Mericitabine + P/R for GT1a and GT1b

	GT1a	GT1b
Mericitabine +P/R	60% (30/50)	50% (12/24)
SOC	35% (24/68)	41% (7/17)

In a monotherapy study [redacted] (b) (4)

(b) (4)

[redacted]

Early Viral Load Decline by Weeks 1, 2, and 4

In an attempt to determine if there were early response predictors of SVR12, SVR12 rates by responses (<25 IU/mL target not detected (TND), <25 IU/mL detected, < and >1,000 IU/mL) at Week 1, Week 2 and Week 4 were analyzed. SVR12 rates were >90% for GT2-infected subjects when Week 1 and 2 viral loads were "<25 IU/mL TND" or "<25 IU/mL detectable" (Table 44). SVR12 rates could be discriminated by "<25 IU/mL TND" and "<25 IU/mL detectable" at Week 4. However, SVR12 rates were still 80% for subjects with viral load <25 IU/mL detectable at Week 4. Notably, subjects with "<25 IU/mL detectable" at Week 4 receiving a longer 16-week duration had an SVR12 rate of 83% which is similar to the SVR rate at 12 weeks. Therefore, it is not clear that changing duration of treatment based on early response rates at a timepoint such as Week 4 would improve SVR12 rates. No subjects had viral load >25 IU/mL at Week 4.

For GT3 infection, SVR12 rates were higher for subjects with viral loads "<25 IU/mL TND" than subjects with viral loads "<25 IU/mL detectable" at both Weeks 1 and 4 (Table 44). The longer 16-week duration improved SVR12 rates for both "<25 IU/mL TND" and "<25 IU/mL detectable" groups. No subjects achieved SVR12 if they had viral loads >25 IU/mL at Week 4 although these subgroups were small.

For GT1a infection, SVR12 rates were >80% regardless of response at Week 1. All GT1a subjects had viral loads <25 IU/mL at Week 4 and SVR12 rates were high - 93% and 89% for subjects with "<25 IU/mL TND" and "<25 IU/mL detectable" at Week 4, respectively (Table 44). For GT1b infection, the subgroups were too small to make conclusions. However, all subjects, except one, had viral loads <25 IU/mL at Week 4 and SVR12 rates were 86% and 75% for subjects with "<25 IU/mL TND" and "<25 IU/mL detectable" at Week 4, respectively (Table 44).

Overall, these data indicate there are no clear recommendations that can be made to increase SVR12 rates based on early responses at Week 1 or 4 for GT1, 2, or 3. However, clearly a longer duration of 16 weeks SOF+RBV increases SVR12 rates for GT3 HCV infection.

Table 44. SVR12 by Week 1, 2, 4 Response

	Week 1		Week 2		Week 4	
	SOF+ RBV	SOF+ RBV Week16	SOF+ RBV	SOF+ RBV Week16	SOF+ RBV	SOF+ RBV Week16
GT2						
<25 IU/mL TND	93% (14/15)	1/1	96% (79/82)	13/13	96% (175/182)	96% (25/26)
<25 IU/mL detected	95% (61/64)	3/3	94% (104/111)		80% (24/30)	83% 5/6
<1,000 IU/mL	94% (122/130)	96% (26/27)	84% (16/19)	75% (3/4)	0	0
>1,000 IU/mL	2/3	0/1	0	0	0	0
GT3						
<25 IU/mL TND	73% (19/26)	60% (3/5)	nd	nd	56% (156/280)	65% (35/54)
<25 IU/mL detected	65% (71/110)	73% (11/15)	nd	nd	43% (23/54)	57% (4/7)
<1,000 IU/mL	46% (85/185)	61% (23/38)	nd	nd	0/4	0/2
>1,000 IU/mL	20% (2/10)	50% (2/4)	nd	nd	0/1	
	Week 1		**Week 2**		**Week 4**	
	SOF+P/R				**SOF+P/R**	
GT1a						
<25 IU/mL TND	100% (16/16)		nd		93% (186/200)	
<25 IU/mL detected	96% (81/84)		nd		89% (17/19)	
<1,000 IU/mL	87% (103/118)		nd		0/1	
>1,000 IU/mL	80% (4/5)		nd		0	
GT1b						
<25 IU/mL TND	75% (3/4)		nd		86% (49/57)	
<25 IU/mL detected	88% (14/16)		nd		75% (6/8)	
<1,000 IU/mL	82% (36/44)		nd		0/1	
>1,000 IU/mL	67% (2/3)		nd		0	

EMERGENT SUBSTITUTIONS

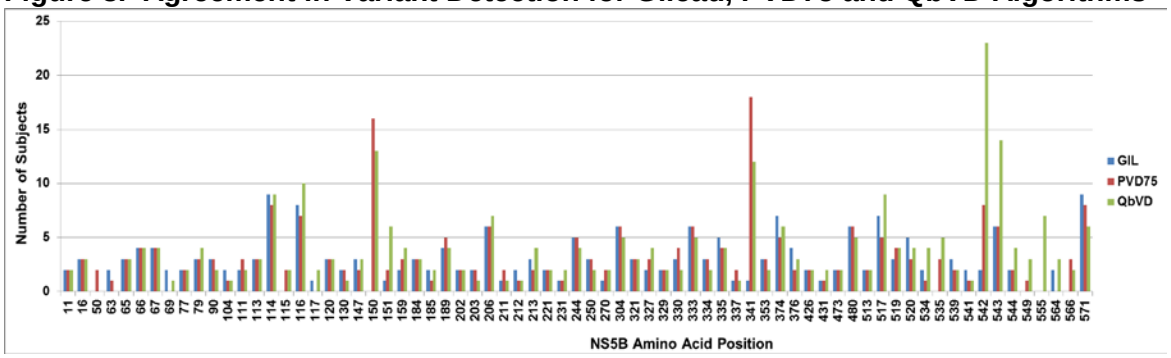
For the analysis of SOF resistant substitution emergence, next generation nucleotide sequencing (NGS) data were submitted for 4 Phase 3 clinical trials. Of the 982 subjects in SOF+RBV or SOF/PEG/RBV groups of the Phase 3 Studies P7977-1231, GS-US-334-0107, GS-US-334-0108 and GSUS-334-110, a total of 224 subjects had NGS data

with 676 files (Table 45). The raw sequence data were submitted in fastq format and resistance analysis tables were populated at a 15% cutoff by Gilead. The number of subjects with NGS data for Studies P7977-1231, 107, 108 and 110 were 78, 41, 76, and 29, respectively (Table 45). The FDA virology analyses of the NGS data was conducted using an internal NGS analysis pipeline that is described in detail in the NDA 204671 SDN 002 review by Clinical Virology Reviewer Eric Donaldson, Ph.D. Briefly, the NGS analysis pipeline mapped the NGS sequence reads for each subject and timepoint to an appropriate reference sequence and used two variant detection methods (i.e., PVD75 and QbVD) to call variants. The variants called by these algorithms were then converted to amino acid frequency tables and resistance analysis tables to facilitate bioinformatics analysis and interpretation of the NGS data. The overall agreement in variant detection between the 2 algorithms the FDA used and the algorithm Gilead used was good (Figure 3). The FDA analysis focused on substitutions identified by at least 2 of the algorithms used (i.e., Gilead, PVD75 and QbVD**)** and identified in 2 or more subjects. The resistance emergence summary table in the FDA analysis used a cutoff of 10% for emergence of substitutions (i.e., substitutions were not present at baseline but were present at a frequency of at least 10% at failure).

Table 45. Next Generation Sequencing Data

Clinical Trial	No. Subjects with NGS data	No. NGS files
P7977-1231 (FISSION)	**78**	**308**
GS-US-334-0107 (POSITRON)	**41**	**115**
GS-US-334-0108 (FUSION)	**76**	**189**
GS-US-334-0110 (NEUTRINO)	**29**	**64**
Totals	**224**	**676**

Figure 3. Agreement in Variant Detection for Gilead, PVD75 and QbVD Algorithms



No S282T substitutions were identified at baseline or at time of relapse in any of the subjects from any of the Phase 3 trials in either the Gilead or FDA analyses. For all NS5B amino acid substitutions observed in >2 subjects, none showed a detectable reduction in susceptibility to SOF or RBV.

Reference ID: 3369468

TREATMENT EMERGENT SUBSTITUTIONS IN SUBJECTS WITH GENOTYPE 3

Study P7977-1231

In Study P7977-1231, there were 78 GT3a virologic failures, 77 of whom had HCV RNA>1,000 IU/mL and were analyzed by NGS. Of these, 75 relapsed by post-treatment Week 4 or 12, one relapsed by post-treatment Week 16 and one experienced viral breakthrough. Gilead identified 29 NS5B substitutions that were observed in >2 subjects using the 15% cutoff. No detectable phenotypic changes to SOF or RBV were observed in subject samples containing these substitutions (chimeric replicons encoding NS5B region derived from subjects' plasma/serum) (Table 46; Report PC-334-2024). Additionally, many of these substitutions were at polymorphic sites.

Table 46. P7977-1231: Phenotypes of Substitutions in NS5B Occurring in Greater than 2 Subjects by Population Sequencing

Amino Acid Change from Baseline Detected	Number of Subjects with Detected Substitution (genotype)	Reference AA	SOF EC_{50} Fold-Change[a]		RBV EC_{50} Fold-Change[a]	
			vs. Reference	vs. Baseline	vs. Reference	vs. Baseline
V11I	4 (3 GT3a; 1 GT1a)	I11 (3a);V11 (1a)	0.92	1.04	0.49	1.13
K65R	3 (GT3a)	K65 (3a)	1.4	1.6	0.92	1.3
I66T	3 (GT3a)	T66 (3a)	1.05	1.03	0.57	1.345
T66I	3 (GT3a)	T66 (3a)	1.08	1.10	0.45	0.64
K79R	3 (GT3a)	K79 (3a)	0.68	0.96	0.37	0.78
K114R	7 (GT3a)	R114 (3a)	1.08	1.26	0.68	1.50
I116V	4 (GT3a)	I116 (3a)	1.23	1.10	0.35	0.46
V116I	7 (GT3a)	I116 (3a)	0.91	1.06	0.44	0.93
M147V	3 (GT3a)	V147 (3a)	1.40	1.55	0.59	0.77
A150T/V; A150T	4 (GT3a)	A150 (3a)	0.70	1.02	0.43	0.87
V150A; I150A; T150A;	8 (GT3a)	A150 (3a)	0.94	1.0	0.63	1.2
A150V	6 (GT3a)	A150 (3a)	0.89	1.07	0.63	0.92
L159F	4 (GT3a)	L159 (3a)	0.71	0.91	0.65	0.80
K304R	4 (GT3a)	K304 (3a)	1.28	0.93	0.56	1.50
V321A	3 (GT3a)	V321 (3a)	1.2	0.75	0.70	0.99
R333K	4 (GT3a)	R333 (3a)	1.03	1.19	0.65	1.03
A335T	3 (GT3a)	A335 (3a)	1.03[b]	n.d[b]	1.12[b]	n.d.[b]
L374Q;R374Q	3 (GT3a)	R374 (3a)	0.92	1.10	0.52	1.05
N376D;G376D	3 (GT3a)	D376 (3a)	1.4	1.6	0.92	1.3
K379R	3 (GT3a)	R379 (3a)	0.88	0.73	0.52	0.83
I480V; A480V	3 (GT3a)	V480 (3a)	0.55	0.70	0.33	0.67
K517R	3 (GT3a)	K517 (3a)	0.72	1.05	0.26	0.60
R517K	4 (GT3a)	K517 (3a)	0.62	0.87	0.27	1.6
T520I; V520I	3 (GT3a)	I520 (3a)	1.30	1.20	0.37	0.89
I520T	3 (GT3a)	I520 (3a)	1.03	1.49	0.86	2.00
S543G	3 (2 GT3a; 1 GT2b)	G543 (3a);R543 (2b)	0.94	1.0	0.63	1.2
G543S	3 (GT3a)	G543 (3a)	1.16	1.30	0.45	0.88
Y571H	5 (GT3a)	H571 (3a)	0.85	0.99	0.52	0.90
H571Y	4 (GT3a)	H571 (3a)	0.65	0.64	0.48	0.74

a EC_{50} fold-change values for more than one patient isolate were averaged

b Phenotypic data from patient isolates not available. SOF and RBV FC in EC_{50} for this mutant derived from comparison of site-directed mutant replicon to reference

The FDA next generation sequencing analysis using the PVD75 and QbVD algorithm identified 41 substitutions, some of which were also identified by Gilead (Table 47).

Table 47. P7977-1231: Phenotype of Substitutions in NS5B Occurring in 2 or More Subjects Identified with 2 or 3 Algorithms

STUDYID	NS5B POS	No. Subjects	SUB10 (+/-)	Ratio	Conservation
P7977-1231	341	10	E341D		99.87%
	66	6	I66T/T66I/N66T/S66T	2-2-1-1	84%
	79	6	K79R/R79K/T79K	3-2-1	96%
	480	6	A480V/V480I/L	4-1-1	81%
	542	6	T542A/A542T	5-1	97%
	116	5	V116I/I116V	4-1	67%
	150	5	T150A/A150V/V150A	2-1-1	42%
	159	4	L159F		100%
	11	3	V11I		78%
	16	3	A16V/S	2-1	99%
	113	3	G113S/S113G	2-1	93%
	114	3	K114R		62%
	189	3	P189S/S189F/S189P	1-1-1	63%
	206	3	K206E/K206Q/Q206K	1-1-1	79%
	250	3	K250R/R250K	2-1	86%
	272	3	A272V/T272A/V272A	1-1-1	91%
	321	3	V321A		100%
	335	3	A335I/A335T/T335A	1-1-1	80%
	374	3	W374R/Q374R	2-1	55%
	376	3	N376D/G376D	2-1	50%
	517	3	R157K		84%
	571	3	Y571H/H571Y	2-1	65%
	82	2	L82M/T82M	1-1	94%
	90	2	A90Q/T	1-1	90%
	106	2	K106R/R106K	1-1	96%
	115	2	A115V		100%
	120	2	H120R/R120C	1-1	93%
	131	2	I131T		94%
	221	2	T221I		100%
	327	2	D327G/N	1-1	88%
	330	2	D330G/E330D	1-1	84%
	333	2	R333K/N	1-1	78%
	513	2	A513G/T513A	1-1	98%

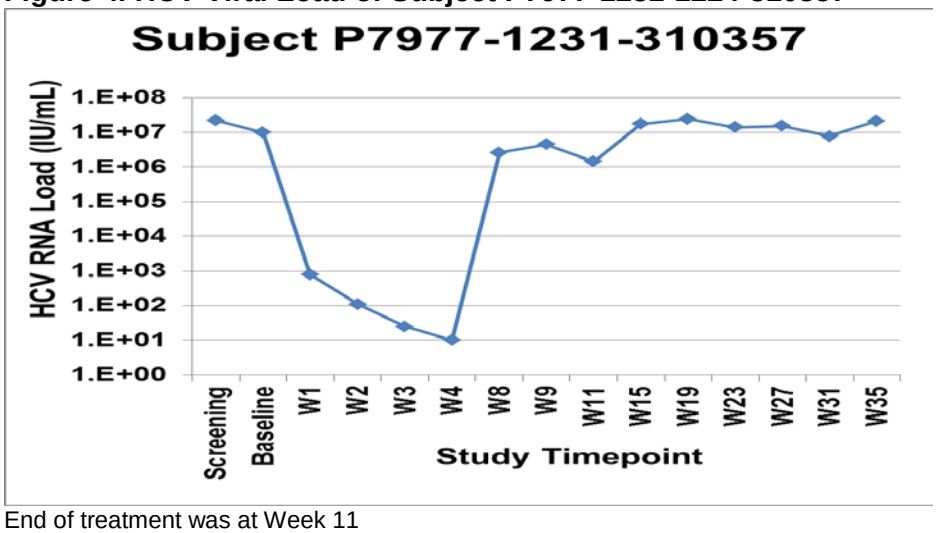
	519	2	R519K		84%
	520	2	T520I		76%
	541	2	A541S/T	1-1	97%
	543	2	G543S/S543G	1-1	81%
	544	2	Q544L/R	1-1	96%
	555	2	I555V/V555I	1-1	96%
	566	2	R566H		93%
	569	2	S569T/T569S	1-1	94%

Bolded positions were identified by Gilead.

Breakthrough

One subject (Subject 1231-1224-310357) in the SOF/RBV group with virologic failure from Study P7977-1231 experienced DAA breakthrough at Week 8 (Figure 4). This subject was infected with GT3a genotype/subtype, had IL28B CT genotype and a baseline viral load of 7 $\log_{10}$ IU/mL. This subject had evidence of noncompliance with low drug levels. At the Week 1 and 4 visits, the subject exhibited expected plasma concentrations of SOF and GS-331007, the predominant metabolite of SOF, at 257 ng/mL and 306 ng/mL, respectively. However, at the Week 8 and EOT visits, the GS-331007 plasma concentrations were below the quantiation limit (i.e., <10 ng/mL), which is indicative of poor compliance with study drug.

Figure 4. HCV Viral Load of Subject P7977-1231-1224-310357



End of treatment was at Week 11

There was only population sequence available from the Week 8 sample for this subject. The substitutions T84S, A150T and E202D emerged in NS5B on treatment (Table 48). The positions T84 and E202 are 95% and 98% conserved, respectively.

Table 48. Baseline and Emergent NS5B Substitutions in Subject who Experienced Breakthrough in Study P7977-1231

PID	Study	GT	Arm	Failure Timepoint	IL28B Genotype	Baseline NS5B substitutions	Emergent NS5B Substitutions
1224-310357	P7977-1231	3a	SOF + RBV	Week 8	CT	R50K K69Q R77K T84T/S R114K D117N A150A/T E202E/D K304R A335T R379K T473S N535K T542A Q544L	**T84S** A150T **E202D** I520I/T (at FU WK12)

Bolded substitutions are conserved sites: T84, 95.5%; E202, 97.7%

Study 107

In Study 107, 41 subjects did not achieve SVR12 and had samples with HCV RNA >1,000 IU/mL; 39 of these were relapsers and were examined by NGS analysis. Five amino acids (V11I, V116I, A150V or I150V, V480I, N543S or G543S) in NS5B were identified by Gilead in GT3a relapsers of Study 107. Some of these amino acids substitutions are at polymorphic sites. The 5 amino acid with substitutions in GT3a relapsers were tested for phenotypic changes to SOF and RBV. None of these substitutions showed detectable evidence of decreased susceptibility from reference or baseline to either SOF or RBV (Table 49; Report PC-334-2024).

Table 49. GS-US-334-0107: Phenotype of Substitutions in NS5B Occurring in Greater than 2 Subjects Identified with 2 or 3 Algorithms

Amino Acid Changes From Baseline Detected	Number of Subjects with Detected Substitution (genotype)	Reference AA	SOF EC_{50} Fold-Change [a]		RBV EC_{50} Fold-Change [a]	
			vs. Reference	vs. Baseline	vs. Reference	vs. Baseline
V11I	3 (GT3a)	I11 (3a)	1.4	1.1	0.56	1.1
V116I	3 (GT3a)	I116 (3a)	0.99	0.82	0.71	1.28
A150V; I150V	3 (GT3a)	A150 (3a)	1.55	1.20	0.89	1.70
V480I	3 (GT3a)	V480 (3a)	1.20	0.94	0.23	0.64
N543S, G543S	3 (2 GT3a; 1 GT2b)	R543 (2b), G543 (3a)	1.8	1.1	0.15	0.65

AA = amino acid; GT = genotype; Ref = wild-type reference of corresponding genotype

a EC_{50} fold-change values for more than one patient isolate were averaged

The QbVD and PVD75 algorithms also identified 3 of these substitutions (11, 116 and 150). Ten additional subsitutions were found in 2 or more subjects using these two algorithms (Table 50).

Table 50. Study GS-US-334-0107: Substitutions Identified in ≥2 Subjects with 2 or 3 Algorithms in the FDA NGS Analysis

STUDYID	NS5A POS	No. Subjects	SUB10 (+/-)	Ratio	Conservation
GS-US-0334-0107	**116**	4	V116I/I116V	3-1	67%
	150	3	A150V/T150A/V150T	1-1-1	42%
	247	3	P247L/P247S/S247P	1-1-1	99.7%
	341	3	E341D		99.87%
	11	2	V11I/I11V	1-1	79%
	156	2	P156S/A156P	1-1	96.5%
	189	2	P189S/S189P	1-1	63%
	207	2	I207M/M207I	1-1	99.5%
	287	2	A287T/T287A	1-1	100%
	310	2	D310N/S310N	1-1	90.3%
	438	2	V438I		89.6%
	520	2	T520I/V520I	1-1	76%
	558	2	N558T/T558N	1-1	98.3%

Bolded substitutions also identified by Gilead.

Study 108

In Study 108, 77 subjects did not achieve SVR12 (relapsers) and had samples with HCV RNA >1,000 IU/mL. Of these, 76 were examined by NGS. In this study, 11 NS5B substitutions (V11I, A67V, R77K, V116I/I116V, M147V, D148N, A150T, R517K, K519R, and G543S) were found by Gilead in the viruses >2 subjects infected with GT3a. These 11 substitutions were tested by Gilead for phenotypic changes to SOF and RBV. None of these substitutions showed detectable evidence of decreased susceptibility from reference or baseline to either SOF or RBV (Table 51).

Table 51. GS-US-334-0108: Phenotype of Emergent Substitutions in NS5B by Consensus Sequencing Occurring in >2 Subjects

Amino Acid Changes From Baseline Detected	Number of Subjects with Detected Substitution (genotype)	Reference AA	SOF EC_{50} Fold-Change[a]		RBV EC_{50} Fold-Change[a]	
			vs. Reference	vs. Baseline	vs. Reference	vs. Baseline
V11I	3 (GT3a)	I11 (3a)	1.1	0.79	0.6	1.4
A67V	3 (GT3a)	A67 (3a)	0.85	1.2	1.7	0.81
R77K	3 (GT3a)	R77 (3a)	1.4	1.6	0.92	1.3
I116V	4 (1 GT1b; 3 GT3a)	I116 (3a), V116 (1b)	1.7	1.2	0.31	0.31
V116I	4 (3 GT3a; 1 GT2b)	I116 (3a), V116 (2b)	1.1	0.79	0.65	0.89
M147V	4 (3 GT3a; 1 GT1b)	V147 (3a, 1b)	1.4	1.6	0.92	1.3
D148N	3 (GT3a)	D148 (3a)	0.44	0.4	0.23	0.45
A150T	4 (GT3a)	A150 (3a)	0.7	0.97	0.4	0.93
R517K	3 (GT3a)	K517 (3a)	0.62	0.87	0.27	1.6
K519R	3 (GT3a)	K519 (3a)	1.2	0.75	0.7	0.99
G543S	3 (2 GT3a; 1 GT1b)	G543 (3a), S543 (1b)	1.4	1.2	0.5	0.66

a EC_{50} fold-change values for more than one patient isolates were averaged
Phenotypic results were obtained from subjects who participated in this study or other previous SOF clinical studies.
Data Source: GS-US-334-0108 Virology Listing 2 and 3;

The QbVD and PVD75 algorithms also identified 9 of these substitutions. Twenty-three additional subsitutions were found in the viruses from 2 or more subjects using these two algorithms (Table 52).

Table 52. Study GS-US-334-0108: Substitutions Identified in ≥2 Subjects with 2 or 3 Algorithms in the FDA NGS Analysis

STUDYID	NS5A POS	No. Subjects	SUB10 (+/-)	Ratio	Conservation
GS-US-334-0108	**116**	7	V116I/T116I	6-1	67%
	542	7	A542T/T542A	4-3	97%
	535	5	K535N/N535K	3-2	52%
	571	5	Y571H/H571Y	3-2	65%
	114	4	K114R/R115K	2-2	62%
	150	4	A150V/T	2-2	42%
	543	4	G543S		81%
	50	3	K50R/R50K	2-1	90%
	66	3	A66T/T66A/T66N	1-1-1	84%
	120	3	R120C/H	2-1	93%
	148	3	D148N/N148D	2-1	99%

	184	3	I184T		84%
	335	3	T335N/A	2-1	80%
	517	3	R517K/K517R	2-1	84%
	11	2	V11I		78%
	47	2	L47Q		94%
	65	2	K65R		96%
	67	2	A67T/T67A		93%
	69	2	Q69K		53%
	71	2	V71I		97%
	100	2	R100K		89%
	147	2	M147V		93%
	159	2	L159F		100%
	211	2	K211R		99%
	250	2	K250R		86%
	304	2	R304K		76%
	305	2	A305V/V305A	1-1	96%
	357	2	T357I		96%
	519	2	R519K		84%
	540	2	P540L		99.9%
	541	2	A541V/T541A	1-1	97%
	564	2	V564M		92%

Bolded substitutions also identified by Gilead.

Summary of Emergent Substitutions in GT3 HCV-Infected Subjects

The substitutions listed in Table 53 were detected in at least 2 subjects and detected by 2 or 3 of the detection algorithms (QbVD, PVD75 and Gilead's algorithm). There were numerous other subjects whose virus had substitutions at these positions identified by only one algorithm.

Table 53. Summary of Treatment Emergent Substitutions in GT3 HCV Identified with 2 or 3 Algorithms in 2 or more Subjects

NS5A POS	No. Subjects	SUB10 (+/-)	Ratio	Conservation	SOF EC50 FC from REF	SOF EC50 FC from BL
341	13	E341D		99.87%		
116	11	V116I/I116V/T116I	8-2-1	67%	1.23	1.1
542	10	T542A/A542T	6-4	97%		
517	7	R517K/K517R	6-1	84%	0.6/0.7	0.9/1.1
535	7	K535N/N535K	5-2	52%		
11	6	V11I		78%	0.92	1.04

159	6	L159F		100%	0.71	0.91
250	6	K250R/R250K	5-1	86%		
114	5	K114R/R114K	4-1	62%	1.08	1.26
321	5	V321A		100%	1.2	0.75
106	4	K106R/R106K	3-1	96%		
113	4	G113S/S113G	3-1	93%		
156	4	A156P/P156S	3-1	96.5%		
520	4	I520V/V520I	3-1	76%	1.3	1.2
147	3	M147V		93%	1.4	1.55
438	3	V438I		89.6%		
519	3	R519K		84%	1.2	0.75

Highlighted positions are potential treatment-emergent substitutions that may be resistance-associated; these substitutions were not detected at baseline

The Subject IDs of subjects who had the key treatment emergent substitutions L159F, V321A, E341D or T542A are listed in Table 54. These sites were conserved and occurred in multiple subjects across studies. Substitution E341D was a treatment emergent substitution that developed in 13 subjects infected with GT3a HCV in two clinical trials (P7977-1231, n=10; GS-US-334-0107, n=3) and it was only found in subjects who relapsed. The L159F substitution emerged in 6 subjects infected with GT3a HCV in two clinical studies (P7977-1231, n=4; GS-US-334-0108, n=2) and is a previously identified NS5B substitution which with L320F was been shown to confer 2- to 5-fold decreased susceptibility to HCV nucleotide inhibitors (Tong X et al., 2012). The L159F and E341D emerged together in one GT3a subject. The phenotypic susceptibility of subject isolates with L159F or V321A was tested by the applicant and the results showed no significant shift in SOF susceptibility. The sponsor did not analyze the phenotypic susceptibility of substitutions E341D and T542A.

(b) (4)

Table 54. Subjects with Key Emergent Substitutions detected by NGS

Substitution	Subject IDs	Genotype/Subtype	N
L159F	GS-US-334-0108-1055-1438 **GS-US-334-0108-5868-1526** **P7977-1231-1017-310257** P7977-1231-1140-310566 **P7977-1231-1228-310637** P7977-1231-1241-310504	3a	6
V321A	GS-US-334-0107-5665-7226 GS-US-334-0108-4238-1508 P7977-1231-1036-310286 P7977-1231-1220-310625 P7977-1231-1230-310369	3a	5

E341D	P7977-1231-310006 P7977-1231-310079 P7977-1231-310013 P7977-1231-310167 GS-US-334-0107-0529-7295 GS-US-334-0107-4262-7294 GS-US-334-0107-5730-7477 P7977-1231-1001-310307 **P7977-1231-1017-310257** P7977-1231-1031-310385 P7977-1231-1042-310013 P7977-1231-1079-310167 P7977-1231-1140-310563 P7977-1231-1216-310472 P7977-1231-1216-310488 P7977-1231-1224-310678 P7977-1231-1225-310195	1a (98.59% conserved) 1b (99.75% conserved) 3a (99.87% conserved)	N=13 15% (2/13) cirrhotics IL28B 15% CC (2/13) 62% CT (8/13) 23% CT (3/13)
T542A	GS-US-334-0108-0057-1482 GS-US-334-0108-0071-1518 **GS-US-334-0108-5868-1526** GS-US-334-0108-6214-1435 P7977-1231-1170-310428 P7977-1231-1171-310448 P7977-1231-1171-310470 P7977-1231-1192-310518 **P7977-1231-1228-310637** P7977-1231-1235-310549	3a (96.79% conserved)	**10**

Bolded subject ID had multiple substitutions

TREATMENT EMERGENT SUBSTITUTIONS IN SUBJECTS WITH GENOTYPE 2

No substitutions were detected by two algorithms in at least two subjects for either GT2a or 2b. This analysis was limited by the small number of GT2 infected subjects failing treatment.

TREATMENT EMERGENT SUBSTITUTIONS IN SUBJECTS WITH GENOTYPE 1

Study 110

In Study 110, of the 32 virologic failures, 29 had HCV RNA >1,000 IU/mL and were analyzed by NGS. Twenty-eight had relapsed by post-treatment Week 4 and 12 and one discontinued. There were 18 relapse subjects with GT1a and 10 relapse subjects with GT1b. Two substitutions (R300Q and F543S/G543S) were identified by Gilead and also by QbVD and PVD75 algorithms (Table 55 and 56). No detectable phenotypic change to SOF or RBV was observed in subject samples containing these subsitutions (chimeric replicons encoding NS5B region derived from subject plasma/serum).

Table 55. GS-US-334-0110: Phenotypes of NS5B Substitutions Occurring in Multiple GT1 Subjects

Amino Acid Changes From Baseline Detected	Number of Subjects with Detected Substitution (genotype)	Reference AA	SOF EC_{50} Fold-Change[a]		RBV EC_{50} Fold-Change[a]	
			vs. Reference	vs. Baseline	vs. Reference	vs. Baseline
R300Q	3 (GT1a)	R300 (1a)	0.71	0.83	0.65	0.81
F543S; G543S	3 (2 GT1a; 1 GT1b)	G543 (1a); S543 (1b)	0.54	0.73	0.71	0.59

AA = amino acid; GT = genotype;
a EC_{50} fold-change values for more than one patient isolates were averaged
Source: GS-US-334-0110 Virology Listing 2 and 3

Table 56. Substitutions Detected by 2 Algorithms in ≥2 subjects for GT1a and 1b

GT1a

STUDYID	NS5A POS	No. Subjects	SUB10 (+/-)	Ratio
GS-US-334-0110	300	2	R300Q/Q300R	1-1
	543	2	G543S/G543S	1-1

GT1b

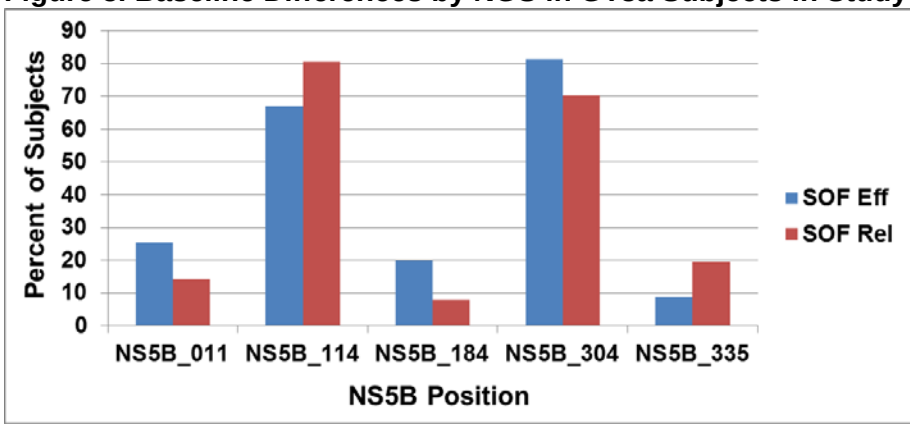
STUDYID	NS5A POS	No. Subjects	SUB10 (+/-)
GS-US-334-0110	556	2	G556S

Baseline Response by Genotype

STUDY P7977-1231 – GENOTYPE 3A

No significant differences between baseline sequences of subjects who achieved SVR12 (n=184) and those who relapsed (n=136) (Fig. 5). The sites shown in the graph are the most variable positions. Notably, S282T was not present in any of the baseline samples.

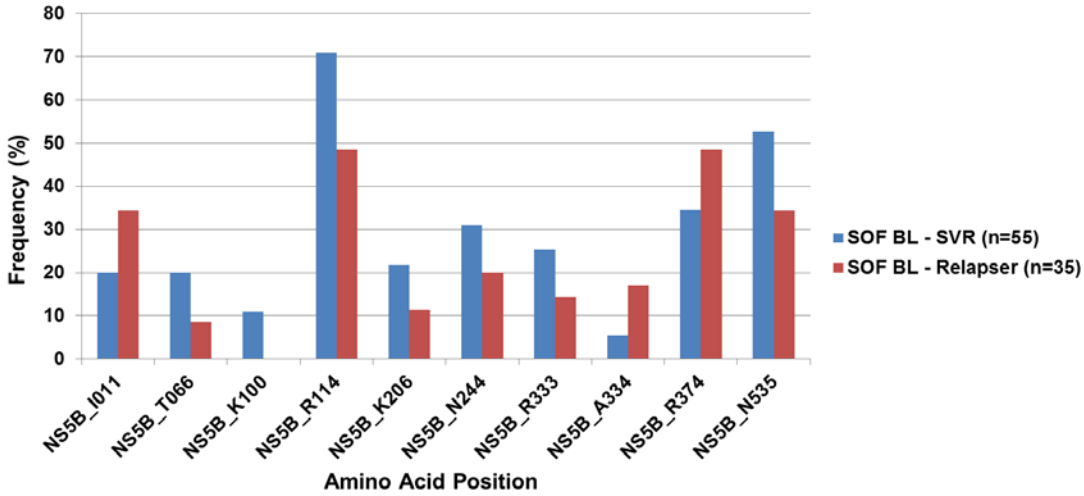
Figure 5. Baseline Differences by NGS in GT3a Subjects in Study P7977-1231



STUDY GS-US-334-0107 – GENOTYPE 3A

There were no significant differences between baseline sequences of subjects who achieved SVR12 (n=55) and those who relapsed (n=35) (Fig. 6). The S282T substitution was not present in any of the baseline samples.

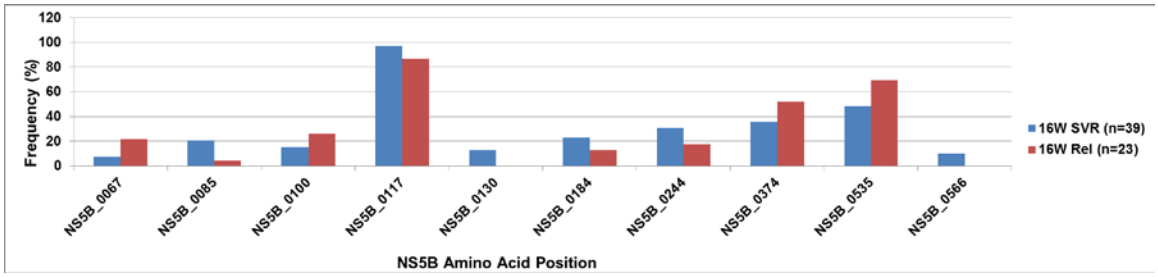
Figure 6. Baseline Differences by NGS in GT3a Subjects in Study 107



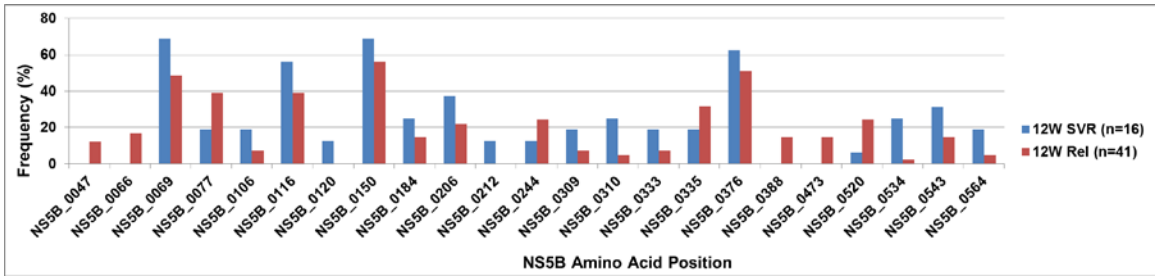
STUDY GS-US-334-0108 – GENOTYPE 3A

There were no major differences between baseline sequences of subjects who achieved SVR12 and those who relapsed in the two SOF+RBV arms in Study 108 (Fig. 7). In addition, there were no trends within or between the 2 arms in this study. The S282T substitution was not present in any of the baseline samples.

Figure 7. Baseline Differences by NGS in GT3a Subjects in Study 108
SOF+RBV 16 Weeks



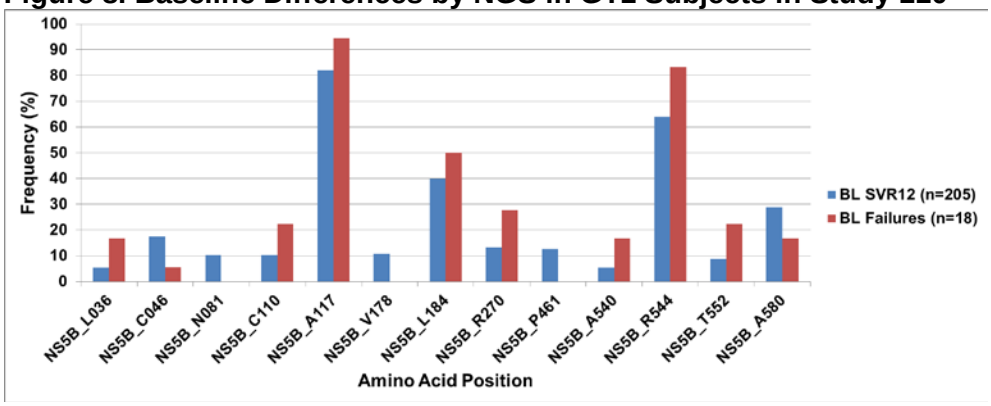
SOF+RBV 12 Weeks



STUDY GS-US-334-0110 – GENOTYPE 1

There were no significant differences between baseline sequences of subjects who achieved SVR12 (n=205) and those who relapsed (n=18) (Fig. 8). The sites shown in the graph are the most variable positions. The S282T substitution was not present in any of the baseline samples.

Figure 8. Baseline Differences by NGS in GT1 Subjects in Study 110



Sites shown in the graph are at the most variable positions.

BASELINE ANALYSIS OF NS5B SUBSTITUTIONS T390I AND F415Y

As SOF was combined with RBV in most treatment regimens, baseline samples were also assessed for the baseline presence of the substitutions NS5B T390I and F415Y, both previously observed in GT1a virologic failure patients from RBV-containing regimens (Young et al., 2003; Ward et al., 2008; Bartels et al., 2011). Of the SOF-

treated subjects, 19 sequenced subjects had detectable T390I (n=11) or F415Y (n=8) at baseline present either as a full mutant or a mixture. Three subjects had the previously reported nucleoside analog polymerase inhibitor associated substitution L159F at baseline with 2/3 subjects achieving SVR12. The cell culture selected nucleoside analog polymerase inhibitor resistance substitutions N142T, M289I or M289L were detected in 24 subjects with 23/24 achieving SVR12. In addition, there were 27 subjects whose virus had substitutions at positions that were previously shown to confer reduced susceptibility to nonnucleoside analog polymerase inhibitors. Twenty-five out of 27 subjects achieved SVR12. No statistical difference in treatment outcome was associated with the presence of these variants at baseline in subjects treated with a SOF containing regimen.

S282T Emergence in One Subject from Phase 2 Study P7977-0523 [ELECTRON]

The S282T substitution was detected by NGS in one GT2b subject (Subject 1030-5068 from Phase 2 Study P7977-0523 [ELECTRON]) who received SOF monotherapy for 24 weeks and relapsed at Week 4 post-treatment. For this subject, the S282T substitution was no longer detectable at Week 12 post-treatment by NGS with an assay cut off of 1%. The sample from this genotype 2b subject with S282T detected displayed a mean 13.5-fold (range 8.99-19.77) reduced susceptibility to SOF.

Overall, S282T mutant replicons from multiple genotypes displayed a decreased replication capacity in cell culture. The replication capacities of S282T mutants were evaluated in GT 1 - 6 replicons. S282T mutants replicated poorly across all genotypes with replication capacity ranging from 1% to 11% as compared to corresponding wild-type replicons. In addition, a significant reduction in replication capacity (1.5 - 1.8% of wild-type) was observed in the replicon clones containing NS5B from the subject with S282T detected. These data are consistent with the short persistence (≤8 weeks) of S282T in the single subject with this mutant detected following SOF treatment.

Phylogenetic Analysis of a Subset of Phase 3 Studies

A phylogenetic analysis of subjects achieving SVR12 and those who failed to achieve SVR12 was performed to determine if subjects who fall within each group cluster or intermingle. The analysis was performed using an alignment of the full-length HCV NS5B nucleotide sequences derived from baseline viral samples of the subjects who participated in the three Phase 3 Studies: GS-US-334-0107, GS-US-334-0108, and P7977-1231. Multiple sequence alignment was performed using Multiple Sequence Comparison and trees were constructed using the maximum likelihood method. In each of these analyses, the verified GT and subtype of the control reference clearly segregated with the GT and subtype assigned to the patient sample. Subjects achieving SVR12 and those that failed to achieve SVR12 were found to be intermingled within each HCV GT2/3 genotype/subtype, without any apparent clustering. This observation is consistent across all three studies and all five treatment arms that were analyzed.

Pre-Transplant Study P7977-2025

After 12 weeks of SOF+RBV treatment, 45 of 48 subjects (93.8%) had HCV RNA <LLOQ. Five subjects (one with HCV RNA ≥LLOQ on the day of transplantation was not discontinued from the study) had on-treatment virologic failure (3 breakthrough and 2 non-response).

- **Subject 0451-7732** had a baseline HCV RNA of 2,350,000 IU/mL. At Week 8, the subject still had detectable HCV RNA at 42 IU/mL. A confirmatory HCV RNA measurement 7 days later reported <25 IU/mL. By Week 12, the subject's HCV RNA was TND; this increased to 215 IU/mL at Week 16, the day of transplantation. Therefore, the subject underwent a transplantation with HCV RNA >LLOQ. At Posttransplant Week 24, the subject had HCV RNA of 21,800,000 IU/mL. The subject is continuing in the follow-up phase of the study.
  Substitutions S/N62S/H, K/E124E, G/S166S and A421A/V were emergent at post-transplant Week 1 and 2. Plasma concentrations of GS-331007 (the predominant metabolite) were comparable to mean concentrations observed in Phase 3 studies.
- **Subject 0585-7751** had a baseline HCV RNA of 700,000 IU/mL. By Week 3, the subject's HCV RNA was <25 IU/mL, which increased to 232 IU/mL at Week 8. The subject discontinued study treatment and HCV RNA was 1510 IU/mL at Posttreatment Week 4. The subject discontinued study treatment and the study due to efficacy failure. Substitutions S300S/T, Q514Q/R, K517R/K and S556S/G were emergent at Follow-up Week 4. Plasma concentrations of GS-331007 (the predominant metabolite) were comparable to mean concentrations observed in Phase 3 studies.
- **Subject 0773-7734** had a baseline HCV RNA of 14,900,000 IU/mL. By Week 2, the subject's HCV RNA was TND. At Week 12, the subject's HCV RNA was 447 IU/mL. A confirmatory HCV RNA measurement 6 days later reported 6200 IU/mL. The subject discontinued study treatment for efficacy failure and discontinued study by withdrawal of consent. Substitutions A80A/T, P/Q130Q, T/A483A, R/H523H and G554G/A were emergent at Week 12. At the Week 1, 4, and 8 visits, plasma concentrations of GS-331007 were comparable to the mean concentrations observed in the Phase 3 studies. However, at the Week 12 visit (sample drawn 26 hours post dose), the GS-331007 plasma concentration was below the quantification limit (i.e., < 10 ng/mL), which is substantially lower than the mean GS-331007 plasma concentration in the Phase 3 population 24 hours post dose [160 ng/mL) indicative of noncompliance.
- **Subject 1249-7720** had a baseline HCV RNA of 1,620,000 IU/mL, which decreased to 268 IU/mL at Week 8. The subject discontinued treatment at Week 8 due to non-response (efficacy failure). The subject's HCV RNA increased to 1,330,000 IU/mL by post-treatment Week 12. Due to progressive liver disease the subject was no longer a transplantation candidate and therefore discontinued the study. No substitutions emerged at the Week 8 timepoint, but S/N189S was present at the post-treatment pre-transplant Week 4 timepoint. Plasma concentrations of GS-331007 (the predominant metabolite) were comparable to mean concentrations observed in Phase 3 studies.
- **Subject 0773-7712** had a baseline HCV RNA of 3,760,000 IU/mL, which decreased to 1490 IU/mL at Week 8. The subject discontinued treatment at Week 12 due to non-response (efficacy failure). The subject's HCV RNA increased to 4,680,000 IU/mL by posttreatment Week 12. The subject discontinued the study due to efficacy failure. Substitutions A252A/V and N273N/S were emergent at Week 8, N273S at Week 12 and I405V/I and F575F/L at post-treatment pre-transplant Week 4. Plasma concentrations of GS-331007 (the predominant metabolite) were comparable to mean concentrations observed in Phase 3 studies.

Reference ID: 3369468

We are requesting the genotypes/subtypes for the subjects who experienced virologic breakthrough or relapsed**.**

For 58 of these subjects, the genotype and subtype results from the LiPA 2.0 assay were confirmed by NS5B sequence alignment. For the 3 subjects whose genotype was not available based on the LiPA 2.0 assay, the genotype was fully determined by NS5B sequencing (Subject 7275-7701, genotype 2a; Subject 7275-7733, genotype 2b; Subject 0773-7760, genotype 4a). Baseline population sequencing of NS5B was performed for all 61 subjects enrolled in the study. A full NS5B population sequence was obtained for 59 subjects and a short NS5B fragment population sequence (amino acids 270-311) for 2 subjects. No S282T was observed at baseline in any subjects.

In the pretransplant phase, 17 of 61 subjects qualified for resistance testing. Of these 17 subjects, on-treatment HCV breakthrough was observed in 3 subjects (Subjects 0773-7734, 0585-7751, and 0451-7732), slower response to treatment was observed in 2 subjects (Subjects 0773-7712 and 1249-7720), early treatment termination occurred in 1 subject (Subject 0585-7758), and post-treatment relapse in 11 subjects (Subjects 0522-7707, 1028-7706, 1657-7704, 1657-7715, 6927-7713, 7585-7710, 0773-7717, 6927-7721, 1028-7724, 1657-7722, and 7585-7731). Full length NS5B at baseline and virologic failure timepoints was analyzed by NGS analsyis with an assay cut-off at 1% in 16 of 17 subjects. Subject 0451-7732 did not qualify for resistance testing prior to transplantation due to HCV RNA levels <1,000 IU/mL. No S282T was observed in any of these 16 subjects. Seven of 11 subjects who experienced viral relapse after 24 weeks of SOF+RBV treatment were retreated with the same regimen (Subjects 1028-7706, 0522-7707, 7585-7710, 6927-7721, 1028-7724, 1657-7722, and 7585-7731). No S282T was observed in these 7 subjects at relapse, however 1–6 substitutions at other NS5B positions were present at relapse in 6 of these subjects before retreatment. HCV RNA was successfully re-suppressed in all 7 retreated subjects, suggesting that the any substitutions observed at the relapse timepoints were not associated with resistance.

A total of 41 subjects received a liver transplantation. One subject received an HCV-infected liver and was excluded from post-transplantation sequencing analysis. Recurrent HCV infection was reported in 9 of 37 subjects who had HCV RNA <LLOQ at last observed measurement prior to transplantation. NS5B NGS analysis was completed at baseline and the post-transplantation virologic failure timepoint in 8 of these subjects (Subjects 0773-7730, 6437-7718, 6437-7723, 6437-7735, 6437-7736, 0522-7737, 0585-7752, and 1651-7748). No S282T was detected above the 1% assay cut-off in any of these 8 subjects. NS5B amplification from 1 subject failed (Subject 7585-7731) due to very low HCV RNA at the reoccurrence timepoint sample available for testing. Recurrent HCV infection was imputed for 3 subjects because no samples for resistance testing were available due to death (Subjects 1028-7705, 1028-7706, and 1028-7724).

5. CONCLUSION

The new DAA, sofusbuvir (SOF), offers a new treatment option for patients with chronic HCV with genotypes 1, 2, 3 and 4. SOF showed antiviral activity across genotypes in stable replicons with EC_{50} values ranging from 14-110 nM [40 nM GT1a; 110 nM GT 1b; 50 nM GT 2a, 15 nM GT2b; 50 nM GT3a; 40 nM GT4a; 15 nM GT5a; 14 nM GT6a]. The data submitted did not exhibit cellular toxicity or mitochondrial toxicity for SOF in cell culture. SOF did not show antagonistic interactions with other classes of DAAs, RBV, or HIV-1 NRTIs. The S282T substitution was the primary substitution selected from SOF selection experiments in cell culture and showed 4- to 24-fold decreases in SOF susceptibility. The S282T substitution is not cross-resistant with NS5A inhibitors, nonnucleoside inhibitors, NS3/4A protease inhibitors, or RBV.

The efficacy and baseline response data from this NDA support a 16-Week duration of SOF+RBV for all subjects infected with GT3 HCV and treatment-experienced subjects infected with GT2 HCV. In addition, the efficacy and baseline response data from this NDA support 12-Week duration of SOF+P/R for subjects with GT1 and 4 HCV.

Next generation nucleotide sequencing (NGS) data were submitted for the 4 Phase 3 clinical trials for the analysis of treatment emergent SOF substitutions. No S282T substitutions were identified at baseline or at time of relapse in any of the subjects from any of the Phase 3 trials in either the Gilead or FDA analyses. Multiple other substitutions in NS5B were identified by two algorithms in ≥2 subjects who relapsed in the phase 3 studies. However, many of these substitutions were at polymorphic sites and none showed a detectable reduction in phenotypic susceptibility to SOF or RBV when tested in replicons by Gilead. Treatment emergent substitutions L159F, V321A, or E341D emerged in multiple subjects across the phase 3 studies and were detected with 2 bioinformatic algorithms. The phenotypic susceptibility of subject isolates with L159F and V321A was tested by Gilead and the results showed no shifts in SOF susceptibility. Gilead did not analyze the phenotypic susceptibility of substitution E341D. One subject in Study P7977-1231 experienced DAA breakthrough at Week 8. This subject had evidence of noncompliance with low drug levels. There was only population sequence available from the Week 8 sample for this subject. The substitutions T84S, A150T and E202D emerged in NS5B on treatment. The positions T84 and E202 are 95% and 98% conserved, respectively. We will request that Gilead analyze substitutions E341D, T84S, E202D, and T84S+A150T+E202D for phenotypic susceptibility.

The S282T substitution was detected by next generation nucleotide sequence analysis in one GT2b subject (Subject 1030-5068 from Phase 2 Study P7977-0523 [ELECTRON]) who received SOF monotherapy for 24 weeks and relapsed at Week 4 post-treatment. For this subject, the S282T substitution was no longer detectable at Week 12 post-treatment by deep sequencing with an assay cut off of 1%. The sample from this genotype 2b subject with S282T detected displayed a mean 13.5-fold reduced susceptibility to SOF.

While S282T was not detected in subjects in the Phase 3 trials, it did arise in at least one subject in the phase 2 trials. Given that this is a known resistance-associated substitution of SOF, the S282T should be included in the resistance section of the label.

6. PACKAGE INSERT

APPLICANT PROPOSED PACKAGE INSERT

12.4. Microbiology

Mechanism of Action

Sofosbuvir is (b) (4) inhibitor of the HCV NS5B RNA-dependent RNA polymerase, which is essential for viral replication. Sofosbuvir is a nucleotide prodrug that undergoes intracellular metabolism to form the pharmacologically active uridine analog triphosphate (GS-461203), which can be incorporated by HCV (b) (4) and acts as a chain terminator. In a biochemical assay, GS-461203 inhibited the polymerase activity of the recombinant NS5B from HCV genotype 1b, 2a, 3a and 4a with an IC_{50} value ranging from 0.7 to 2.6 μM. GS-461203 is not an inhibitor of human DNA and RNA polymerases nor an inhibitor of mitochondrial RNA polymerase.

Antiviral Activity

In HCV replicon assays, the EC_{50} values of sofosbuvir against full-length replicons from genotype 1a, 1b, 2a, 3a and 4a, and chimeric 1b replicons encoding NS5B from genotype 2b, 5a or 6a ranged from 0.014 to 0.11 μM. The (b) (4) EC_{50} of sofosbuvir against chimeric replicons encoding NS5B sequences from clinical isolates was (b) (4) for genotype 1a (N=67), (b) (4) for genotype 1b (N=29), (b) (4) for genotype 2 (N=15) and (b) (4) for genotype 3a (N=106). In infectious virus assays, the EC_{50} values of sofosbuvir against genotype 1a and 2a were 0.03 and 0.02 μM, respectively. The presence of 40% human serum had no effect on the anti-HCV activity of sofosbuvir. Evaluation of sofosbuvir in combination with interferon alpha or ribavirin showed no antagonistic effect in reducing HCV-RNA levels in replicon cells.

Resistance

In Cell Culture

HCV replicons with reduced susceptibility to sofosbuvir have been selected in cell culture for multiple genotypes including 1b, 2a, 2b, 3a, 4a, 5a and 6a. Reduced susceptibility to sofosbuvir was associated with the primary NS5B substitution S282T in all replicon genotypes examined. Site-directed mutagenesis of the S282T substitution in replicons of 8 genotypes conferred 2- to 18-fold reduced susceptibility to sofosbuvir and reduced the replication viral capacity by 89% to 99% compared to the corresponding wild-type. In biochemical assays, recombinant NS5B polymerase from genotypes 1b, 2a, 3a and 4a expressing the S282T substitution showed reduced susceptibility to GS-461203 compared to respective wild-types.

In Clinical Trials

(b) (4)

Reference ID: 3369468

[redacted] (b) (4)

[redacted] (b) (4)

[redacted]

Cross Resistance

HCV replicons expressing the sofosbuvir-associated resistance substitution S282T were [redacted] (b) (4) susceptible to [redacted] (b) (4) (b) (4) (b) (4)

FDA PROPOSED PACKAGE INSERT

[redacted] (b) (4)

12.1 Mechanism of Action

Sofosbuvir is a direct-acting antiviral agent against the hepatitis C virus *[See Microbiology (12.4)]*.

12.4. Microbiology

Mechanism of Action

Sofosbuvir is [redacted] (b) (4) inhibitor of the HCV NS5B RNA-dependent RNA polymerase, which is essential for viral replication. Sofosbuvir is a nucleotide prodrug that undergoes intracellular metabolism to form the pharmacologically active uridine analog triphosphate (GS-461203), which can be incorporated [redacted] (b) (4) into HCV RNA by the [redacted] (b) (4) -NS5B polymerase 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 IC_{50} values ranging from 0.7 to 2.6 μM. GS-461203 is not an inhibitor of human [redacted] (b) (4) polymerases nor an inhibitor of mitochondrial RNA polymerase.

Antiviral Activity

In HCV replicon assays, the EC_{50} values of sofosbuvir against full-length replicons from genotype 1a, 1b, 2a, 3a and 4a, and chimeric 1b replicons encoding NS5B from genotype 2b, 5a or 6a ranged from 0.014 to 0.11 μM. (b) (4) The (b) (4)-median (b) (4)-EC_{50} value of sofosbuvir against chimeric replicons encoding NS5B sequences from clinical isolates was (b) (4)-for genotype 1a (N=67) (b) (4)-for genotype 1b (N=29), (b) (4) μM for genotype 2 (N=15) and (b) (4)-μM for genotype 3a (N=106). In infectious virus assays, the EC_{50} values of sofosbuvir against genotype 1a and 2a were 0.03 and 0.02 μM, respectively. The presence of 40% human serum had no effect on the anti-HCV activity of sofosbuvir. Evaluation of sofosbuvir in combination with interferon alpha or ribavirin showed no antagonistic effect in reducing HCV-RNA levels in replicon cells.

Resistance

In Cell Culture

HCV replicons with reduced susceptibility to sofosbuvir have been selected in cell culture for multiple genotypes including 1b, 2a, 2b, 3a, 4a, 5a and 6a. Reduced susceptibility to sofosbuvir was associated with the primary NS5B substitution S282T in all replicon genotypes examined. (b) (4) an M289L substitution developed along with the S282T substitution in genotype 2a, 5 and 6 replicons. Site-directed mutagenesis of the S282T substitution in replicons of 8 genotypes conferred 2- to 18-fold reduced susceptibility to sofosbuvir and reduced the replication viral capacity by 89% to 99% compared to the corresponding wild-type. In biochemical assays, recombinant NS5B polymerase from genotypes 1b, 2a, 3a and 4a expressing the S282T substitution showed reduced susceptibility to GS-461203 compared to respective wild-types.

In Clinical Trials

(b) (4)

(b) (4)

(b) (4)

Cross Resistance

HCV replicons expressing the sofosbuvir-associated resistance substitution S282T were (b) (4) susceptible to (b) (4)

(b) (4)

(b) (4)

7. REFERENCES

Bartels DJ, Tigges AM, Sullivan JC, Henshaw J, Jiang M, Zhang EZ, et al. Enrichment of the NS5B Polymerase Variant F415Y Following Failure of Ribavirin Containing Regimens in Patients with Subtype 1a HCV. 6th International Workshop on Hepatitis C- Resistance & New Compounds; 2011 June 23-24; Boston, MA.

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/s/

LINDA C ONAGA
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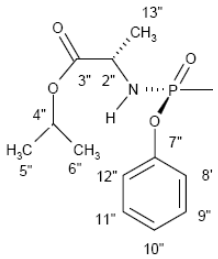
DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 204671 **SDN** 002 **DATE REVIEWED:** 08/27/2013

Reviewer: Eric F. Donaldson, Ph.D.
Date Submitted: 4/08/13 **Date Assigned:** 4/09/13
Date Received: 4/09/13

Sponsor: Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA, 94404

Product Names: GS-7977, PSI-7977, Sofosubvir
Chemical Name: (S)-Isopropyl 2-((*S*)-(((2*R*,3*R*,4*R*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy) phosphorylamino) propanoate

Structure:



GS-7977

Molecular formula: $C_{22}H_{29}FN_3O_9P$
Molecular weight: 529.46
Drug category: Antiviral against Hepatitis C Virus
Indication: Treatment of chronic HCV infection
Dosage Form/Route of administration: Capsules/Oral
Supporting documents: IND106739; IND106739 SDN 286
Abbreviations: DAA, HCV direct antivirals; GT, genotype; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IFN, interferon; LLOQ, lower limit of quantification; LOD, limit of detection; NGS, next generation sequencing; PEG, pegylated interferon alpha; RBV, ribavirin; SOC, standard-of-care; SOF, sofosbuvir; SVR, sustained virologic response

Reference ID: 3369467

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Reference ID: 3369467

EXECUTIVE SUMMARY

Sofosbuvir (SOF) is a nucleotide analog prodrug that targets the NS5B polymerase of hepatitis C virus (HCV) and inhibits HCV replication. When used in combination with ribavirin (RBV) with or without pegylated interferon (PEG), SOF produced high sustained virologic response (SVR) rates in subjects with chronic HCV infection. The proposed indication for SOF is for use in combination with other agents for the treatment of chronic hepatitis C (CHC) virus infection in adults. The sponsor provided four pivotal Phase 3 studies in support of SOF:

1. GS-US-334-0107 (POSITRON) was a Phase 3, randomized, double-blind, placebo-controlled study evaluating the efficacy and safety of 12 weeks of SOF+RBV in subjects with chronic genotype 2 or 3 HCV infection who were IFN intolerant, IFN ineligible, or unwilling to take IFN.
2. GS-US-334-0108 (FUSION) was a Phase 3, randomized, double-blind study evaluating the efficacy and safety of 12 or 16 weeks of SOF+RBV in treatment-experienced subjects with chronic genotype 2 or 3 HCV infection.
3. GS-US-334-0110 (NEUTRINO) was a Phase 3, single-arm, open-label study evaluating the efficacy and safety of SOF+PEG+RBV for 12 weeks in treatment-naive subjects with chronic genotype 1, 4, 5, or 6 HCV infection.
4. P7977-1231 (FISSION) was a Phase 3, randomized, open-label, active-controlled study evaluating the efficacy and safety of 12 weeks of SOF+RBV compared with 24 weeks of PEG+RBV in treatment-naive subjects with chronic genotype 2 or 3 HCV infection.

Gilead submitted Next Generation Nucleotide Sequencing (NGS; also referred to as deep sequencing) data for four Phase 3 and five Phase 2 clinical trials that were provided to support the NDA for GS-7977 (SOF). Of the 982 subjects in SOF+RBV or SOF/PEG/RBV groups of the Phase 3 studies P7977-1231, GS-US-334-0107, GS-US-334-0108 and GSUS-334-110, a total of 224 subjects had NGS data for a total of 676 files. The raw nucleotide sequence data were submitted in fastq format and resistance analysis tables were populated at a 15% cutoff by Gilead. The number of virologic failure subjects with NGS data for studies P7977-1231, GS-US-334-107, -108 and -110 were 78, 41, 76, and 29, respectively. The Division of Antiviral Products (DAVP) conducted an independent assessment of the NGS data using an internal NGS analysis pipeline that is described in detail in this review.

Gilead concluded that no genotypic resistance to SOF or RBV was identified by NGS or Sanger population nucleotide sequence analysis and no phenotypic resistance was identified by phenotypic analyses for 299 of 300 subjects not achieving SVR12 in the Phase 2 and 3 clinical trials. Further, baseline NS5B sequences (Sanger population sequencing) were obtained for 1994 of 2016 subjects from the Phase 2 and 3 clinical studies and none of the 1994 subjects had the SOF-associated substitution S282T at baseline. A total of 39 subjects treated with SOF had detectable GT1a RBV-associated substitutions T390I or F415Y at baseline but there were no statistically significant correlations between the presence of any variant at baseline and treatment outcome.

Gilead reported that the S282T substitution was only detected in one subject infected with HCV genotype (GT) 2b who received SOF monotherapy in a Phase 2 study. A total of 95 NS5B substitutions were observed in >2 subjects, with 94/95 of these substitutions reported to occur at polymorphic sites. The one additional substitution occurred at a conserved site and was a change to the wild-type amino acid. Gilead reported that none of the substitutions were associated with a phenotypic change in SOF or RBV susceptibility. In addition, the sponsor stated that no reduction in susceptibility to SOF or RBV was observed in HCV variants from 173 subjects with a total of 344 substitutions detected at 202 different amino acid positions at post-baseline among these samples. The sponsor reported that the NS5B sequence from the HCV GT2b subject with S282T displayed reduced susceptibility to SOF.

Clinical Virology discussed the results of the independent analysis of the NGS data and compared the results with those submitted by Gilead. For the most part, there was good agreement on the overall results and conclusions; however, after discussion with the Clinical Virology Reviewer for this application, Lisa Naeger,

Ph.D., it was agreed that the following modifications should be made to the label and/or proposed as post-marketing actions:

1. While S282T was not detected in most subjects, it did arise in at least one GT2a infected subject in a Phase 2 trial. Given that this is a known resistance-associated substitution of SOF, this should be included in the resistance section of the label.
2. Substitution E341D was a treatment-emergent substitution at a highly conserved position that developed in 13 subjects infected with HCV GT3a in two clinical trials (P7977-1231, n=10; GS-US-334-0107, n=3) and it was only found in subjects who relapsed. Therefore, this substitution should be characterized in phenotypic assays to determine if its presence in NS5B impacts SOF susceptibility.
3. The L159F substitution is a previously identified resistance-associated NS5B substitution which with L320F has been shown to confer low-level resistance to HCV nucleotide inhibitors (Tong X et al., 2012). L159F emerged in 6 GT3a infected relapse subjects and with E341D in one of those subjects. L159F should be characterized in combination with E341D in a phenotypic assay to determine if these substitutions in combination in NS5B results in an increased EC_{50} value.
4. Three substitutions at highly conserved positions (T84S, E202D, and T473S) were enriched in the one subject infected with HCV GT3a who experienced breakthrough in clinical trial P7977-1231. We recommend assessing T84S+E202D+T473S in combination in a GT3a phenotypic assay.
5. Additional substitutions at highly conserved positions may be associated with resistance to SOF and should be further characterized in a GT3a phenotypic assay: K211R, V321A, P540L, and T542A.

BACKGROUND AND SUMMARY

GS-7977 (sofosbuvir, SOF) is a prodrug of a uridine nucleotide analog polymerase inhibitor with EC_{50} values in the H77 (GT1a) and JFH-1 (GT2a) infectious systems of 0.03 µM and 0.02 µM, respectively. The NS5B substitution S282T, which is selected by SOF in cell culture and confers reduced susceptibility to multiple nucleos(t)ide analog polymerase inhibitors in development, has been detected in one GT2b subject who relapsed after receiving 12 weeks of GS-7977 monotherapy.

NDA205123 was submitted on April 8, 2013 and received priority review status. The sponsor provided next generation nucleotide sequencing data (NGS) used in the resistance analysis of four Phase 3 clinical trials:

1. **GS-US-334-0107 (POSITRON)** was a Phase 3, randomized, double-blind, placebo-controlled study evaluating the efficacy and safety of 12 weeks of SOF+RBV in subjects with chronic genotype 2 or 3 HCV infection who were IFN intolerant, IFN ineligible, or unwilling to take IFN.
2. **GS-US-334-0108 (FUSION)** was a Phase 3, randomized, double-blind study evaluating the efficacy and safety of 12 or 16 weeks of SOF+RBV in treatment-experienced subjects with chronic genotype 2 or 3 HCV infection.
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4. **P7977-1231 (FISSION)** was a Phase 3, randomized, open-label, active-controlled study evaluating the efficacy and safety of 12 weeks of SOF+RBV compared with 24 weeks of PEG+RBV in treatment-naive subjects with chronic genotype 2 or 3 HCV infection.

In addition, the sponsor submitted NGS data for five Phase 2 clinical trials; however, this review of the NGS data focused on the pivotal Phase 3 trials. The sponsor provided the NGS data on a hard drive, including 1) frequency tables showing amino acid variation that occurred at each position of NS3/4A for each failure sample that was successfully sequenced using Illumina; 2) raw sequence data in fastq format for all samples that were deep sequenced; and 3) summary resistance data for each study.

Given that next generation sequencing is an emerging technology with no current standards for analysis, the division requested raw data so that an independent analysis could be performed on the NGS data. The sponsor's summary NGS data was compared to the results generated by the Division of Antiviral Products (DAVP) following these criteria:

1. The sponsor's frequency tables were used to generate a summary and do a direct comparison of the results reported by the sponsor;
2. Frequency tables were generated by DAVP using an independent mapping of reads to a reference for each sample and using two different variant detection algorithms and the results were compared with those reported by the sponsor and those generated using the sponsor's frequency table; and
3. The conclusions from the NGS data were compared to the results reported by the sponsor using Sanger population sequence analysis.

RATIONALE FOR REQUESTING AND ANALYZING NGS DATA

In general, the FDA does not analyze raw nucleotide sequence data in conjunction with new drug applications (NDAs); however, when the technology used to generate the data is relatively new, it is necessary to perform independent assessments of the data to confirm that the review division understands how the data are interpreted by the sponsor. Next Generation Sequencing (NGS) is an emerging technology that presents many potential data integrity issues that must be considered upon careful review:

1. There are currently multiple sequencing platforms available for resistance analysis by NGS (454, Illumina, Ion Torrent, PacBio), and these technologies are continuously emerging. Each platform has different error rates and chemistries that contribute to unique types of base calling errors.
2. There are currently no standardized analysis pipelines with which to analyze NGS data and more than 200 algorithms can be used to generate an assembly of small reads, with each algorithm employing unique strategies and using unique parameters. Comparison of different platforms and algorithms has shown that often differences in data interpretation are attributed to the bioinformatics analysis and not the sequencing platform.
3. To date, each sponsor submitting NGS data has generated data with unique NGS analysis pipelines that use internal scripts and programs that are not currently available in the public domain.

The Division of Antiviral Products reviews Investigational New Drugs (IND) and New Drug Applications (NDA) with indications for the treatment of a variety of viral diseases. In nearly all cases, these drugs select for resistant viruses that escape antiviral therapies. Therefore, viral resistance has important implications for the durability of a new drug and the potential impact on future treatment options for patients infected with the virus. DAVP performs independent analyses of all resistance data associated with antivirals drugs being developed to ensure that the emergence of resistance is carefully characterized and explained in the label of newly approved antiviral drug products. Providing accurate resistance information is imperative for protecting public health to prevent emergence of novel resistant and cross-resistant viral variants that have the potential to infect others and cause major outbreaks of disease that cannot be controlled by approved drugs. In addition, the resistance information provides important guidance for health care professionals who oversee the use of these therapeutics and is included in the drug product information approved by DAVP.

Until recently, the genotypic resistance data reviewed by DAVP virology reviewers were generated using the Sanger DNA sequencing method. However, NGS is an emerging technology that provides an inexpensive and comprehensive approach for performing high throughput nucleotide sequence analysis and is being employed more and more frequently by sponsors when performing resistance analyses. Because it determines the sequence for all RNAs or DNAs in a clinical sample, NGS adds complexity to the resistance analysis process while reducing sequencing costs. In contrast to Sanger DNA sequencing which provides an average sequence of the virus population, NGS provides nucleotide sequence information for individual viruses within a virus population, potentially providing millions of short sequences per sample. The complexity of the data makes it challenging for virology reviewers to analyze and validate the sequence information, which is complicated by the fact, as mentioned above, that there are currently no standard bioinformatics analysis approaches for analyzing these large datasets. Moreover, nearly every sponsor performing NGS has developed their own proprietary bioinformatics analysis pipeline. Given that there are over two hundred assembly algorithms alone, it is expected that each pipeline will provide a unique interpretation of the data.

Currently, industry is rapidly adopting the use of NGS technology in support of product development and application submissions. This has created unique review challenges for CDER where no NGS data analysis/review capabilities had previously existed. To address this gap in the review process which could have a significant impact on public health, DAVP teamed up with CDER's Computational Science Center to develop an independent NGS analysis pipeline that would allow virology reviewers to perform a robust and independent analysis of NGS resistance datasets submitted in support of antiviral drugs in development.

NGS DATA ANALYSIS PIPELINE

The Division of Antiviral Products worked with the Office of Scientific Computing within CDER to acquire the resources to analyze NGS data for review purposes. The CLC Genomics Workbench was installed for use on the High Performance Computer at CDRH and was used to establish an analysis pipeline for independently analyzing NGS data. CLC Genomics was used to evaluate each of the sequence runs, trim and filter the sequences prior to mapping, and to map the sequences to HCV GT1, GT1b, GT2a, GT2b and GT3a reference sequences. Two independent variant detection algorithms were used to call variants from each mapping, and the variant tables were exported from CLC Genomics Workbench and combined to generate frequency tables and resistance summary tables (Figure 1).

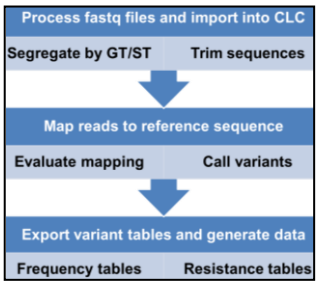

Figure 1. An overview of the NGS Analysis Pipeline using CLC Genomics Workbench.

NGS Analysis Parameters and Overview of Data Analysis

Each step of the analysis process is described in detail below.

1. **Processing fastq files with CLC Genomics Workbench**. Data were received on a portable hard drive, which included fastq files for each subject and timepoint that was sequenced using the Illumina platform. The sequences were uploaded via the CLC Genomics interface, using the Illumina specific criteria. Failed reads were removed, read names were discarded, and Quality scores were calculated using the NCBI/Sanger (Illumina Pipeline 1.8) option.
2. **Segregating sequences by HCV genotype and trimming the sequence reads prior to mapping.** The fastq files were separated by genotype and subtype and the NS5B genes for HCV GT1a (H77), GT1b (Con1), GT2a (JFH1), GT2b (HC−J8), GT3a (S52) were imported and annotated as coding sequences and used as the reference sequences. The individual reads from each fastq file were subjected to trimming using the default parameters for CLC Genomics Workbench and these are defined in Figure 2.

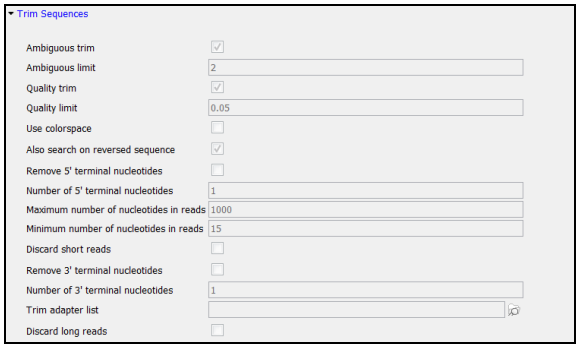

Figure 2. CLC Genomics Workbench default trimming parameters.

3. **Mapping reads to the appropriate reference sequence for each HCV genotype and subtype.** The reads from each fastq file were aligned to the appropriate reference sequence to generate a mapping for each timepoint (Figure 3). The mapping contained the target of interest (the NS5B gene sequence) and was used to generate a consensus sequence for each sequence run. The consensus sequences were conceptually translated to amino acid sequences to compare changes that occurred at the amino acid level.

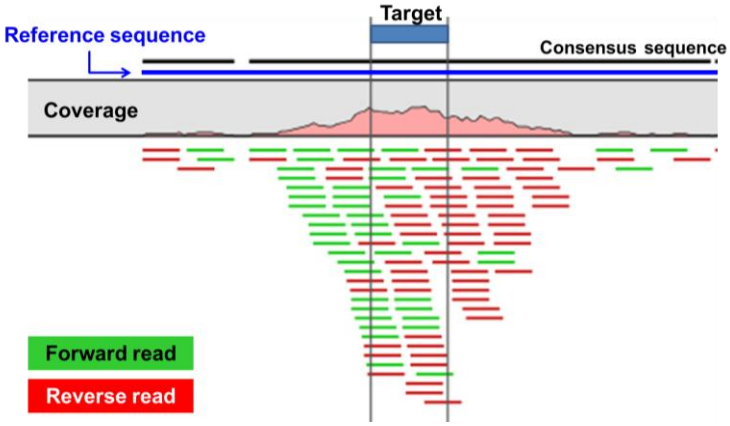

Figure 3. Schematic representation of a sequence mapping.

In general, the mappings were assessed to determine the depth of coverage at each nucleotide position and to evaluate read directionality (ratio of forward to reverse reads) to identify regions of bias. The parameters used for the mapping are shown in Figure 4.

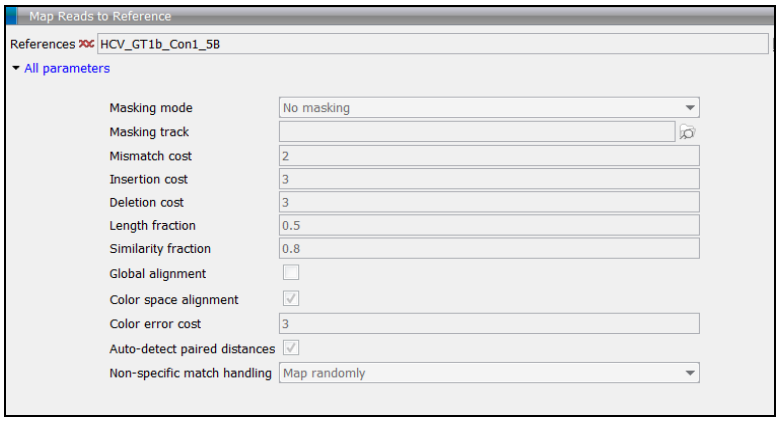

Figure 4. Parameters for mapping sequence reads to the reference sequence.

NGS Analysis Pipeline Output

4. **Generating frequency tables of amino acid substitutions.** From the read mappings, two algorithms were used to call variants based on independent criteria, and variant tables were generated for each sequence run and variant detection method. The variant tables included the following column headers: Reference Position, Type, Length Reference, Allele Linkage, Zygosity, Count Coverage, Frequency, Forward/reverse balance, Average quality, Overlapping annotations, Coding region change, and Amino acid change. The two variant detection systems employed different strategies for calling variants, and the variant detection parameters were relaxed from default to maximize the number of variants called, given that true variants would likely be

identified in multiple subjects, allowing those that were of low quality or probability to be filtered out at the analysis stage. The two detection methods were:

1. **Probabilistic Variant detection (PVD75)** – calls variants from a read mapping using a probabilistic model (combines a Bayesian model and a Maximum Likelihood approach to calculate prior and error probabilities). Parameters are calculated on the mapped reads without considering the reference sequence. The variant probability parameter was reduced from a default value of 90 to 75 to increase the number of variant calls, given that false calls would likely be filtered during data analysis.

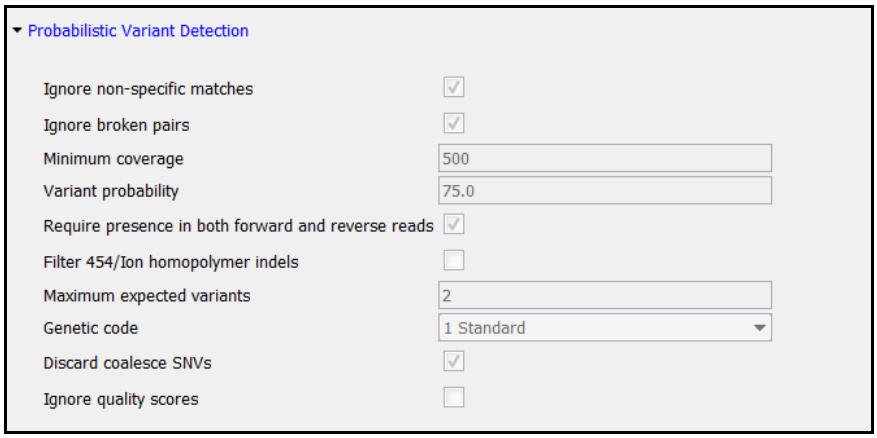

Figure 5. Parameter settings used for Probabilistic Variant Detection. The Variant Probability was set to 75% in order to increase the likelihood of a variant call, to provide a comprehensive view of potential resistance-associated substitutions.

2. **Quality-based variant detection (QbVD)** - based on the Neighborhood Quality Standard algorithm, it uses a combination of quality filters and user-specified thresholds for coverage and frequency to call variants covered by aligned reads. The parameters used for this algorithm are shown in Figure 6.

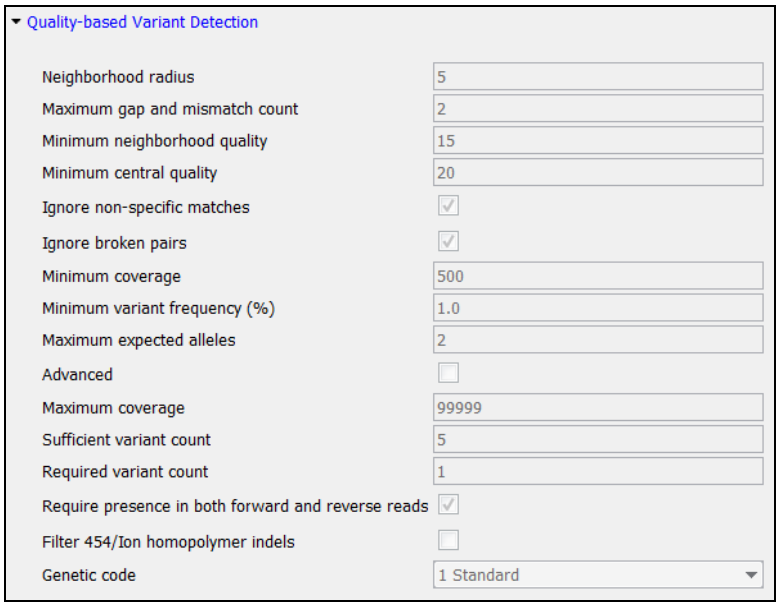

Figure 6. Parameter settings used for Quality based Variant Detection. The minimum variant frequency (%) was set at 1% in order to increase the likelihood of a variant call, to provide a comprehensive view of potential resistance-associated substitutions.

Frequency tables were generated by exporting the variant tables for both variant detection methods (PVD75 and QBVD) for each mapping and then reformatting the data to reflect variation at the amino acid level with these pertinent changes (Figure 7):

1. The variant tables were combined by genotype/subtype and study
2. The variant tables were filtered to remove synonymous substitutions
3. The variant tables were reformatted to be directly comparable to the frequency tables submitted by the sponsor

Reference Position	Type	Length	Reference	Allele	Linkage	Zygosity	Count	Coverage	Frequency	Forward/reverse balance	Average quality	Overlapping annotations	Coding region change	Amino acid change
876	SNV	1	A	T		Homozygous	20821	20861	99.81	0.49	35.62	CDS: NS5B	NS5B:c.876A>T	
898	SNV	1	A	G		Homozygous	19843	19907	99.68	0.50	34.49	CDS: NS5B	NS5B:c.898A>G	NS5B:p.Thr300Ala
906	SNV	1	C	T		Homozygous	19783	19861	99.61	0.50	35.22	CDS: NS5B	NS5B:c.906C>T	
909	SNV	1	G	A		Heterozygous	19537	20325	96.12	0.48	36.59	CDS: NS5B	NS5B:c.909G>A	
911	SNV	1	A	G		Heterozygous	18571	18928	98.11	0.48	36.05	CDS: NS5B	NS5B:c.911A>G	NS5B:p.Lys304Arg
915	SNV	1	C	T		Heterozygous	884	20985	4.21	0.50	35.81	CDS: NS5B	NS5B:c.915C>T	
931	SNV	1	C	T		Homozygous	20592	20634	99.80	0.48	35.36	CDS: NS5B	NS5B:c.931C>T	NS5B:p.Pro311Ser
954	SNV	1	T	C		Homozygous	20129	20171	99.79	0.49	34.98	CDS: NS5B	NS5B:c.954T>C	
958	SNV	1	T	C	5	Homozygous	18696	18751	99.71	0.49	35.72	CDS: NS5B	NS5B:c.958T>C	
960	SNV	1	G	A	5	Homozygous	18696	18751	99.71	0.49	35.72	CDS: NS5B	NS5B:c.960G>A	
990	SNV	1	T	C		Homozygous	18935	18950	99.92	0.47	34.14	CDS: NS5B	NS5B:c.990T>C	
1003	SNV	1	G	A		Homozygous	20859	20995	99.35	0.49	35.41	CDS: NS5B	NS5B:c.1003G>A	NS5B:p.Ala335Thr
1053	SNV	1	A	G		Homozygous	21449	21469	99.91	0.49	35.89	CDS: NS5B	NS5B:c.1053A>G	
1056	SNV	1	T	C		Heterozygous	18948	19321	98.07	0.50	34.00	CDS: NS5B	NS5B:c.1056T>C	
1059	SNV	1	C	T		Homozygous	20312	20373	99.70	0.48	34.73	CDS: NS5B	NS5B:c.1059C>T	
1065	SNV	1	G	A		Homozygous	21661	21708	99.78	0.50	35.65	CDS: NS5B	NS5B:c.1065G>A	
1068	SNV	1	C	T		Homozygous	20078	20121	99.79	0.49	33.91	CDS: NS5B	NS5B:c.1068C>T	

USUBJID	NGSPL	VISIT	NS5B AAPOS	TCOV	AAREF	AASUB	VCOV	AAFREQ
GS-US-334-0108-1594	ILLUMINA	FUWK8	206	27626	K	E	27492	0.995
GS-US-334-0108-1594	ILLUMINA	FUWK4	300	19907	T	A	19843	0.997
GS-US-334-0108-1594	ILLUMINA	FUWK8	300	25788	T	A	25623	0.994
GS-US-334-0108-1594	ILLUMINA	Baseline	304	19599	K	R	17480	0.892
GS-US-334-0108-1594	ILLUMINA	FUWK4	304	18928	K	R	18571	0.981
GS-US-334-0108-1594	ILLUMINA	FUWK8	304	25025	K	R	24630	0.984
GS-US-334-0108-1594	ILLUMINA	FUWK4	311	20634	P	S	20592	0.998
GS-US-334-0108-1594	ILLUMINA	FUWK8	311	27175	P	S	27081	0.997
GS-US-334-0108-1594	ILLUMINA	Baseline	335	23605	A	T	23334	0.989
GS-US-334-0108-1594	ILLUMINA	FUWK4	335	20995	A	T	20859	0.994
GS-US-334-0108-1594	ILLUMINA	FUWK8	335	27805	A	T	27679	0.995
GS-US-334-0108-1594	ILLUMINA	Baseline	376	23285	D	N	15191	0.652
GS-US-334-0108-1594	ILLUMINA	Baseline	376	23285	D	S	7902	0.339
GS-US-334-0108-1594	ILLUMINA	FUWK4	376	20022	D	S	19907	0.994
GS-US-334-0108-1594	ILLUMINA	FUWK8	376	26563	D	S	26379	0.993
GS-US-334-0108-1594	ILLUMINA	Baseline	379	24452	R	K	24328	0.995
GS-US-334-0108-1594	ILLUMINA	FUWK4	379	21710	R	K	21621	0.996

Figure 7. Comparing the QbVD table to the frequency table. The variant detection table for the Quality based Variant Detection (QbVD) method for subject GS-US-334-0108-1594 of clinical trial GS-US-334-0108 is shown in the top panel. This table shows information at the nucleotide level, with variation defined as differences from the reference sequence. The frequency table (bottom panel) contains the appropriate information for each subject at the amino acid level. **USUBJID**, Unique Subject ID; **NGSPL**, NGS Sequencing platform; **VISIT**, visit timepoint; **NS5B AAPOS**, gene name and amino acid position; **AAREF**, amino acid present in the reference sequence; **AASUB**, amino acid substitution; **TCOV**, total coverage; **VCOV**, variant coverage (number of reads that contain the nucleotides encoding the substitution); and **AAFREQ**, the frequency of the variant.

5. **Generating resistance analysis tables.** An ETL/Kettle script was developed to convert the frequency tables into resistance analysis tables, allowing the resistance tables to be populated using different frequency thresholds. For example, the frequency tables generated from CLC Genomics Workbench output or submitted by the sponsor contained all variants with a frequency

greater than or equal to 1%, and this tool allowed resistance analysis tables to be generated showing variants at different levels of sensitivity (5%, 15%, 25%, etc.) as defined by the user. Figure 8 shows a frequency table and three resistance analysis tables generated from the same frequency table at different levels of sensitivity (0, 0.05, and 0.20). A background threshold of 1% was chosen as preliminary analysis of the sponsor's "mock" dataset found cross-contamination of genotypes at this level (IND106739 SDN 286).

USUBJID	NGSPL	VISIT	NS5B AAPOS	TCOV	AAREF	AASUB	VCOV	AAFREQ
P7977-1231-310601	ILLUMINA	Baseline	376	14686	D	N	14610	0.995
P7977-1231-310601	ILLUMINA	FUWK12	376	18250	D	N	18157	0.995
P7977-1231-310601	ILLUMINA	FUWK8	376	14198	D	N	14145	0.996
P7977-1231-310601	ILLUMINA	Baseline	377	13984	K	R	2419	0.173
P7977-1231-310601	ILLUMINA	Baseline	377	13984	K	G	11428	0.817
P7977-1231-310601	ILLUMINA	FUWK12	377	17418	K	G	17345	0.996
P7977-1231-310601	ILLUMINA	FUWK8	377	13477	K	G	13442	0.997
P7977-1231-310601	ILLUMINA	Baseline	379	15582	R	K	15508	0.995
P7977-1231-310601	ILLUMINA	FUWK12	379	19465	R	K	19365	0.995
P7977-1231-310601	ILLUMINA	FUWK8	379	15418	R	K	15354	0.996
P7977-1231-310604	ILLUMINA	Baseline	374	13210	R	W	412	0.031
P7977-1231-310604	ILLUMINA	Baseline	374	13152	R	Q	758	0.058
P7977-1231-310604	ILLUMINA	Baseline	374	13152	R	L	10287	0.782
P7977-1231-310604	ILLUMINA	FUWK12	374	16965	R	L	14471	0.853
P7977-1231-310604	ILLUMINA	FUWK4	374	17387	R	L	14787	0.850
P7977-1231-310604	ILLUMINA	Baseline	376	13553	D	N	13419	0.990
P7977-1231-310604	ILLUMINA	FUWK12	376	18848	D	N	18741	0.994
P7977-1231-310604	ILLUMINA	FUWK4	376	19796	D	N	19719	0.996
P7977-1231-310604	ILLUMINA	Baseline	379	15368	R	K	15106	0.983
P7977-1231-310604	ILLUMINA	FUWK12	379	18855	R	K	18741	0.994
P7977-1231-310604	ILLUMINA	FUWK4	379	20314	R	K	20216	0.995

$\omega_t = 0$

USUBJID	VISIT	NGSPL	NS5B 373	NS5B 374	NS5B 375	NS5B 376	NS5B 377	NS5B 378	NS5B 379	NS5B 380	NS5B 381	NS5B 382
P7977-1231-310601	Baseline	ILLUMINA				N	G,R		K			
P7977-1231-310601	Baseline	ILLUMINA										
P7977-1231-310601	FUWK12	ILLUMINA				N	G		K			
P7977-1231-310601	FUWK8	ILLUMINA				N	G		K			
P7977-1231-310604	Baseline	ILLUMINA		L,Q		N			K			
P7977-1231-310604	Baseline	ILLUMINA		W								
P7977-1231-310604	FUWK12	ILLUMINA		L		N			K			
P7977-1231-310604	FUWK4	ILLUMINA		L		N			K			

$\omega_t = 0.05$

USUBJID	VISIT	NGSPL	NS5B 373	NS5B 374	NS5B 375	NS5B 376	NS5B 377	NS5B 378	NS5B 379	NS5B 380	NS5B 381	NS5B 382
P7977-1231-310601	Baseline	ILLUMINA				N	G,R		K			
P7977-1231-310601	Baseline	ILLUMINA										
P7977-1231-310601	FUWK12	ILLUMINA				N	G		K			
P7977-1231-310601	FUWK8	ILLUMINA				N	G		K			
P7977-1231-310604	Baseline	ILLUMINA		L,Q		N			K			
P7977-1231-310604	Baseline	ILLUMINA										
P7977-1231-310604	FUWK12	ILLUMINA		L		N			K			
P7977-1231-310604	FUWK4	ILLUMINA		L		N			K			

$\omega_t = 0.20$

USUBJID	VISIT	NGSPL	NS5B 373	NS5B 374	NS5B 375	NS5B 376	NS5B 377	NS5B 378	NS5B 379	NS5B 380	NS5B 381	NS5B 382
P7977-1231-310601	Baseline	ILLUMINA				N	G		K			
P7977-1231-310601	Baseline	ILLUMINA										
P7977-1231-310601	FUWK12	ILLUMINA				N	G		K			
P7977-1231-310601	FUWK8	ILLUMINA				N	G		K			
P7977-1231-310604	Baseline	ILLUMINA		L		N			K			
P7977-1231-310604	Baseline	ILLUMINA										
P7977-1231-310604	FUWK12	ILLUMINA		L		N			K			
P7977-1231-310604	FUWK4	ILLUMINA		L		N			K			

Figure 8. Generating resistance analysis tables from frequency tables at different levels of sensitivity. A frequency table is shown for subjects P7977-1231-310601 and -310604 at NS5B amino acid positions 374 to 379 (top panel). Resistance analysis tables were generated at frequency thresholds of 0, 0.05 and 0.20 (bottom panels) for these subjects at NS5B amino acid positions 373 to 382. Blank spaces represent amino acid positions that had no variation for a given timepoint as compared to the reference sequence. ω_t = frequency threshold.

6. **Conducting independent resistance analysis.** The frequency tables and resistance analysis tables were then analyzed to identify substitutions that occurred above an arbitrary frequency threshold. Three filtering thresholds were tested, including:

   a. **SUBS20** – Identified all substitutions that were different by a frequency of 0.20 compared to baseline (i.e., 0.10 at baseline to 0.30 at a later timepoint or not present at

baseline but present at 0.20 or greater at later timepoints or present at baseline at 0.20 but not present at later timepoints).

b. **SUBS75** - Identified all substitutions that were different by a frequency of 0.75 compared to baseline (using the same criteria as for SUBS20).

c. **SUBS10+-** – Identified all substitutions that were absent at baseline (<0.01 frequency) but present at a frequency of 0.10 or greater at later timepoints or present at baseline at 0.10 and disappeared at later timepoints (Figure 9).

All three filtering thresholds identified the major substitutions associated with resistance and showed good overlap; however, SUBS10+- identified substitutions that were more likely to be associated with resistance and reduced the amount of background noise (substitutions at polymorphic sites, substitutions occurring in only one subject, etc.).

USUBJID	NGSPL	VISIT	NS5B AAPOS	TCOV	AAREF	AASUB	VCOV	AAFREQ	DIFF	SUB20	SUBS75	SUBS10+-	VARDECT
P7977-1231-310578	LLUM NA	FUWK8	50	29040	R	K	28952	0.997	0 000				QbVD
P7977-1231-310578	LLUM NA	FUWK4	66	31422	T	I	29707	0.945	0 945	**T66I**	**T66I**	**T66I**	QbVD
P7977-1231-310578	LLUM NA	Baseline	114	10421	R	K	450	0.043					QbVD
P7977-1231-310578	LLUM NA	Baseline	116	10522	I	V	1995	0.190					QbVD
P7977-1231-310578	LLUM NA	FUWK8	116	17631	I	V	15506	0.879	0 690	**I116V**			QbVD
P7977-1231-310578	LLUM NA	Baseline	117	10676	D	N	10657	0.998					QbVD
P7977-1231-310578	LLUM NA	FUWK4	117	9081	D	N	9073	0.999	0 001				QbVD
P7977-1231-310578	LLUM NA	FUWK8	117	17903	D	N	17891	0.999	0 001				QbVD
P7977-1231-310578	LLUM NA	Baseline	130	12669	T	N	158	0.012					QbVD
P7977-1231-310578	LLUM NA	Baseline	150	13867	A	V	6702	0.483					QbVD
P7977-1231-310578	LLUM NA	FUWK8	150	18234	A	V	16066	0.881	0 398	**A150V**			QbVD
P7977-1231-310578	LLUM NA	Baseline	185	15723	E	A	15571	0.990					QbVD
P7977-1231-310578	LLUM NA	FUWK4	185	13099	E	A	13058	0.997	0 007				QbVD
P7977-1231-310578	LLUM NA	FUWK8	185	18772	E	A	18600	0.991	0 001				QbVD
P7977-1231-310578	LLUM NA	Baseline	189	16684	S	P	326	0.020					QbVD
P7977-1231-310578	LLUM NA	Baseline	215	15342	L	M	254	0.017					QbVD
P7977-1231-310578	LLUM NA	FUWK4	221	12930	T	I	12308	0.952	0 952	**T221I**	**T221I**	**T221I**	QbVD

Figure 9. Identifying substitutions in the fequency tables. For emergence analysis, amino acid substitutions were compared to the values present at baseline and the differences are shown in the Diff column. SUBS20 identified substitutions using a 0.20 threshold, SUBS75 identified substitutions using a 0.75 threshold and SUBS10+- identified substitutions using 0.10 threshold (described in the main text). VARDECT, reports the variant detection method used to identify the substitutions. This is a portion of the frequency table generated by QbVD for subject P7977-1231-310578. Green, frequencies that are greater than or equal to 0.10; yellow, shows baseline amino acid frequencies.

Specific sites of interest were also plotted to determine how the frequency of a substitution changed over time (Figure 10). It should be noted that these frequency changes do not necessarily reflect changes in the absolute levels of the specific HCV RNA expressing the indicated variant, but could change as a result of an increase or decrease in other HCV RNA variants.

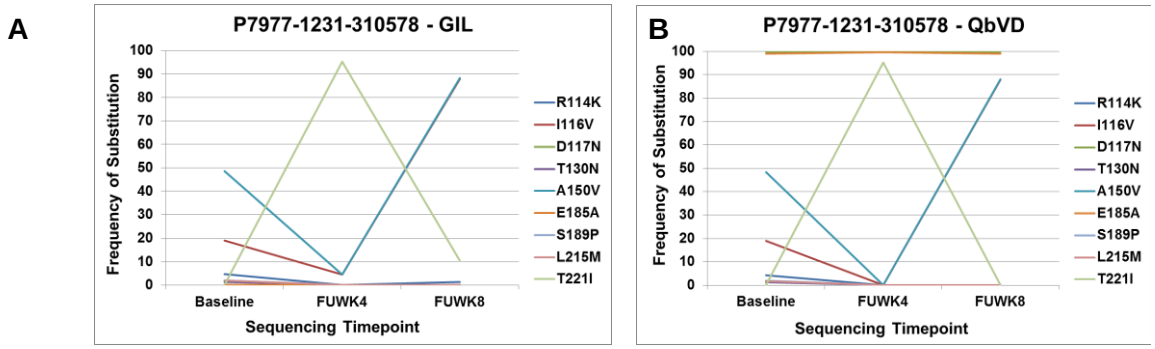

Figure 10. Comparing resistance profiles at NS5B amino acid positions 114-221 for subject P7977-1231-310578. There was reasonably good agreement between frequencies reported by the sponsor (panel A) and those calculated using the QbVD variant detection method (panel B). Similar results were obtained with the PVD75 method.

NGS Data Comparison

7. **Comparing results to those submitted by the sponsor.** The remainder of this review provides details on how the NGS data submitted by the sponsor was independently evaluated using the above described NGS analysis pipeline. In general, the NGS data analysis was performed using data generated in this pipeline and provided by the sponsor, and the results were compared as follows:
    a. Frequency and resistance analysis tables were compared directly and major differences were noted
    b. Amino acid substitutions were identified by the three algorithms (the sponsor's algorithm (GIL) and QbVD and PVD75 used by DAVP) and major differences between algorithms were reported
    c. Novel resistance-associated amino acid substitutions reported by different NGS analysis approaches were compared and major differences were reported
    d. NGS analysis results were compared to results obtained and reported by the sponsor using Sanger population sequencing
    e. Novel resistance-associated substitutions identified by the independent analysis were noted and discussed with the review team for potential labeling/post-marketing actions

CLINICAL STUDIES

The sponsor submitted NGS data for four Phase 3 clinical trials and five Phase 2 clinical trials that were provided to support the NDA for GS-7977 (sofosbuvir; SOF). This portion of the NDA review focuses exclusively on the independent analysis of NGS data from the four Phase 3 clinical trials. The primary review of the pivotal Phase 3 trials and overall conclusions drawn from Phase 2 and Phase 3 resistance data can be found in the review of Clinical Virology Reviewer Lisa Naeger, Ph.D.

The NGS data submitted for four Phase 3 clinical trials included:

1. Raw sequence data in fastq format
2. Frequency tables showing the frequency of amino acid substitutions at each timepoint
3. Resistance analysis tables populated at a 15% cutoff (see Figure 8 for details)
4. Summary of resistance associated substitutions

There were a total of 676 NGS files for the four Phase 3 studies for 224 subjects, with study P7977-1231 (FISSION) containing the most subjects sequenced (n=78) and the most NGS files (n=308) (Table 1).

Table 1. NGS data files submitted for the four pivotal Phase 3 trials for sofosbuvir.

Clinical Trial	No. Subjects with NGS data	No. NGS files
P7977-1231 (FISSION)	78	308
GS-US-334-0107 (POSITRON)	41	115
GS-US-334-0108 (FUSION)	76	189
GS-US-334-0110 (NEUTRINO)	29	64
Totals	224	676

REVIEW OF GS-US-334-0107 (POSITRON)

GS-US-334-0107 (POSITRON) was a Phase 3, randomized, double-blind, placebo-controlled study evaluating the efficacy and safety of 12 weeks of SOF+RBV in subjects with chronic GT2 or GT3 HCV infection who were IFN intolerant, IFN ineligible, or unwilling to take IFN. This trial enrolled subjects infected with HCV GT2

(n=109) and HCV GT3 (n=98) and compared the SVR12 rates to a placebo arm (n=71). The sponsor reported an overall SVR rate of 77.8% for this trial, with HCV GT2 subjects achieving an SVR12 rate of 92.7% and HCV GT3 subjects achieving a SVR12 rate of 77.8% (Table 2).

Table 2. Summary of baseline characteristics and viral response in GS-US-334-0107 (Table 8, page 26, Integrated Virology Study Report for the Phase 2/3 Clinical Studies of Sofosbuvir).

	n/total number of subjects (% of total)	
	SOF+RBV (n = 207)	Placebo (n = 71)
SVR 12 Overall	161/207 (77.8%)	0/71
SVR12 Genotype 2	101/109 (92.7%)	0/34
SVR12 Genotype 3	60/98 (61.2%)	0/37

Among the 47 failures in this trial, 41 subjects who did not achieve SVR12 had samples with HCV RNA >1,000 IU/mL, qualifying them for resistance analysis. Of these, 39 virologic failures were examined by NGS, including 35 from subjects infected with GT3a and six subjects infected with HCV GT2 of various subtypes (Figure 11). There were no on-treatment failures in this trial, so all of the NGS data was for subjects who relapsed after treatment with SOF in trial GS-US-334-0107 (predominantly GT3a).

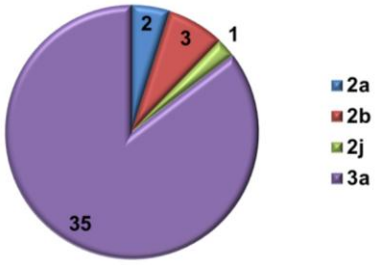

Figure 11. HCV GT distribution of failures with NGS data for GS-US-334-0107.

GS-US-334-0107 Baseline Sequence Data

The sponsor reported that Sanger population nucleotide sequence analysis of the HCV NS5B gene was successfully obtained at baseline for all 278 subjects with a partial NS5B fragment from 12 subjects. As a result of the NS5B sequence and phylogenetic analysis, further refinement or correction of the subtype assignment that was determined at screening by the LiPA assay was determined for 45 (45 of 278, 16.2%) subjects. All of the discrepancies were in HCV GT2 and GT3 and were mostly due to subtype assignment. Sequencing results were compared to the appropriate reference sequence based on genotype/subtype to identify the prevalence of amino acid variants that occurred at baseline. DAVP used the genotype/subtype determined by sequencing for determining the appropriate reference sequence for resistance analysis.

Conclusions reached by the sponsor for baseline sequence analysis using Sanger population sequencing were:

- S282T was not detected in the baseline sequences of any of the 278 subjects in GS-US-334-0107
- T390I, associated with RBV resistance in HCV GT1a (Bartels et al., 2011), was detected in 1 subject at baseline who achieved SVR12
- GT1a RBV associated substitution, F415Y (Young et al., 2003; Ward et al., 2008; Bartels et al., 2011), was not detected at baseline in any subjects
- Known HCV GT2a, 5 and 6 nucleos(t)ide analog polymerase inhibitor (NAPI) resistance substitutions M289L and M289I were observed in 1 and 8 subjects, respectively. All 9 subjects achieved SVR12
- Known HCV GT1 non-nucleos(t)ide analog polymerase inhibitor (NNAPI) substitutions, M423I and Y448H, were detected in one subject each with each subject achieving SVR12

- The presence of T390I, M289L/I, M423I, Y448H, and S556G variants at baseline did not appear to have a negative impact on the viral response of GT2 and GT3 HCV infected subjects treated with SOF+RBV

DAVP compared the baseline sequences, determined by Sanger population sequencing, of subjects achieving SVR12 and subjects who relapsed in the SOF+RBV arm of GS-US-344-0107 (Figure 12). There were no significant differences between baseline sequences of subjects who achieved SVR12 and those who relapsed.

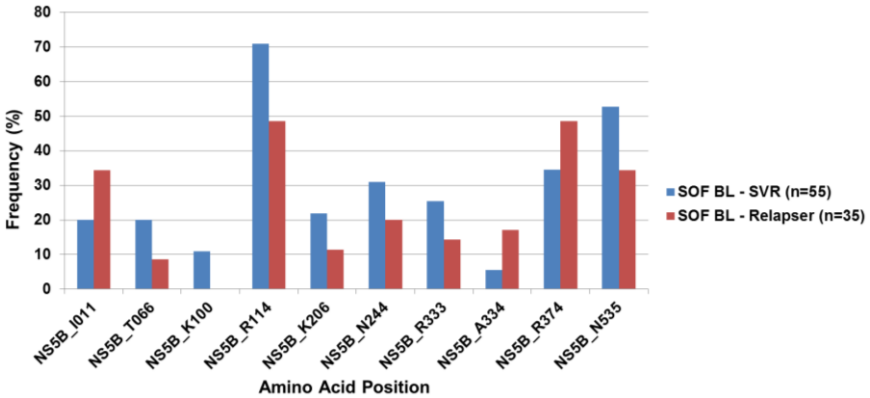

Figure 12. Comparison of baseline sequences between subjects who achieved SVR12 and those that relapsed in the SOF+RBV arm of GS-US-334-0107(DAVP analysis). Only the most variable positions are shown.

GS-US-334-0107 Resistance Analyses in Subjects Experiencing Virologic Failure

The sponsor reported that there were no on-treatment virologic failures observed in any SOF+RBV-treated subjects in this study. Resistance analysis was performed for subjects in the SOF+RBV group who did not achieve SVR12, had HCV RNA >1,000 IU/mL at the last on-treatment time point or at relapse, and had plasma samples available for analysis. There were 41 subjects in the resistance analysis population (RAP)(Figure 11). Thirty five of these were GT3a, 2 were GT2a, 3 were GT2b, and 1 was GT2j.

Deep sequencing of NS5B using the Illumina platform (described in Methods) with a 1% assay cut-off was attempted at both baseline and virologic failure time points for subjects in the RAP. The sponsor reported that full-length NS5B was successfully amplified and NGS results were obtained from 34 (4 GT2 infected subjects, 30 GT3a infected subjects) of 41 subjects at post-treatment Week 4 or Week 12. In addition, the sponsor used an alternative strategy for 6 of the 7 samples that failed to amplify full-length NS5B. For these, a short fragment spanning the coding sequence for amino acid residue 282 was generated and deep sequenced for 5 subjects (4 GT3a infected subjects, 1 GT2 infected subject) and population sequenced for 1 subject (GT3a). Thus, a total of 40 subjects had HCV NS5B sequence data post-treatment covering amino acid 282, 39 of which had NGS data and only 34 subjects had the full length polymerase gene sequenced.

The sponsor identified five substitutions that were detected in ≥2 subjects (Table 3) and arrived at the following conclusions using the combined NGS and population sequence data generated for these subjects:

- 5 substitutions were observed in >2 subjects by consensus sequences: V11I, V116I, A150V, V480I, and G543S (Table 3)
- These amino acids substitutions were at polymorphic sites and did not cause phenotypic changes to SOF or RBV (Table 3)
- These substitutions appear to be natural variations of the wild-type HCV population
- No clinically significant resistance substitutions, including the S282T substitution in NS5B, were detected among subjects not achieving SVR12 by deep or population sequence analysis

Table 3. Summary of substitutions found by the sponsor in two or more subjects in GS-US-334-0107 (Table 11, page 30, Integrated Virology Study Report for the Phase 2/3 Clinical Studies of Sofosbuvir).

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a]
Sourc

DAVP performed an independent analysis of the NGS data for GS-US-334-0107, using the raw sequence data (fastq sequences) submitted by the sponsor in the NGS Analysis Pipeline described above. Briefly, the reads for each sample were aligned to the appropriate HCV reference sequence for the NS5B polymerase gene. Variants were called using two algorithms, PVD75 and QbVD, and the variant tables were combined to generate frequency tables showing all amino acid substitutions that occurred above a frequency ≥1% for a given position within a given sample for each subject. Resistance analysis tables were generated for each frequency table for each variant detection algorithm using a threshold of 0.05, and the three resistance analysis tables were compared to determine how closely the three algorithms agreed with one another. To further assess the overall population-based results generated using the resistance analysis tables, DAVP used the SUBS10+- filtering threshold to look at individual changes occurring at each position in the frequency tables. SUBS10+- used the following criteria to identify substitutions of interest:

1. Identify all substitutions that were detected at a frequency of 0.10 or greater at a later timepoint but were not detected at baseline (<1% at baseline)
2. Ignore all substitutions that were detected with a total coverage of less than 1,000

Using this approach, the resistance analysis tables were used to compare algorithms and identify population based positions of interest and the SUBS10+- threshold for analyzing the frequency tables provided the exact substitutions of each individual subject that met this threshold. This provided a more robust prediction of substitutions that were likely to be associated with resistance. DAVP considered all substitutions to be potential emerging resistance-associated substitutions if they met these criteria: 1) met the SUBS10+- threshold, 2) were detected by 2-of-3 variant detection methods (QbVD, PVD75, or variants calls made by the sponsor), and 3) occurred in 2 or more subjects. This approach was used for all four Phase 3 trials.

Overall, the results obtained by DAVP were comparable to the results reported by the sponsor. DAVP identified 13 substitutions (Table 4, Appendix 1:GS-US-334-0107) that occurred in at least two subjects; however, 2 of the 5 substitutions reported by the sponsor were not detected (V480 in GT3a and R/G543 in GT3a and GT2b, respectively).

Table 4. Potential resistance-associated substitutions identified by DAVP among relapsers in GS-US-334-0107 (DAVP analysis).

STUDYID	NS5B POS	No. Subjects	SUB10 (+/-)	Ratio
GS-US-0334-0107	116	4	V116I/I116V	3-1
	150	3	A150V/T150A/V150T	1-1-1
	247	3	P247L/P247S/S247P	1-1-1
	341	3	E341D	
	11	2	V11I/I11V	1-1
	156	2	P156S/A156P	1-1
	189	2	P189S/S189P	1-1
	207	2	I207M/M207I	1-1
	287	2	A287T/T287A	1-1
	310	2	D310N/S310N	1-1
	438	2	V438I	
	520	2	T520I/V520I	1-1
	558	2	N558T/T558N	1-1

SUBS10(+/-) – Substitutions detected if present at 0.10 or greater frequency at failure timepoint but not detected at baseline.
Ratio – ratio of different substitutions at a position in the order or most to least

Given that most of these substitutions were variable at baseline, occurred at sites known to be polymorphic, substitutions occurred in both directions, and were detected at a low frequency of occurrence, most of these were considered to not be related to virologic failure. However, one of these substitutions, E341D, was detected in 3 HCV GT3a infected subjects and occurs at a position that is 99.87% conserved (as reported by the sponsor), indicating that this may be a substitution of interest. V438I occurred in two subjects, but is only 89.7% conserved and, therefore, a specific conclusion could not be drawn from this analysis.

GS-US-334-0107 Phenotype Analyses

The sponsor performed population phenotypic analyses for 19 of 41 subjects and reported that compared to baseline sequences, 41 substitutions were observed at 38 amino acid positions among the virologic failure timepoints in these samples. The sponsor reported that there was no change in susceptibility to SOF or RBV compared with the corresponding baseline samples or wild-type references observed (Table 5).

Table 5. Summary of phenotypic testing of 19 subjects in GS-US-334-0107 (Table 12, page 31, Integrated Virology Study Report for the Phase 2/3 Clinical Studies of Sofosbuvir).

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Note: DAVP uses phenotypic assessments to confirm resistance associations but lack of a phenotype does not exclude a role in resistance. Modest shifts in susceptibility have been seen for known resistance substitutions of other nucleoside polymerase inhibitors.

GS-US-334-0107 Resistance Analyses Conclusions

The sponsor concluded that no clinically significant substitutions arose during treatment with SOF in clinical trial GS-US-334-0107, in particular, S282T was not detected by NGS or Sanger population sequencing. DAVP largely agrees with the conclusion reached by the sponsor with the exception of E341D in GT3a. This substitution may be resistance-associated and should be further characterized.

REVIEW OF GS-US-334-0108 (FUSION)

GS-US-334-0108 (FUSION) was a Phase 3, randomized, double-blind study evaluating the efficacy and safety of 12 or 16 weeks of SOF+RBV in treatment-experienced subjects with chronic GT2 or GT3 HCV infection. This ongoing Phase 3 study assessed the efficacy and safety of 12 or 16 weeks of SOF+RBV treatment in subjects with chronic GT2 or GT3 HCV infection who had failed prior treatment with an interferon-based regimen. Subjects were randomized in a 1:1 ratio to one of the following 2 treatment groups:

1. SOF+RBV 12 Week group: SOF 400 mg administered once daily + RBV total daily dose of 1000 to 1200 mg administered in a divided daily dose for 12 weeks; followed by SOF placebo administered once daily + RBV placebo administered in a divided daily dose for 4 weeks
2. SOF+RBV 16 Week group: SOF 400 mg administered once daily + RBV total daily dose of 1000 to 1200 mg administered in a divided daily dose for 16 weeks

Out of 201 treated subjects, 195 HCV GT2 or GT3 infected subjects received SOF+RBV and were included in the efficacy analysis. In addition, 6 HCV GT1 infected subjects received SOF+RBV due to incorrect genotyping by the LiPa assay. HCV GT1 infected subjects were not included in the efficacy analysis, but subjects infected with HCV GT1 who failed to achieve SVR12 were included in the resistance analysis population. The sponsor reported that the SVR12 rates for the 12 week treatment arm were 86.1% for HCV GT2 infected subjects and 29.7% for HCV GT3 infected subjects. For the 16 week treatment arm, SVR12 rates were 93.8% for HCV GT2 infection and 61.9% for HCV GT3 infection (Table 6).

Table 6. Summary of viral response data in GS-US-334-0108 (Table 13, page 33, Integrated Virology Study Report for the Phase 2/3 Clinical Studies of Sofosbuvir).

	n/total number of subjects (% of total)	
	SOF+RBV 12 Weeks + Placebo 4 Weeks (n=100)	SOF+RBV 16 Weeks (n=95)
SVR12 Overall	50/100 (50.0%)	69/95 (72.6%)
SVR12 GT 2	31/36 (86.1%)	30/32 (93.8%)
SVR12 GT 3	19/64 (29.7%)	39/63 (61.9%)

The sponsor reported that a total of 77 subjects who relapsed had samples with HCV RNA >1,000 IU/mL and were included in the resistance analysis population (2 for HCV GT1a, 2 for HCV GT1b, 2 for HCV GT2a, 5 for HCV GT2b, 65 for HCV GT3a, and 1 for HCV GT3b), including subjects who were genotyped incorrectly or incompletely by the LiPa assay. Among these 77 subjects, 73 were HCV GT2 and GT3 virologic failures who experienced viral relapse at SVR4 or SVR12 time points. There were no on-treatment failures reported in this trial. Of note, 4 HCV GT1 infected subjects who were mis-genotyped were included in the resistance analysis although these subjects were excluded from the efficacy analysis set (Figure 13).

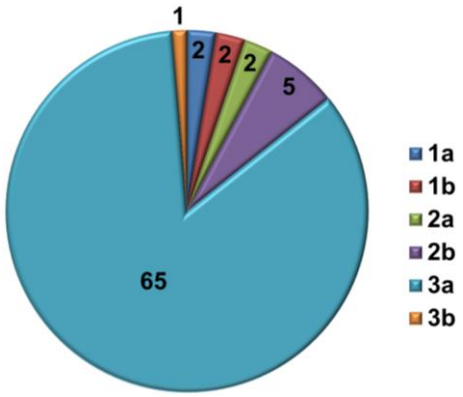

Figure 13. HCV GT distribution of failures analyzed for resistance for GS-US-334-0108.

GS-US-334-0108 Baseline Sequence Data

Population nucleotide sequence analysis of the HCV NS5B gene was performed at baseline for the 201 subjects and the baseline sequences of NS5B were analyzed to determine the number of subjects who had a baseline substitution that was known to associated with resistance to sofosbuvir. Successful sequencing results were obtained in 193 subjects as full-length NS5B sequences and in 8 subjects as a short fragment covering amino acid position 282.

Conclusions reached by the sponsor for baseline sequence analysis using Sanger population sequencing were:

- S282T was not detected in the baseline sequences of any of the 201 subjects in GS-US-334-0108
- T390I, associated in GT1a with failure of a RBV containing regimen (Bartels et al., 2011), was detected in 3 subjects at baseline, and 2 out of 3 achieved SVR12
- Known HCV GT2a, 5 and 6 nucleos(t)ide analog polymerase inhibitor (NAPI) resistance-associated substitution, M289I, was detected in 9 subjects with 8/9 achieving SVR12
- One subject had a substitution at position 419, which was previously shown to be associated with resistance to GT1 non-nucleos(t)ide analog polymerase thumb II inhibitors. This subject achieved SVR12
- The pre-existence of these variants at baseline did not have a negative impact on the viral response of the subjects treated with SOF+RBV

DAVP compared the baseline sequences, determined by Sanger population sequencing, of subjects achieving SVR12 and subjects who relapsed in the SOF+RBV 12 week and 16 week arms of GS-US-334-0108 (Figures 14 and 15).

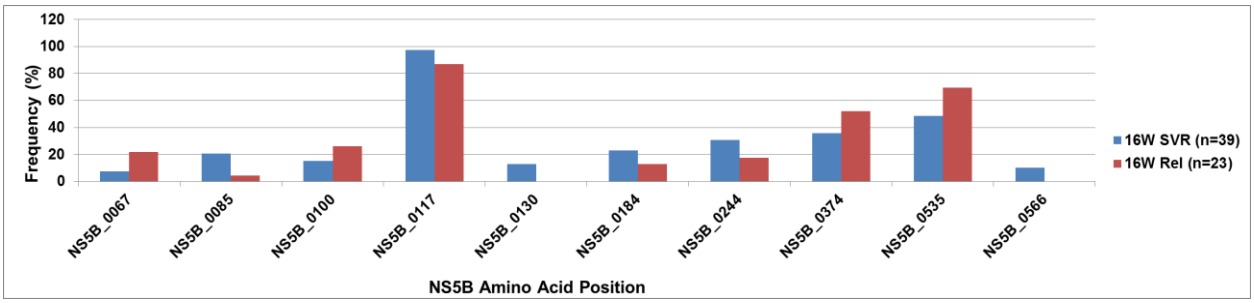

Figure 14. Comparison of baseline sequences between subjects who achieved SVR12 and those that relapsed in the SOF+RBV 16 week arm of GS-US-334-0108(DAVP analysis). Only the most variable positions are shown.

There were no significant differences between baseline sequences of subjects who achieved SVR12 and those who relapsed in the 16 Week arm of GS-US-334-0108. However, 4 substitutions were detected among failures in the 12 Week arm that were not detected in those who achieved SVR12, including substitutions at positions 47, 66, 388, and 473. These occurred at low frequency (<20%) and are unlikely to contribute to failure.

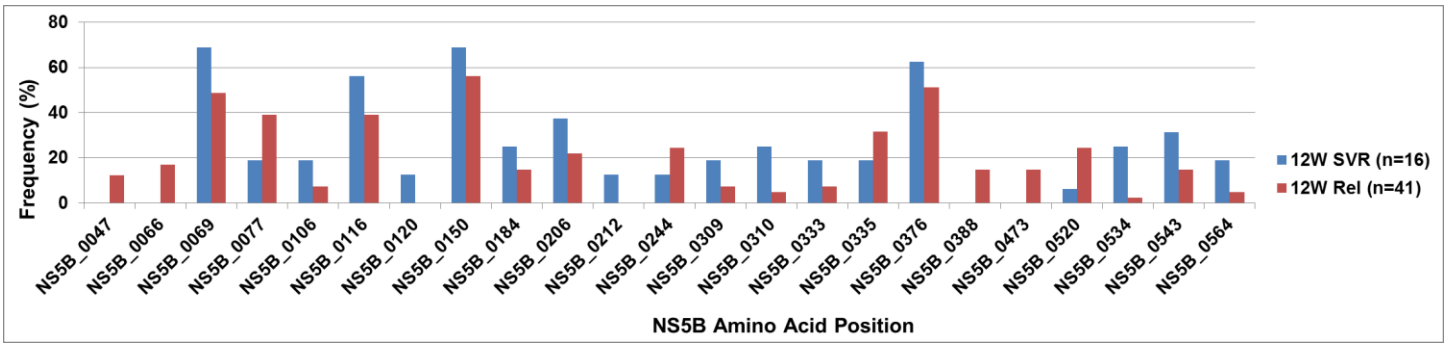

Figure 15. Comparison of baseline sequences between subjects who achieved SVR12 and those that relapsed in the SOF+RBV 12 week arm of GS-US-334-0108 (DAVP analysis). Only the most variable positions are shown.

GS-US-334-0108 Resistance Analyses in Subjects Experiencing Virologic Failure

NGS analysis of NS5B with a 1% assay cut-off (described in Methods) was attempted at both baseline and virologic failure timepoints for the 77 subjects that qualified for resistance analysis (Figure 13). The sponsor reported that full-length NS5B genes were successfully amplified and deep sequencing results were obtained from 70 (61 GT3a infected, 5 GT2 infected, and 4 GT1 infected subjects) out of 77 subjects at post-treatment Week 4 or 12. For the remaining subjects, a short NS5B fragment spanning amino acid position 282 was successfully deep sequenced for 5 other subjects (GT3a) or population sequenced for 2 subjects (both GT3a). The sponsor reported 11 NS5B substitutions that were detected in >2 subjects infected with GT3a (using a 15% cutoff), including V11I, A67V, R77K, V116I/I116V, M147V, D148N, A150T, R517K, K519R, and G543S. The previously identified SOF-associated resistance substitution, S282T, was not detected in any of the 77 subjects' virus at relapse (Table 7).

Table 7. Summary of substitutions found by the sponsor in two or more subjects in GS-US-334-0108 (Table 16, page 37, Integrated Virology Study Report for the Phase 2/3 Clinical Studies of Sofosbuvir).

M147V	
D148N	
A150T	
R517K	
K519R	
G543S	

a EC_{50} fold-
Phenotypic resu
Data Source: G

DAVP performed an independent analysis of the NGS data for GS-US-334-0108, using the raw sequence data (fastq sequences) submitted by the sponsor in the NGS Analysis Pipeline and using the filtering criteria described in resistance analysis section of study GS-US-334-0107 above. Briefly, the reads for each sample were aligned to the appropriate HCV reference sequence for the NS5B polymerase gene. Variants were called using two algorithms, PVD75 and QbVD, and the variant tables were combined to generate frequency tables showing all amino acid substitutions that occurred above a frequency ≥1% for a given position within a given sample for each subject. DAVP used the SUBS10+- filtering threshold to look at individual changes occurring at each position in the frequency tables and considered all substitutions to be potential emerging resistance-associated substitutions if they met these criteria: 1) met the SUBS10+- threshold, 2) were detected by 2-of-3 variant detection methods (QbVD, PVD75, or variants calls made by the sponsor), and 3) occurred in 2 or more subjects.

Overall, the results obtained by DAVP were more comprehensive than the results reported by the sponsor. DAVP identified 32 substitutions (Appendix 2: GS-US-334-0108) that occurred in at least two subjects; including 10 of the 11 substitutions reported by the sponsor. The only substitution that was not detected was R77K. A total of 15 of the 32 substitutions had multiple variants detected at baseline indicating that these were polymorphic sites. Among the remaining 17 substitutions, 3 occurred at highly conserved positions of NS5B amino acid sequence, including positions L159, K211 and P540. Of note, one of these substitutions, L159F, is a known resistance-associated substitution of mericitabine (b) (4) Tong X et al., 2012). This substitution was not reported as a potential resistance-associated substitution by the sponsor nor is it listed in the label. Furthermore, the two additional substitutions may be resistance-associated as these occur in two or more subjects at highly conserved positions of NS5B (Table 8).

Table 8. Potential resistance-associated substitutions identified by DAVP among relapsers in GS-US-334-0108 (DAVP analysis). Only substitutions that occurred in one direction are shown in this table.

STUDYID	NS5B POS	No. Subjects	SUB10 (+/-)	Ratio	Conservation	Substitutions
GS-US-334-0108	543	4	G543S		81.3%	G/S
	184	3	I184T		83.7%	I/T
	11	2	V11I		77.8%	I/V
	47	2	L47Q		94.0%	
	65	2	K65R		95.7%	
	69	2	Q69K		53.1%	K/Q
	71	2	V71I		96.8%	
	100	2	R100K		89.3%	K/R
	147	2	M147V		93.0%	M/V
	159	2	L159F		100.0%	
	211	2	K211R		98.9%	
	250	2	K250R		86.4%	K/R
	304	2	R304K		75.53%	K/R
	357	2	T357I		95.6%	
	519	2	R519K		84.1%	K/R
	540	2	P540L		99.87%	
	564	2	V564M		92.37%	M/V

SUBS10(+/-) – Substitutions detected if present at 0.10 or greater frequency at failure timepoint but not detected at baseline.
Ratio – ratio of different substitutions at a position in the order or most to least
Green – highly conserved positions

GS-US-334-0108 Phenotype Analyses

The sponsor attempted population phenotypic analyses for 6 of 77 subjects whose viruses were analyzed for resistance. Drug susceptibility results are available from 6 of 6 subjects. Compared to baseline sequences, 42 different substitutions over 38 amino acid positions were observed in post-baseline samples. The sponsor did not observe any change in susceptibility to SOF or RBV compared with the corresponding baselines or wild-type reference for any of the 6 subjects at the virologic failure time point (Table 9).

Table 9. Table 1 of phenotypic testing of 6 subjects in GS-US-334-0108 (Table 17, page 38, Integrated Virology rt for the Phase 2/3 Clinical Studies of Sofosbuvir).

Numbe Tes	
a	Phenc

Of note, substitutions L159F, K211R, and P540L were not phenotyped in this study, but L159F was phenotyped in study P7977-1231 (Table 15).

GS-US-334-0108 Resistance Analyses Conclusions

The sponsor concluded that no SOF on-treatment viral breakthroughs were observed in any of the subjects in study GS-US-334-0108 and no resistance-associated substitutions to SOF or RBV were identified by deep or population sequencing in relapse isolates treatment with SOF. Moreover, S282T was not observed at baseline or at time of failure in any subject.

DAVP identified three potential resistance-associated substitutions, including L159F, R211K, and P540L. These substitutions may be resistance-associated and should be further characterized.

REVIEW OF GS-US-334-0110 (NEUTRINO)

GS-US-334-0110 (NEUTRINO) was a Phase 3, single-arm, open-label study evaluating the efficacy and safety of SOF+PEG+RBV for 12 weeks in treatment-naive subjects with chronic GT1, GT4, GT5, or GT6 HCV infection. Subjects were treated for 12 weeks with SOF (400 mg once daily) + PEG (180 µg/week) + RBV (1000 or 1200 mg/day). According to the sponsor, the 327 subjects receiving study drugs had the following characteristics: 225 subjects (68.8%) were infected with HCV GT1a, 66 (20.2%) with HCV GT1b, 28 with HCV GT4 (8.6%), 1 with HCV GT5 (0.3%), and 5 with GT6 (1.8%). One additional subject was infected with both HCV GT1a and GT1b. In addition, 232 subjects (70.9%) had a non-CC (CT or TT) IL28B genotype. The SVR12 rate for overall population (GT1, GT4, GT5 and GT6) was 90.2% while 89.4% of subjects infected with HCV GT1 (GT1a, GT1b, or GT1a/1b) achieved SVR12 (Table 10).

Table 10. Summary of baseline characteristics and viral response in GS-US-334-0110 (Table 18, page 39, Integrated Virology Study Report for the Phase 2/3 Clinical Studies of Sofosbuvir).

	n/total number of subjects (% of total)
	SOF+PEG+RBV (N=327)
SVR12 Overall	295/327 (90.2%)
SVR12 Genotype 1 (1a, 1b, 1a/1b)	261/292 (89.4%)
SVR12 Genotype 1a	206/225 (91.6%)
SVR12 Genotype 1b	54/66 (81.8%)

Among the 32 failures in this trial, 29 subjects who did not achieve SVR12 had samples with HCV RNA >1,000 IU/mL, qualifying them for resistance analysis. Of these, 29 virologic failures were examined by NGS, including 18 from subjects infected with HCV GT1a, 10 from subjects infected with GT1b and 1 subject who was infected with HCV GT4 (Figure 16).

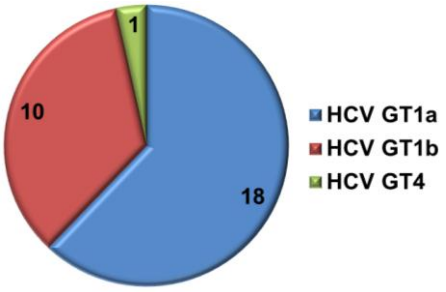

Figure 16. HCV GT distribution of failures with NGS data for GS-US-334-0110.

GS-US-334-0110 Baseline Sequence Data

The sponsor performed Sanger population sequencing of the HCV NS5B gene at baseline for 324 of 327 subjects. Sequences were not available from 3 subjects (2 GT1a and 1 GT1b) due to assay failure. As a result of the NS5B nucleotide sequence analysis, further refinement or correction of the subtype assignment at screening by the LiPA assay was determined for 35 subjects. Reference sequences for each sample were selected based on the HCV GT identified by Sanger population sequencing.

The sponsor arrived at the following conclusions regarding its analysis of baseline sequences:

- S282T was not observed in any of the 324 subjects at baseline
- NS5B substitutions T390I and F415Y, associated with failure of a RBV containing regimen in GT1a subjects (Young et al., 2003; Ward et al., 2008; Bartels et al., 2011), were each detected in 4 subjects at baseline. All 8 subjects achieved SVR12
- Nucleos(t)ide analog polymerase inhibitor (NAPI) resistance-associated substitution, L159F, was observed in 3 subjects infected with HCV GT3a, two of whom achieved SVR12
- Known HCV GT1 non-nucleos(t)ide analog polymerase inhibitor resistance-associated substitutions M414L (n=1), R422K (n=1), M423I (n=3), M423V (n=1), S556G (n=6) and C316N (n=3) were also observed. Except 1 subject with C316N, all subjects achieved SVR12
- Overall, the sponsor concluded that the pre-existence of the RBV (T390I and F415Y) or the NAPI (L159F) or NNAPI (M414L, R422K, M423I, M423V, S556G and C316N) resistance-associated substitutions at baseline did not have a negative impact on the viral response of the subjects to treatment

DAVP compared the baseline sequences, determined by Sanger population sequencing, of subjects achieving SVR12 and subjects who relapsed in the SOF+RBV arm of GS-US-344-0110 (Figure 17). There were no significant differences between baseline sequences of subjects who achieved SVR12 and those who relapsed.

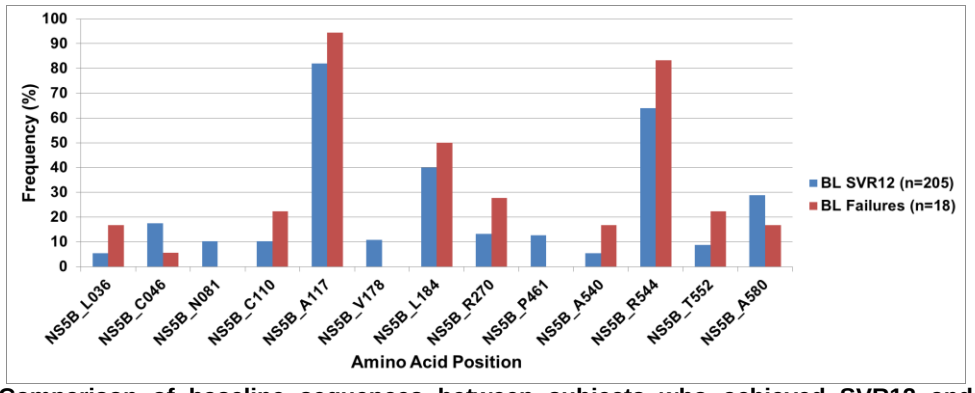

Figure 17. Comparison of baseline sequences between subjects who achieved SVR12 and those that relapsed in the SOF+RBV arm of GS-US-334-0110 (DAVP analysis). Only the most variable positions are shown.

GS-US-334-0110 Resistance Analyses in Subjects Experiencing Virologic Failure

The sponsor performed resistance analysis on the 29 subjects (18 infected with GT1a, 10 infected with GT1b and 1 infected with GT4) who met the criteria, including 28 subjects who had relapsed at post-treatment Week 4 or 12 and 1 subject who discontinued study treatment after 1 week on treatment with HCV RNA of 70 IU/mL at Week 1 and 2,010,000 IU/mL at the post-treatment Week 4 visit). NGS analysis of NS5B with a 1% assay cut-off (described in the Methods) was attempted at both baseline and virologic failure or post-treatment follow-up time points for these subjects. The sponsor was able to obtain full-length NS5B from all 29 subjects at post-treatment Week 4 or 12.

Sanger population sequencing and NGS analyses detected amino acid changes at a number of residues in the NS5B region at the failure timepoints compared with baseline. Most of these amino acid substitutions were identified in only 1 or 2 subjects. Two substitutions were detected in >2 subjects at positions R300 in GT1a and S543 (GT1b) or G543 (GT1a) (Table 11). However, the sponsor determined that no change in susceptibility was observed in the subject isolate containing either substitution and concluded that these substitutions are a natural variation of the wild-type HCV population. The sponsor also reported that S282T was not detected by NGS or Sanger population sequencing at baseline or at failure for any subject in this study.

Table 11. Summary of substitutions found by the sponsor in two or more subjects in GS-US-334-0110 (Table 21, page 42, Integrated Virology Study Report for the Phase 2/3 Clinical Studies of Sofosbuvir).

Acid Changes From Baseline Detected
R300Q
F543S; G543S

AA = amino a
a EC_{50} fold
Source: GS-U

DAVP performed an independent analysis of the NGS data for GS-US-334-0110, using the raw sequence data (fastq sequences) submitted by the sponsor in the NGS Analysis Pipeline and using the filtering criteria described in resistance analysis section of study GS-US-334-0107 above. Briefly, the reads for each sample were aligned to the appropriate HCV reference sequence for the NS5B polymerase gene. Variants were called using two algorithms, PVD75 and QbVD, and the variant tables were combined to generate frequency tables showing all amino acid substitutions that occurred above a frequency ≥1% for a given position within a given sample for each subject. DAVP used the SUBS10+- filtering threshold to look at individual changes occurring at each position in the frequency tables and considered all substitutions to be potential emerging resistance-associated substitutions if they met these criteria: 1) met the SUBS10+- threshold, 2) were detected by 2-of-3 variant detection methods (QbVD, PVD75, or variants calls made by the sponsor), and 3) occurred in 2 or more subjects.

Overall, the results obtained by DAVP were similar to the results reported by the sponsor. DAVP identified 3 substitutions (Appendix 3: GS-US-334-0110) that occurred in at least two subjects; including both substitutions reported by the sponsor (Table 12). However, none of these substitutions is likely to be associated with resistance because in the cases of substitutions R300Q and G543S, both amino acids associated with these positions were observed at baseline in multiple subjects and G556 is 86.73% conserved with S being the most common substitution.

Table 12. Potential resistance-associated substitutions identified by DAVP among relapsers in GS-US-334-0110 (DAVP analysis).

STUDYID	GT/ST	NS5B POS	No. Subjects	SUB10 (+/-)	Ratio
GS-US-334-0110	1a	300	2	R300Q/Q300R	1-1
		543	2	G543S/G543S	1-1
	1b	556	2	G556S	

SUBS10(+/-) – Substitutions detected if present at 0.10 or greater frequency at failure timepoint but not detected at baseline.
Ratio – ratio of different substitutions at a position in the order or most to least

GS-US-334-0110 Phenotype Analyses

The sponsor performed population phenotypic analyses for the NS5B genes of 27 subjects who experienced viral relapse and 1 subject with HCV GT4 infection who relapsed at Week 4 post-treatment but did not have any change from baseline was analyzed as well and did not show any reduced susceptibility to SOF. Drug susceptibility results were obtained for all of these 27 subjects. In total, there were 73 different amino acid substitutions over 62 different positions in the NS5B protein sequences of these subjects. No change in susceptibility to SOF, IFN, or RBV compared with their corresponding baselines or wild-type reference was observed with any of the 27 subjects at the virologic failure time point (Table 13).

Tabl Table 22 **nary of phenotypic testing of 27 subjects in GS-US-334-0110** (Table 22, page 48, Integrated Virol eport for the Phase 2/3 Clinical Studies of Sofosbuvir).

Number Teste
1
1

a No con

GS-US-334-0110 Resistance Analyses Conclusions

Of the 327 subjects enrolled in GS-US-334-0110, 29 met the criteria for resistance analysis with NGS data successfully obtained for all 29 subjects. No resistance-associated substitutions to SOF or RBV were identified by NGS or by phenotypic analyses among subjects not achieving SVR12.

REVIEW OF P7977-1231 (FISSION)

P7977-1231 (FISSION) was a Phase 3, randomized, open-label, active-controlled study evaluating the efficacy and safety of 12 weeks of SOF+RBV compared with 24 weeks of PEG+RBV in treatment-naive subjects with chronic GT2 or GT3 HCV infection. Subjects with GT2 or GT3 HCV infection were randomized in a 1:1 ratio to one of following treatment groups:

1. SOF+RBV: SOF 400 mg + RBV 1000 mg or 1200 mg (based on baseline body weight) daily for 12 weeks
2. PEG+RBV: PEG 180 µg weekly + RBV 800 mg daily for 24 weeks

Of the 499 treated subjects, 253 GT2 or GT3 infected subjects received SOF+RBV while 243 subjects received PEG+RBV. All of the GT2 and GT3 infected subjects were included in the efficacy analysis. In addition, 3 GT1 infected subjects received SOF+RBV, but these subjects were not included in the efficacy analysis. However, they were included in the resistance analysis population. The baseline characteristics were similar between the 2 treatment groups, with 214 subjects bearing the IL28B CC allele (43.1%) and 282 (56.9%) having a non-CC (CT or TT) genotype. Overall, 27.7% of subjects treated with SOF were infected with HCV GT2 and 72.3% were infected with GT3. The SVR12 rates for subjects treated with SOF were 97.1% for GT2 and 55.7% for GT3 (Table 14) compared to 77.6% and 62.5% in the PEG+RBV arm, respectively.

Table 14. Summary of baseline characteristics and viral response in P7977-1231 (Table 3, page 26, Integrated Virology Study Report for the Phase 2/3 Clinical Studies of Sofosbuvir).

	n/total number of subjects (% of total)	
	SOF+RBV (n =253)	PEG+RBV (n =243)
SVR12 Overall	170/253 (67.2%)	162/243 (66.7%)
SVR12 GT 2	68/70 (97.1%)	52/67 (77.6%)
SVR12 GT 3	102/183 (55.7%)	110/176 (62.5%)

Of the 79 virologic failures from the SOF+RBV arm of P7977-1231, 77 subjects had HCV RNA >1,000 IU/mL and were included in the resistance analysis population. Among these treatment failures, 75 had relapsed by post-treatment Week 4 or 12, one relapsed by post-treatment Week 16 and one subject experienced viral breakthrough. The subjects included in the resistance analysis were predominantly infected with HCV GT3a (Figure 18).

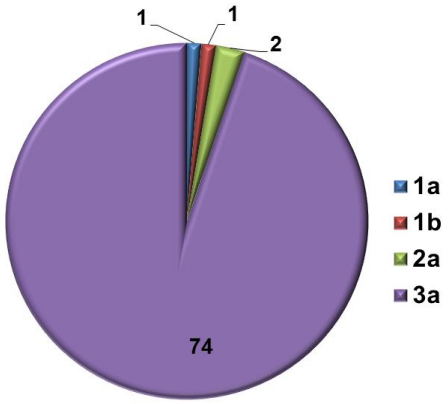

Figure 18. HCV GT distribution of failures with NGS data for P7977-1231.

P7977-1231 Baseline Sequence Data

The sponsor performed Sanger population sequencing of the HCV NS5B gene for baseline samples from 499 subjects participating across the 2 treatment groups of the study, and successfully obtained baseline sequences from 489 subjects (2 GT1a, 1 GT1b, 28 GT2a, 107 GT2b, 349 GT3a, 1 GT3b, and 1 GT3g). The NS5B sequence analysis resulted in a further refinement or correction of the subtype or GT assignment determined at screening by the LiPa assay for 52 (52/489, 10.7%) subjects. Reference sequences for mappings and comparisons were determined based on the Sanger sequence information.

The sponsor's conclusions for baseline sequence analysis using Sanger population sequencing were:

- S282T was not detected in the baseline sequences of any of the 489 subjects in P7977-1231

- T390I, associated in GT1a with failure of a RBV containing regimen (Bartels et al., 2011), was detected in 3 subjects at baseline (2/3 achieved SVR12)
- GT1a RBV associated substitution, F415Y (Young et al., 2003; Ward et al., 2008; Bartels et al., 2011), was not detected at baseline in any subjects
- Known HCV GT2a, 5 and 6 nucleos(t)ide analog polymerase inhibitor (NAPI) resistance substitutions M289L and M289I were observed in 5 and 1 subjects, respectively. All 6 subjects achieved SVR12
- The presence of T390I, M289L/I, and N142T variants at baseline did not appear to have a negative impact on the viral response of subjects treated with SOF+RBV

DAVP compared the baseline sequences, determined by Sanger population sequencing, of subjects achieving SVR12 and subjects who relapsed in the SOF+RBV arm of P7977-1231 (Figure 19). There were no significant differences between baseline sequences of subjects who achieved SVR12 and those who relapsed.

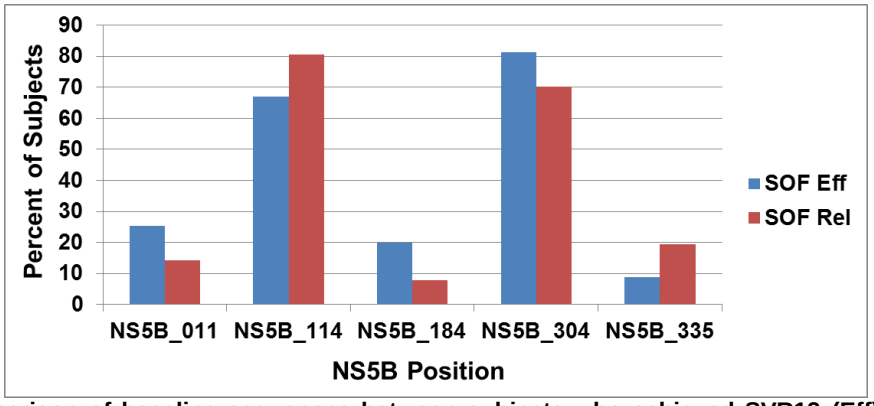

Figure 19. Comparison of baseline sequences between subjects who achieved SVR12 (Eff) and those that relapsed (Rel) in the SOF+RBV arm of P7977-1231 (DAVP analysis). Only the most variable positions are shown.

P7977-1231 Resistance Analyses in Subjects Experiencing Virologic Failure

The sponsor reported that there was one on-treatment virologic failure observed among SOF+RBV-treated subjects in this study. Resistance analysis was performed for subjects in the SOF+RBV group who did not achieve SVR12, had HCV RNA >1,000 IU/mL at the last on-treatment time point or at relapse, and had plasma samples available for analysis. There were 78 subjects in the resistance analysis population (Figure 18).

NGS analysis of NS5B with a 1% assay cut-off (described in Methods) was attempted at both baseline and virologic failure time points for subjects. Full-length NS5B was successfully amplified and NGS results were obtained from 75 of 79 subjects at post-treatment Weeks 4, 12, 16, or 24 time points. For 4 samples from which full-length NS5B failed to amplify, a short fragment covering amino acid residue 282 was generated and deep sequenced for 3 subject samples and population sequenced for 1 subject sample. The sponsor reported that substitution S282T was not detected in any of the 79 subjects in the resistance analysis population by NGS or Sanger population sequencing.

The sponsor reported that there were several amino acid changes at a number of residues in the NS5B region at the failure time points compared with baseline that were detected by Sanger population sequence or NGS analyses. The majority of these amino acid substitutions were identified in only 1 or 2 subjects, with 29 substitutions being observed in >2 subjects by consensus sequences. The sponsor reported that these amino acids substitutions were at polymorphic sites and none of these 29 substitutions caused phenotypic changes to SOF or RBV. Therefore, the sponsor claimed that all 29 substitutions appear to be natural variations of the wild-type HCV population (Table 15).

Table 15. Summary of substitutions found by the sponsor in two or more subjects in P7977-1231 (Table 6, page 23, Integrated Virology Study Report for the Phase 2/3 Clinical Studies of Sofosbuvir).

	H571Y
a	EC_{50} fol
b	Phenoty of site-d

DAVP performed an independent analysis of the NGS data for P7977-1231, using the raw sequence data (fastq sequences) submitted by the sponsor in the NGS Analysis Pipeline and using the filtering criteria described in resistance analysis section of study GS-US-334-0107 above. Briefly, the reads for each sample were aligned to the appropriate HCV reference sequence for the NS5B polymerase gene. Variants were called using two algorithms, PVD75 and QbVD, and the variant tables were combined to generate Frequency tables showing all amino acid substitutions that occurred above a frequency ≥1% for a given position within a given sample for each subject. DAVP used the SUBS10+- filtering threshold to look at individual changes occurring at each position in the Frequency tables and considered all substitutions to be potential emerging resistance-associated substitutions if they met these criteria: 1) met the SUBS10+- threshold, 2) were detected by 2-of-3 variant detection methods (QbVD, PVD75, or variants calls made by the sponsor), and 3) occurred in 2 or more subjects.

Overall, the results obtained by DAVP were similar to the results reported by the sponsor. DAVP identified 41 emergent substitutions overall (Appendix 4: P7977-1231) that occurred in at least two subjects infected with HCV GT3a; including many of the same substitutions reported by the sponsor (Table 16). At least five of these

could be resistance-associated and should be further characterized, including E341D, L159F, K114R, V321A, and R517K. Later analyses indicated that K114R and R517K are likely polymorphic sites (Table 20).

Table 16. Potential resistance-associated substitutions identified by DAVP among virologic failures in P7977-1231 (DAVP analysis). Only substitutions that occur in one direction are shown.

STUDYID	NS5B POS	No. Subjects	SUB10 (+/-)	Ratio
P7977-1231	341	10	E341D	
	159	4	L159F	
	114	3	K114R	
	321	3	V321A	
	517	3	R517K	
	90	2	A90Q/T	1-1
	115	2	A115V	
	131	2	I131T	
	221	2	T221I	
	519	2	R519K	
	520	2	T520I	
	541	2	A541S/T	1-1
	544	2	Q544L/R	1-1
	566	2	R566H	

SUBS10(+/-) – Substitutions detected if present at 0.10 or greater frequency at failure timepoint but not detected at baseline.
Ratio – ratio of different substitutions at a position in the order or most to least

P7977-1231 Phenotype Analyses

The sponsor attempted population phenotypic analyses for 74 of the 79 subjects in the resistance analysis population and drug susceptibility results were successfully obtained from 58 (56 infected with GT3a, 1 infected with GT2 and 1 infected with GT1) of 74 subjects (Table 17). A total of 129 amino acid substitutions compared to baseline at 93 different amino acid positions were assessed among these 58 subject samples. No change in susceptibility to SOF or RBV compared with their corresponding baseline or wild-type reference sequences was observed with any of the 58 subjects at the virologic failure time points. The sponsor concluded that this observation confirmed that these substitutions were not associated with a reduced susceptibility to SOF or RBV.

Table 17. Summary of phenotypic testing of 78 subjects in GS-US-334-0107 (Table 7, page 24, Integrated Virology Study Report for the Phase 2/3 Clinical Studies of Sofosbuvir).

Table 7.

Number o Tested
37/
13/
5/5
1/5
2/5

a No corre
b No basel

P7977-1231 Resistance Analyses Conclusions

The sponsor stated that of 253 subjects treated with SOF+RBV, 79 qualified for resistance analysis. No resistance-associated substitutions to SOF or RBV were identified by deep sequencing (78/79) or population sequencing (1/79) and by phenotypic analyses among subjects not achieving SVR12 or 16. The sponsor concluded that overall, no clinically significant resistance substitutions, including the S282T substitution, in NS5B were detected among subjects with virologic failure by NGS or Sanger population sequencing.

However, DAVP identified six substitutions of interest that should be further characterized to determine if these are associated with resistance to sofosbuvir. Of particular interest are substitutions E341D, L159F, and V321A.

P7977-1231 - Viral Breakthrough of subject P7977-1231-310357

One subject in the SOF+RBV arm of clinical trial P7977-1231 experienced virologic breakthrough between on-treatment Week 4 and Week 8 (Figure 20). However, the sponsor reported that the concentration of GS-331007 (the predominant metabolite of SOF) for this subject was below the quantitation limit of the assay (i.e., <10 ng/mL), which they stated was indicative of noncompliance with study drug.

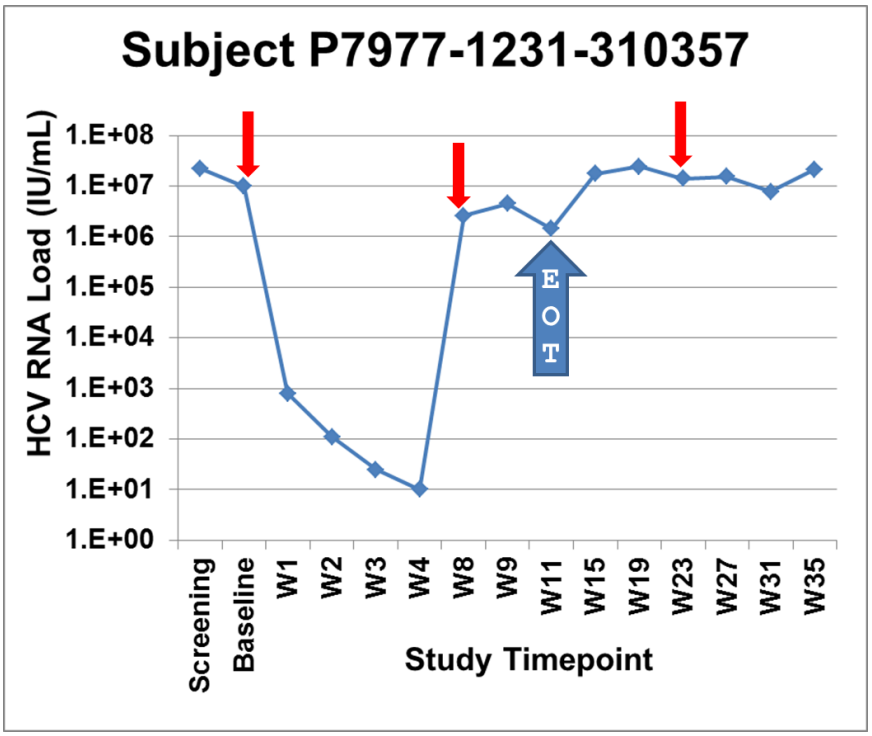

Figure 20. HCV RNA levels for subject P7977-1231-310357 who experienced virologic breakthrough in clinical trial P7977-1231. EOT, end of treatment with SOF; red arrows, timepoints that were analyzed by NGS.

DAVP analyzed the NGS data for this subject to determine if resistance-associated substitutions could be identified. There were 16 amino acid substitutions that differed from the NS5B amino acid reference sequence (HCV GT3a strain S52, Accession no.: GU814263) and 12 of these were present at baseline, at Week 8, and at Follow Up Week (FU-) 12 (Figure 21).

	NS5B_050	NS5B_069	NS5B_077	NS5B_084	NS5B_114	NS5B_117	NS5B_150	NS5B_202	NS5B_304	NS5B_335	NS5B_379	NS5B_473	NS5B_520	NS5B_535	NS5B_542	NS5B_544
3a Reference	R	K	R	T	R	D	A	E	K	A	R	T	I	N	T	Q
Conservation	90.24%	53.07%	59.76%	95.45%	62.17%	93.18%	42.11%	97.73%	75.53%	80.21%	84.49%	94.51%	75.50%	52.07%	96.79%	96.39%
Variants	K/R	K/Q	G/K/R	T	K/R	D/N	A/T/V	E	K/R	A/T	K/R	T	I/T	K/N/R	A	Q
Baseline	K	Q	K	T/S	K	N	A/T	E/D	R	T	K	S		K	A	L
Week 8	K	Q	K	**S**	K	N	**T**	**D**	R	T	K	S		K	A	L
FU-12	K	Q	K	T/S	K	N	**T**	E/D	R	T	K	S	**I/T**	K	A	L

Figure 21. Amino acid differences compared to the GT3a NS5B reference sequence in the HCV GT3a NS5B protein at baseline, Week 8 and FU-12 for subject P7977-1231-310357. 3a Reference, HCV GT3a strain S52 (GU814263); **Conservation**, percent conservation reported by sponsor; **Variants**, most common substitutions observed at that position; **red**, amino acid replacements that were different from baseline; **blank cells** represent agreement with the reference amino acid.

Difference from the reference NS5B peptide sequence that were observed at all timepoints occurred at positions R50K, K69Q, R77K, R114K, D117N, K304R, A335T, R379K, T473S, N535K, T542A and Q544L. Of note, positions 84, 542 and 544 were reported to be greater than 95% conserved by the sponsor (Figure 21). Differences from baseline occurred at positions T84S, A150T, E202D, and I520T. E202D was reported to be highly conserved 97.73%, Figure 21). The frequency tables of all three variant detection methods were analyzed for all amino acid substitutions that differed from baseline, with good agreement observed between all three variant detection approaches. Therefore, only the frequency table and a plot of the substitution frequencies generated using the NGS data provided by the sponsor are shown in Figure 22. Overall, there were no novel substitutions that occurred in post-baseline samples, and only two amino acid positions showed enrichment of ≥20% (T84S increased by 27% frequency by Week 8 compared to baseline and E202D increased by 29.3% frequency by Week 8 compared to baseline). Of note, no substitutions met the criteria of the SUBS10+- threshold. Lower frequency differences were detected at positions M82T, A150T, S473T, and I520T (Figure 22).

USUBJID	NGSPL	VISIT	AAPOS	TCOV	AAREF	AASUB	VCOV	PERCENT	Diff	Subs	Analysis
P7977-1231-310357	Ilumina	Baseline	82	8399	M	T	281	0.033			**97%M/3%T**
P7977-1231-310357	Ilumina	WEEK8	82	9841	M	T	814	0.083	0.049	**M82T**	**92%M/8%T**
P7977-1231-310357	Ilumina	FU-12	82	9012	M	T	1152	0.128	0.094		**87%M/13%T**
P7977-1231-310357	Ilumina	Baseline	84	7519	T	S	4427	0.589			**59%S/41%T**
P7977-1231-310357	Ilumina	WEEK8	84	9037	T	S	7776	0.860	0.272	**T84S**	**86%S/14%T**
P7977-1231-310357	Ilumina	FU-12	84	8480	T	S	6869	0.810	0.221		**81%S/19%T**
P7977-1231-310357	Ilumina	Baseline	150	6192	A	T	5020	0.811			**81%T/19%A**
P7977-1231-310357	Ilumina	WEEK8	150	9170	A	I	109	0.012	0.012		
P7977-1231-310357	Ilumina	WEEK8	150	9170	A	T	8831	0.963	0.152	**A150T**	**96%T/4%A/1%I**
P7977-1231-310357	Ilumina	FU-12	150	10006	A	I	145	0.014	0.014		
P7977-1231-310357	Ilumina	FU-12	150	10006	A	T	9579	0.957	0.147		**96%T/4%A/1%T**
P7977-1231-310357	Ilumina	Baseline	202	6847	E	D	3720	0.543			**54%D/46%E**
P7977-1231-310357	Ilumina	WEEK8	202	10338	E	D	8650	0.837	0.293	**E202D**	**84%D/16%E**
P7977-1231-310357	Ilumina	FU-12	202	10544	E	D	8336	0.791	0.247		**79%D/21%E**
P7977-1231-310357	Ilumina	Baseline	473	4002	S	T	788	0.197			**20%T/80%S**
P7977-1231-310357	Ilumina	WEEK8	473	6331	S	L	95	0.015	0.015		
P7977-1231-310357	Ilumina	WEEK8	473	6331	S	T	102	0.016	-0.181	**S473T**	**2%T/2%L/96%S**
P7977-1231-310357	Ilumina	FU-12	473	5454	S	T	96	0.018	-0.179		**2%T/98%S**
P7977-1231-310357	Ilumina	Baseline	520	8937	I	T	521	0.058			**6%T/94%I**
P7977-1231-310357	Ilumina	WEEK8	520	16564	I	T	1778	0.107	0.049	**I520T**	**11%T/89%I**
P7977-1231-310357	Ilumina	FU-12	520	13012	I	T	2247	0.173	0.114		**17%T/83%I**
P7977-1231-310357	Ilumina	Baseline	572	11698	L	V	1303	0.111			**11%V/89%L to 100%L**

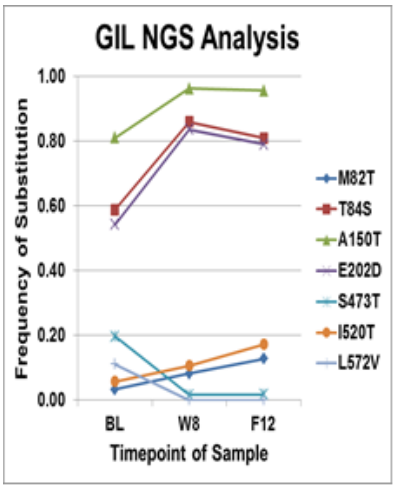

Figure 22. Frequencies of amino acid differences (as compared to baseline) that occurred in post-baseline samples for subject P7977-1231-310357 who experienced virologic breakthrough in clinical trial P7977-1231. Left panel, frequency table showing all substitutions that were detected by the variant detection method employed by the sponsor. Right panel, a plot showing an increase and/or decrease in frequencies for the amino acids that differed from baseline.

This analysis indicated that there were no novel substitutions that appeared to be associated with resistance. However, of the six substitutions that were identified, including two that were enriched at greater than 20% (T84S and E202D) and four that increased below 20% (M82T, A150T, S473T, and I520T), three of these were

enriched at higher frequencies than would be expected based on the conservation reported by the sponsor (Table 19): T84S, E202D, and T473S.

Table 19. Potential resistance-associated substitutions detected in subject P7977-1231-310357 who was infected with HCV GT3a and who experienced virologic breakthrough.

Substitution	GIL Max Var	PVD75 Max Var	QbVD Max Var	Nat Var	Status
M82T	9.4%	ND	9.4%	5.88%	Marginal
T84S	**27.2%**	**27.2%**	**27.2%**	**4.55%**	**RAS**
A150T	15.2%	16.0%	15.0%	57.89%	Natural
E202D	**29.3%**	**28.9%**	**29.0%**	**2.27%**	**RAS**
K304R	ND	16.4%	ND	24.47%	Natural*
T473S	**18.1%**	**18.3%**	**17.8%**	**5.49%**	**RAS**
I520T	11.4%	ND	11.7%	24.5%	Natural*
G543/S543G	ND	ND	98.3%/97.3%	18.74%	Seq error*

GIL Max Var, maximum post-baseline increase in frequency detected by the sponsor's variant detection method
QbVD Max Var, maximum post-baseline increase in frequency detected by the QbVD variant detection method
PVD75 Max Var, maximum post-baseline increase in frequency detected by the PVD75 variant detection method
Nat Var, natural variation which is the difference between the conservation reported by the sponsor and the maximum variation possible
RAS, potential resistance-associated substitution
Marginal, increased frequency is not significantly different from Natural Variation reported by the sponsor
Natural, increase is frequency could be explained by natural variation
*, Differences noted in frequencies reported by the different variant detection methods
Red, potential resistance-associated substitutions

While 20-30% increases in frequency could be associated with resistance, the collection of subject blood samples for resistance analysis was not conducive to determining if a substitution was present at a high enough level to be associated with a several $\log_{10}$ decrease in HCV viral load. Therefore, while it is possible that T84S, E202D, and T473S are potentially associated with resistance that led to breakthrough, the data are inconclusive. Moreover, these substitutions were found in other subjects infected with HCV GT3a who went on to relapse; however, in all cases the frequency of these substitutions at baseline were similar to the frequency at later timepoints and there were no major differences in SVR rates for subjects infected with HCV GT3a who had these substitutions at baseline. We recommend assessing T84S+E202D+T473S in a phenotypic assay.

COMBINED RESISTANCE ANALYSIS

In addition to analyzing all of the data for each clinical trial independently, DAVP also performed a meta-analysis that combined all GTs from all four Phase 3 studies to determine if there were novel substitutions present in any of the Phase 3 trials and to determine the predominant substitutions and low frequency substitutions that were likely be treatment emergent and possibly contributing to SOF resistance. There were no substitutions of interest for GT1 and GT2 subtypes, and this was largely due to the high SVR rates and low numbers of failures. For HCV GT3a, 17 substitutions were detected that were potentially resistance-associated, including L159F and V321A. L159F has been reported to be associated with resistance to mericitabine [redacted] (b) (4) Tong X et al., 2012) (Table 20). All of the substitutions that occurred in two or more subjects across all subjects infected with HCV GT3a across all phase 3 studies are shown in Appendix 5: Combined Resistance Analysis for HCV GT3a. In addition, substitution E341D was detected in 13 subjects from studies P7977-1231 (n=10) and GS-US-334-0107 (n=3). Of note, this substitution was detected at nearly equivalent numbers by the sponsor (n=9 in study P7977-1231; Table 40, page 87, Integrated Virology Study Report for the Phase 2/3 Clinical Studies of Sofosbuvir); however, it was not considered to be resistance-associated. Moreover, while this was reported in the appendix, D341E was not discussed in the summary for P7977-1231 or GS-US-334-0107. Moreover, it was detected by the sponsor in 7 subjects in study

P7677-1231(although 4 of these were at frequencies below 10%, but with no presence at baseline). By contrast, DAVP detected E341D in a total of 13 subjects at much higher frequencies.

Table 20. Potential resistance-associated substitutions for sofosbuvir.

STUDYID	NS5B AAPOS	No. Subjects	SUB10 (+/-)	Ratio	Conservation	Substitutions	Baseline	Conclusion
All 3as from all trials	**341**	**13**	**E341D**		**99.87%**		**Not deteceted at BL**	**Potential RAS**
	116	11	V116I/I116V/T116I	8-2-1	67.11%	I/V	Equal BL vs. Fail	Not likely
	542	10	T542A/A542T	6-4	96.79%	A	Equal BL vs. Fail	Not likely
	517	7	R517K/K517R	6-1	83.67%	K/R	Equal BL vs. Fail	Not likely
	535	7	K535N/N535K	5-2	52.07%	K/N/R	Equal BL vs. Fail	Not likely
	11	6	V11I		77.81%	I/V	Equal BL vs. Fail	Not likely
	159	**6**	**L159F**		**100%**		**Not deteceted at BL**	**Known RAS**
	250	6	K250R/R250K	5-1	86.38%	K/R	Equal BL vs. Fail	Not likely
	114	5	K114R/R114K	4-1	62.17%	K/R	Equal BL vs. Fail	Not likely
	321	**5**	**V321A**		**100%**		**Not deteceted at BL**	**Known RAS**
	106	4	K106R/R106K	3-1	96.39%		Equal BL vs. Fail	Not likely
	113	4	G113S/S113G	3-1	93.32%	S/G	Equal BL vs. Fail	Not likely
	156	4	A156P/P156S	3-1	96.52%	P	Equal BL vs. Fail	Not likely
	520	4	R520K/V520I	3-1	75.50%	I/T	Equal BL vs. Fail	Not likely
	147	3	M147V		92.78%	M/V	Equal BL vs. Fail	Not likely
	438	3	V438I		89.57%	I	Equal BL vs. Fail	Not likely
	519	3	R519K		84.07%	K/R	Equal BL vs. Fail	Not likely

SUBS10(+/-) – Substitutions detected if present at 0.10 or greater frequency at failure timepoint but not detected at baseline.
Ratio – ratio of different substitutions at a position in the order or most to least
Conservation – percent conservation reported by sponsor
Substitutions – common variants at that position
Baseline – comparison of subjects who had the substitution at baseline
Equal BL vs. Fail – present at baseline at equivalent frequencies for subjects that achieved SVR12 and those who failed
RAS – potentially a resistance-associated substitu ion

Combined Resistance Analysis Conclusions

Overall, there was insufficient data to draw any conclusions on resistance-associated substitutions for HCV GT1 and GT2. In addition, only one subject infected with HCV GT3a experienced on-treatment failure, and while there were no clear resistance-associated substitutions, three substitutions, T84S, E202D, and T473S, were enriched. We recommend assessing T84S+E202D+T473S in combination in a GT3a phenotypic assay. Moreover, the sponsor reported that there were no clinically significant resistance-associated substitutions detected for any Phase 2 or 3 clinical trial, and Clinical Virology disagrees with this overall assessment. The following five items should be addressed in the label and considered as post marketing actions:

1. While S282T was not detected in most subjects, it did arise in at least one GT2a infected subject in a Phase 2 trial. Given that this is a known resistance-associated substitution of SOF, this should be included in the resistance section of the label.
2. Substitution E341D was a treatment-emergent substitution at a highly conserved position that developed in 13 subjects infected with HCV GT3a in two clinical trials (P7977-1231, n=10; GS-US-334-0107, n=3) and it was only found in subjects who relapsed. Therefore, this substitution should be characterized in phenotypic assays to determine if its presence in NS5B impacts SOF susceptibility.
3. The L159F substitution is a previously identified resistance-associated NS5B substitution which with L320F has been shown to confer low-level resistance to HCV nucleotide inhibitors (Tong X et al., 2012). L159F emerged in 6 GT3a infected relapse subjects and with E341D in one of those subjects. L159F should be characterized in combination with E341D in a phenotypic assay to determine if these substitutions in combination in NS5B results in an increased EC_{50} value.
4. Three substitutions at highly conserved positions (T84S, E202D, and T473S) were enriched in the one subject infected with HCV GT3a who experienced breakthrough in clinical trial P7977-1231. We recommend assessing T84S+E202D+T473S in combination in a GT3a phenotypic assay.
5. Additional substitutions at highly conserved positions may be associated with resistance to SOF and should be further characterized in a GT3a phenotypic assay: K211R, V321A, P540L, and T542A.

METHODS

The text in this section was copied from pages 14-18 of the Integrated Virology Study Report for the Phase 2/3 Clinical Studies of Sofosbuvir. The methods used had been previously submitted and reviewed as part of the Virology Analysis Plan (PC-334-2008).

Resistance Analysis Population

The Virology Analysis Plan for all Phase 3 studies have been previously described (PC-334-2008). Population sequencing of NS5B was performed at baseline for all subjects enrolled in all of the Phase 2 and 3 studies. Resistance testing was performed for subjects who met the criteria of the resistance analysis population (RAP). The RAP includes any subjects who received at least one dose of SOF-containing regimens, but did not achieve SVR12 due to virologic failure or early discontinuation, and had HCV RNA ≥1,000 IU/ml and had a plasma sample available for analysis. Virologic failure was defined as follows:

1. On-treatment virologic failure
    a. Breakthrough: HCV RNA ≥LLOQ after having previously had HCV RNA <LLOQ, while on treatment, confirmed with 2 consecutive values (note, second confirmation value could be post-treatment), or last available on-treatment measurement with no subsequent follow-up values
    b. Rebound: > 1 $\log_{10}$ IU/mL increase in HCV RNA from nadir while on treatment, confirmed with 2 consecutive values (note, second confirmation value could be posttreatment), or last available on-treatment measurement with no subsequent follow-up values
    c. Non-responsive: HCV RNA persistently ≥LLOQ through 8 weeks of treatment
2. Relapse
    a. HCV RNA ≥LLOQ during the post treatment period having achieved HCV RNA <LLOQ at end of treatment, confirmed with 2 consecutive values or last available post treatment measurement

Resistance analyses included NS5B deep sequencing which was performed at failure and phenotypic analysis which was attempted for the majority of virologic failure subjects and was successful for a subset of subjects.

Resistance Analysis Methodologies

A full description of the resistance analysis methodologies can be found in the Virology Analysis Plan PC-334-2008. A brief description is below.

Population Sequencing

Baseline population sequencing of the full-length HCV NS5B coding region was performed by (b) (4) using RT PCR and standard Sanger population sequencing. The obtained NS5B sequence was also used to confirm the results of genotyping/subtyping by the INNO-LiPA assay performed at screening. For post-baseline resistance analyses of subjects with virologic failure, the HCV NS5B gene was amplified from subject samples if the serum/plasma level of HCV RNA was ≥1,000 IU/mL.

Deep Sequencing

For virologic failures, NS5B PCR amplicons at baseline and post-baseline timepoints were subjected to deep sequencing using the Illumina MiSeq deep sequencing platform. A high level summary of the predominant substitutions for each study was summarized for each individual study and the details of the approach were reviewed as part of the viral resistance analysis plan.

CONCLUSIONS

DAVP performed an independent analysis of the NGS data submitted for four pivotal Phase 3 clinical trials and compared the results to those reported by the sponsor. In general, there was good agreement between these results. However, the different analysis pipelines used much different default filtering and mapping criteria and this was apparent when comparing frequency table values such as Total Coverage, Variant Coverage, and Amino Acid Frequency. However, despite these differences, the general trends observed were very similar.

Overall, the DAVP assessment of the NGS data for the four pivotal Phase 3 trials determined that there was insufficient data to draw any conclusions on resistance-associated substitutions for HCV GT1 and GT2. In addition, only one subject infected with HCV GT3a experienced on-treatment failure, and while there were no clear resistance-associated substitutions, three substitutions, T84S, E202D, and T473S, were enriched. We recommend assessing T84S+E202D+T473S in combination in a GT3a phenotypic assay. Moreover, the sponsor reported that there were no clinically significant resistance-associated substitutions detected for any Phase 2 or 3 clinical trials. Clinical Virology discussed the results of the independent analysis of the NGS data and compared the results with those submitted by Gilead. For the most part, there was good agreement on the overall results and conclusions; however, after discussion with the Clinical Virology Reviewer for this application, Lisa Naeger, Ph.D., it was agreed that the following modifications should be made to the label and/or proposed as post-marketing actions:

1. While S282T was not detected in most subjects, it did arise in at least one GT2a infected subject in a Phase 2 trial. Given that this is a known resistance-associated substitution of SOF, this should be included in the resistance section of the label.
2. Substitution E341D was a treatment-emergent substitution at a highly conserved position that developed in 13 subjects infected with HCV GT3a in two clinical trials (P7977-1231, n=10; GS-US-334-0107, n=3) and it was only found in subjects who relapsed. Therefore, this substitution should be characterized in phenotypic assays to determine if its presence in NS5B impacts SOF susceptibility.
3. The L159F substitution is a previously identified resistance-associated NS5B substitution which with L320F has been shown to confer low-level resistance to HCV nucleotide inhibitors (Tong X et al., 2012). L159F emerged in 6 GT3a infected relapse subjects and with E341D in one of those subjects. L159F should be characterized in combination with E341D in a phenotypic assay to determine if these substitutions in combination in NS5B results in an increased EC_{50} value.
4. Three substitutions at highly conserved positions (T84S, E202D, and T473S) were enriched in the one subject infected with HCV GT3a who experienced breakthrough in clinical trial P7977-1231. We recommend assessing T84S+E202D+T473S in combination in a GT3a phenotypic assay.
5. Additional substitutions at highly conserved positions may be associated with resistance to SOF and should be further characterized in a GT3a phenotypic assay: K211R, V321A, P540L, and T542A..

For complete labeling details, please see the review of NDA 204671 SDN 000 by Dr. Lisa Naeger, Senior Clinical Virology Reviewer.

POST MARKETING RECOMMENDATIONS

1. Evaluate the K211R, V321A, E341D, P540L, and T542A substitutions in a GT3a phenotypic assay.
2. Evaluate the L159F substitution separately and in combination with an E341D substitution in a GT3a phenotypic assay.
3. Evaluate the T84S+E202D+T473S substitutions separately and as a combination in a GT3a phenotypic assay.

Eric F. Donaldson, Ph.D.
Clinical Virology Reviewer

CONCURRENCES

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cc:
HFD-530/IND
HFD-530/Division File
HFD-530/RPM/Onaga

APPENDICES

APPENDIX 1: GS-US-334-0107

This appendix contains all SUBS10+- substitutions identified in GS-US-334-0107 that were detected by at least two variant detection methods (QbVD, PVD75 or the sponsor's variant call) and in at least two subjects.

STUDYID	NS5B POS	No. Subjects	SUB10 (+/-)	Ratio
GS-US-0334-0107	116	4	V116I/I116V	3-1
	150	3	A150V/T150A/V150T	1-1-1
	247	3	P247L/P247S/S247P	1-1-1
	341	3	E341D	
	11	2	V11I/I11V	1-1
	156	2	P156S/A156P	1-1
	189	2	P189S/S189P	1-1
	207	2	I207M/M207I	1-1
	287	2	A287T/T287A	1-1
	310	2	D310N/S310N	1-1
	438	2	V438I	
	520	2	T520I/V520I	1-1
	558	2	N558T/T558N	1-1

SUBS10(+/-) – Substitutions detected if present at 0.10 or greater frequency at failure timepoint but not detected at baseline.
Ratio – ratio of different substitutions at a position in the order or most to least

APPENDIX 2: GS-US-334-0108

This appendix contains all SUBS10+- substitutions identified in GS-US-334-0108 that were detected by at least two variant detection methods (QbVD, PVD75 or the sponsor's variant call) and in at least two subjects.

STUDYID	NS5B POS	No. Subjects	SUB10 (+/-)	Ratio
GS-US-334-0108	116	7	V116I/T116I	6-1
	542	7	A542T/T542A	4-3
	535	5	K535N/N535K	3-2
	571	5	Y571H/H571Y	3-2
	114	4	K114R/R115K	2-2
	150	4	A150V/T	2-2
	543	4	G543S	
	50	3	K50R/R50K	2-1
	66	3	A66T/T66A/T66N	1-1-1
	120	3	R120C/H	2-1
	148	3	D148N/N148D	2-1
	184	3	I184T	
	335	3	T335N/A	2-1
	517	3	R517K/K517R	2-1
	11	2	V11I	
	47	2	L47Q	
	65	2	K65R	
	67	2	A67T/T67A	
	69	2	Q69K	
	71	2	V71I	
	100	2	R100K	
	147	2	M147V	
	159	2	L159F	
	211	2	K211R	
	250	2	K250R	
	304	2	R304K	
	305	2	A305V/V305A	1-1
	357	2	T357I	
	519	2	R519K	
	540	2	P540L	
	541	2	A541V/T541A	1-1
	564	2	V564M	

SUBS10(+/-) – Substitutions detected if present at 0.10 or greater frequency at failure timepoint but not detected at baseline.
Ratio – ratio of different substitutions at a position in the order or most to least

APPENDIX 3: GS-US-334-0110

This appendix contains all SUBS10+- substitutions identified in GS-US-334-0110 that were detected by at least two variant detection methods (QbVD, PVD75 or the sponsor's variant call) and in at least two subjects.

STUDYID	GT/ST	NS5B POS	No. Subjects	SUB10 (+/-)	Ratio
GS-US-334-0110	1a	300	2	R300Q/Q300R	1-1
		543	2	G543S/G543S	1-1
	1b	556	2	G556S	

APPENDIX 4: P7977-1231

This appendix contains all SUBS10+- substitutions identified in P7977-1231 that were detected by at least two variant detection methods (QbVD, PVD75 or the sponsor's variant call) and in at least two subjects.

STUDYID	NS5B POS	No. Subjects	SUB10 (+/-)	Ratio
P7977-1231	341	10	E341D	
	66	6	I66T/T66I/N66T/S66T	2-2-1-1
	79	6	K79R/R79K/T79K	3-2-1
	480	6	A480V/V480I/L	4-1-1
	542	6	T542A/A542T	5-1
	116	5	V116I/I116V	4-1
	150	5	T150A/A150V/V150A	2-1-1
	159	4	L159F	
	11	3	V11I	
	16	3	A16V/S	2-1
	113	3	G113S/S113G	2-1
	114	3	K114R	
	189	3	P189S/S189F/S189P	1-1-1
	206	3	K206E/K206Q/Q206K	1-1-1
	250	3	K250R/R250K	2-1
	272	3	A272V/T272A/V272A	1-1-1
	321	3	V321A	
	335	3	A335I/A335T/T335A	1-1-1
	374	3	W374R/Q374R	2-1
	376	3	N376D/G376D	2-1
	517	3	R157K	
	571	3	Y571H/H571Y	2-1
	82	2	L82M/T82M	1-1
	90	2	A90Q/T	1-1
	106	2	K106R/R106K	1-1
	115	2	A115V	
	120	2	H120R/R120C	1-1
	131	2	I131T	
	221	2	T221I	
	327	2	D327G/N	1-1
	330	2	D330G/E330D	1-1
	333	2	R333K/N	1-1
	513	2	A513G/T513A	1-1
	519	2	R519K	
	520	2	T520I	
	541	2	A541S/T	1-1
	543	2	G543S/S543G	1-1
	544	2	Q544L/R	1-1
	555	2	I555V/V555I	1-1
	566	2	R566H	
	569	2	S569T/T569S	1-1

APPENDIX 5: COMBINED RESISTANCE ANALYSIS FOR HCV GT3a

STUDYID	NS5B POS	No. Subjects	SUB10 (+/-)	Ratio
All 3as from all trials	341	13	E341D	
	116	11	V116I/I116V/T116I	8-2-1
	542	10	T542A/A542T	6-4
	66	9	I66T/T66I/A66T/N66T/S66T/T66A/N	2-2-1-1-1-1-1
	79	9	K79R/R79K/T79K/N79K	4-3-1-1
	150	8	A150V/T150A/V150A/T	3-3-1-1
	480	7	A480V/V480I/L	4-2-1
	517	7	R517K/K517R	6-1
	535	7	K535N/N535K	5-2
	11	6	V11I	
	159	6	L159F	6
	189	6	P189S/S189P/F	3-2-1
	250	6	K250R/R250K	5-1
	114	5	K114R/R114K	4-1
	120	5	R120C/H/H120R	3-1-1
	247	5	P247L/P247S/S247P	3-1-1
	321	5	V321A	
	541	5	T541A/A541S/T/V	2-1-1-1
	571	5	Y571H/H571Y	3-2
	16	4	A16V/S/V16A	2-1-1
	82	4	M82I/T/T82M/L82M	1-1-1-1
	90	4	A90T/Q/V	2-1-1
	106	4	K106R/R106K	3-1
	113	4	G113S/S113G	3-1
	156	4	A156P/P156S	3-1
	206	4	K206E/Q/T/Q206K	1-1-1-1
	335	4	T335A/A335I/T	2-1-1
	520	4	R519K/V520I	3-1
	543	4	G543S/S543G	2-2
	67	3	A67T/T67A/V67A	1-1-1
	71	3	V71I/I71V	2-1
	147	3	M147V	
	148	3	D148N/N148D	2-1
	184	3	I184T/T184I/V184I	1-1-1
	207	3	I207M/M207I/M207V	1-1-1
	272	3	A272V/T272A/V272A	1-1-1
	374	3	W374R/Q374R	2-1
	376	3	N376D/G376D	2-1
	438	3	V438I	
	513	3	A513G/T513A/V513A	1-1-1
	519	3	R519K	
	555	3	I555V/V555I	2-1
	564	3	V564M/M546V	2-1
	566	3	R566H/H566R	2-1
	569	3	S569T/T569S	2-1
	50	2	K50R/R50K	1-1
	73	2	D73E/G73E	1-1
	77	2	K77R/R77K	1-1
	92	2	V92I	
	104	2	C104S/T104S	1-1
	115	2	A115V	
	117	2	N117D	
	131	2	I131T	2
	203	2	H203R/R203E	1-1
	221	2	T221I	
	244	2	D244N	
	287	2	A287T/T287A	1-1
	304	2	K304R/R304K	1-1
	305	2	A305V/V305A	1-1
	310	2	D310N/S310N	1-1
	311	2	P311S	
	327	2	D327G/N	1-1
	330	2	D330G/E330D	1-1
	333	2	R333K/N	1-1
	334	2	T334A/V334A	1-1
	353	2	A353T/V353A	1-1
	379	2	G379R/K379R	1-1
	381	2	Y381C/H	1-1
	388	2	A388S/P388A	1-1
	440	2	D440E/E440D	1-1
	441	2	R441C/R441Q	1-1
	475	2	H475Y	
	534	2	A534T/I534T	1-1
	540	2	P540L	
	544	2	Q544L/R	1-1
	558	2	N558T/T558N	1-1

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/s/

ERIC F DONALDSON
09/06/2013

JULIAN J O REAR
09/06/2013

Product Quality Microbiology Review

6/13/2013

NDA: 204671

Drug Product Name
Proprietary: (pending)
Non-proprietary: Sofosbuvir

Review Number: 1

Dates of Submission(s) Covered by this Review

Submit	Received	Review Request	Assigned to Reviewer
4/06/2013	4/08/2013	5/09/2013	5/13/2013
7/05/2013	7/05/2013	N/A	N/A

Submission History (for 2nd Reviews or higher)
None

Applicant/Sponsor
Name: Gilead Sciences, Inc.
Address: 333 Lakeside Drive, Foster City, CA 94404
Representative: Shalini Gidwani, M.Sc., RAC
Telephone: 650-522-2454

Name of Reviewer: Steven P. Donald, M.S.

Conclusion: Recommended for Approval

Product Quality Microbiology Data Sheet

A. **1.** **TYPE OF SUBMISSION:** Original NDA

2. **SUBMISSION PROVIDES FOR:** Manufacture and marketing of an oral drug product

3. **MANUFACTURING SITE:**

(b) (4)

2. Gilead Sciences Limited, IDA Business and Technology Park, Carrigtohill, Co. Cork, Ireland

4. **DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY:** Oral tablet, 400 mg

5. **METHOD(S) OF STERILIZATION:** N/A

6. **PHARMACOLOGICAL CATEGORY:** Anti-viral

B. **SUPPORTING/RELATED DOCUMENTS:** None

C. **REMARKS:** An information request for a commitment microbial limits testing on the annual stability batches was conveyed to the sponsor in the 74-day letter, dated 6/7/2013. An acceptable response was received on 7/05/2013.

filename: N204671r1.doc

Reference ID: 3339517

Executive Summary

I. Recommendations

A. Recommendation on Approvability - Recommended for Approval.

B. Recommendations on Phase 4 Commitments and/or Agreements, if Approvable – N/A

II. Summary of Microbiology Assessments

A. Brief Description of the Manufacturing Processes that relate to Product Quality Microbiology – (b) (4)

B. Brief Description of Microbiology Deficiencies –
No product quality microbiology deficiencies were identified based upon the information provided.

C. Assessment of Risk Due to Microbiology Deficiencies – N/A

D. Contains Potential Precedent Decision(s)- ☐ **Yes** ☒ **No**

III. Administrative

A. Reviewer's Signature _____
Steven P. Donald, M.S.
Microbiology Reviewer

B. Endorsement Block_____
Stephen Langille, Ph.D.
Senior Microbiology Reviewer

C. CC Block
N/A

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Reference ID: 3339517

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/s/

STEVEN P DONALD
07/11/2013

STEPHEN E LANGILLE
07/11/2013

PRODUCT QUALITY MICROBIOLOGY FILING CHECKLIST

NDA Number: 204671 **Applicant:** Gilead Sciences, Inc. **Letter Date:** 4/06/2013

Drug Name: Sofobuvir Tablets **NDA Type:** 505(b)(1) **Stamp Date:** 4/08/2013

The following are necessary to initiate a review of the NDA application:

	Content Parameter	Yes	No	Comments
1	Is the product quality microbiology information described in the NDA and organized in a manner to allow substantive review to begin? Is it legible, indexed, and/or paginated adequately?	X		Submission is in eCTD format.
2	Has the applicant submitted an overall description of the manufacturing processes and microbiological controls used in the manufacture of the drug product?		X	Process is described but microbiological controls are not specifically addressed. See 3.2.P.3.3. Microbial issues are addressed in Justification of Specifications
3	Has the applicant submitted protocols and results of validation studies concerning microbiological control processes used in the manufacture of the drug product?		X	Validation for microbiology studies are not presented
4	Are any study reports or published articles in a foreign language? If yes, has the translated version been included in the submission for review?		X	
5	Has the applicant submitted preservative effectiveness studies (if applicable) and container-closure integrity studies?			N/A

	Content Parameter	Yes	No	Comments
6	Has the applicant submitted microbiological specifications for the drug product and a description of the test methods?	X		Microbiological limits are not included in the proposed specifications although they were included in product development and stability; see batch analysis summary, batch_analysis.pdf, pg. 9/23 and stability studies. Also see Justification of Specifications, pg. 19
7	Has the applicant submitted the results of analytical method verification studies?	X		Verification studies are not presented, however USP <61> and <62> are cited for the development studies and stability studies (see above reference).
8	Has the applicant submitted all special/critical studies/data requested during pre-submission meetings and/or discussions?			N/A
9	If sterile, are extended post-constitution and/or post-dilution hold times in the draft labeling supported by microbiological data?			N/A
10	Is this NDA fileable? If not, then describe why.	X		

Additional Comments: The protocol for the primary stability studies includes microbial limits assay on batches under two long term conditions (25 ^{0}C and 30 ^{0}C) at release and yearly up to 60 months. The first three post approval batches, but not the annual commitment batches, include microbial limits testing using the same conditions and schedule. In Section 2.3.P.2, Microbial Attributes, it states that all drug product lots, including the primary stability batches demonstrate that the product consistently meets the proposed acceptance criteria, showing a total aerobic microbial count of (b) (4) and total combined yeast and mold count of (b) (4) as well as the absence of *E. coli*. Based on the consistency of these results, the applicant proposes reduced microbiological testing and references ICH guideline Q6A. To further support reduced microbial limits testing, the drug product is (b) (4) (b) (4) is included in the release specifications at

NMT (b) (4). Also the applicant states that a (b) (4) dilution of the drug is required to permit microbial growth.

The only part of the manufacturing process that may be of concern is (b) (4) This step in the manufacturing process is the only (b) (4) The batch records (3800004034.pdf) indicate that the (b) (4) is held anywhere from (b) (4) (b) (4) The hold time for the (b) (4) is NMT (b) (4). A previous study associated with NDA 203469 (see 203469r1, 10/16/12 submission) references a study investigating the microbial limits of (b) (4) No bacteria or mold were recovered. This reviewer concludes that the (b) (4)

Steven P. Donald	CDER/OPS/NDMS	5/24/2013
Reviewing Microbiologist		Date
Stephen Langille	CDER/OPS/NDMS	5/24/2013
Microbiology Secondary Reviewer/Team Leader		Date

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/s/

STEVEN P DONALD
06/03/2013

STEPHEN E LANGILLE
06/03/2013

MICROBIOLOGY FILING CHECKLIST FOR NDA

NDA Number: 204,671 **Applicant: Gilead Sciences** **Stamp Date: 04/08/2013**

Drug Name: Sofusbuvir **NDA Type: Original**

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

	Content Parameter	Yes	No	Comments
1	Is the virology information (nonclinical and clinical) provided and described in different sections of the NDA organized in a manner to allow substantive review to begin?	X		
2	Is the virology information (nonclinical and clinical) indexed, paginated and/or linked in a manner to allow substantive review to begin?	X		
3	Is the virology information (nonclinical and clinical) legible so that substantive review can begin?	X		
4	On its face, has the applicant submitted cell culture data in necessary quantity, using necessary clinical and non-clinical strains/isolates, and using necessary numbers of approved current divisional standard of approvability of the submitted draft labeling?	X		
5	Has the applicant submitted any required animal model studies necessary for approvability of the product based on the submitted draft labeling?			n/a
6	Has the applicant submitted all special/critical studies/data requested by the Division during pre-submission discussions?	X		
7	Has the applicant submitted the clinical virology datasets in the appropriate format as described in the relevant guidance documents and are the datasets complete?	X		
8	Has the applicant used standardized or nonstandardized methods for virologic outcome measures? If nonstandardized methods were used, has the applicant included complete details of the method, the name of the laboratory where actual testing was done and performance characteristics of the assay in the laboratory where the actual testing was done?	X		standardized
9	Has the applicant submitted draft labeling consistent with current regulation, divisional and Center policy, and the design of the development package?	X		
10	Has the applicant submitted annotated microbiology draft labeling consistent with current divisional policy, and the design of the development package?	X		
11	Have all the study reports, published articles, and other references been included and cross-referenced in the	X		

File name: 5_Microbiology Filing Checklist for a NDA or Supplement 010908

MICROBIOLOGY FILING CHECKLIST FOR NDA

	Content Parameter	Yes	No	Comments
	annotated draft labeling or summary section of the submission?			
12	Are any study reports or published articles in a foreign language? If yes, has the translated version been included in the submission for review?		X	

IS THE MICROBIOLOGY SECTION OF THE APPLICATION FILEABLE? __Yes__

If the NDA is not fileable from the microbiology 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**

Lisa K. Naeger, Ph.D. 05/07/2013
Reviewing Virologist Date

Jules O'Rear 05/08/2013
Virology Team Leader Date

File name: 5_Microbiology Filing Checklist for a NDA or Supplement 010908

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/s/

LISA K NAEGER
05/08/2013

ERIC F DONALDSON
05/08/2013

JULIAN J O REAR
05/08/2013