CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

205834Orig1s007, s008, s009

MICROBIOLOGY/VIROLOGY REVIEW(S)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW
NDA: 205834 S7-9 **SDN**: 194, 195, 196
DATE REVIEWED: 01/20/2016
Virology Reviewer: Lisa K. Naeger, Ph.D.

NDA#: 205,834 Supplements 7-9 **Serial #:** 194, 195, 196
Reviewer's Name(s): Lisa K. Naeger, Ph.D.

Sponsor's Name and Address:
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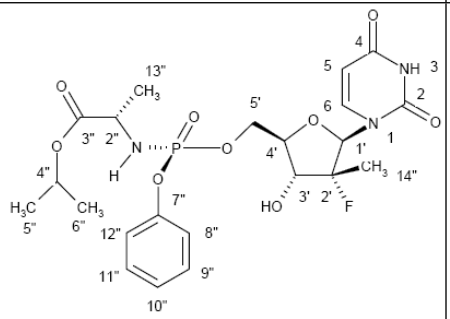
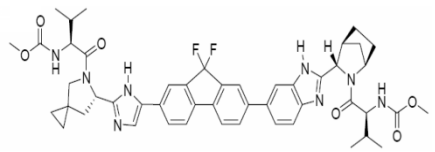
Related/Supporting Documents: IND115268, IND106739, NDA204671

Product Names	**Sofosbuvir (GS-7977)**	**Ledipasvir (GS-5885)**
Structures		
Chemical Names	(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	Methyl [(2*S*)-1-{(6*S*)-6-[5-(9,9-difluoro-7-{2-[(1*R*,3*S*,4*S*)-2-{(2*S*)-2-[(methoxycarbonyl) amino]-3-methylbutanoyl}-2-azabicyclo[2.2.1]hept-3-yl]-1*H*-benzimidazol-6-yl}-9*H*-fluoren-2-yl)-1*H*-imidazol-2-yl]-5-azaspiro[2.4]hept-5-yl}-3-methyl-1-oxobutan-2-yl]carbamate
Molecular formula	$C_{22}H_{29}FN_3O_9P$	$C_{49}H_{54}F_2N_8O_6$
Molecular weight	529.4 (b) (4)	889.00

Reference ID: 3878352

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW
NDA: 205834 S7-9 **SDN**: 194, 195, 196
DATE REVIEWED: 01/20/2016
Virology Reviewer: Lisa K. Naeger, Ph.D.

Drug category: Antiviral
Indication: Fixed-dose combination of ledipasvir, a hepatitis C virus (HCV) NS5A inhibitor and sofosbuvir, an HCV nucleotide analog NS5B polymerase inhibitor and is indicated for the treatment of chronic hepatitis C (CHC) genotype 1, 4, 5, and 6 infection

Dosage Form/Route of administration: Oral

Dispensed: Rx

Abbreviations: BL, baseline; DAA, direct acting antiviral; EC_{50}, effective concentration at 50%; FC, fold-change; FDA, Food and Drug Administration; FDC, fixed-dose combination; GT, genotype; HCV, hepatitis C virus; IC_{50}, inhibitory concentration at 50%; IFN, recombinant human interferon; LDV, ledipasvir; NGS, next generation sequencing; NRTIs, nucleoside reverse transcriptase inhibitors; PBL, peripheral blood lymphocytes; PDVF, protocol defined virologic failure; PI, NS3/4A protease inhibitor; P/R, pegylated interferon/ribavirin; RBV, ribavirin; SDM, site-directed mutants; SOF, sofosbuvir; SVR, sustained virologic response; SVR12, sustained virologic response at 12 week after end of treatment; WT, wild-type

TABLE OF CONTENTS

Executive Summary…….....*Page 3*

1 *Recommendations*
- ***1.1*** *Recommendations on Approvability*…….....*Page 4*
- ***1.2*** *Recommendation on Phase 4 Commitments*……*Page 4*

2 *Summary of Virology Assessments*
- ***2.1*** *Non-Clinical Virology*……....*Page 5*
- ***2.2*** *Clinical Virology* …….…..*Page 6*

3 *Administrative signatures*…….....*Page 8*

4 *Virology Review*
- ***4.1*** *Important Milestones in Development*…….....*Page 9*
- ***4.2*** *Methodology*…….....*Page 9*
- **4.3** *Prior FDA Reviews*….…….....*Page 11*
- **4.4** *State of antivirals used for the indication sought.*…….....*Page 11*
- ***4.5*** *Non-Clinical Virology* ……..*Page 14*
- ***4.6*** *Clinical Studies*……....*Page 14*
- ***4.7*** *Clinical Virology*…….....*Page 16*

5 *Conclusion*……..…..*Page 24*

6 *Package Insert*
- ***6.1*** *Applicant-Proposed*…….....*Page 26*
- ***6.2*** *FDA-Proposed* …….....*Page 30*

Appendix A…… … *Page 35*

Reference ID: 3878352

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW
NDA: 205834 S7-9 **SDN**: 194, 195, 196
DATE REVIEWED: 01/20/2016
Virology Reviewer: Lisa K. Naeger, Ph.D.

EXECUTIVE SUMMARY

This supplemental NDA for HARVONI®, a fixed-dose combination (FDC) of ledipasvir (LDV) and sofosbuvir (SOF) seeks indications for liver transplant recipients without cirrhosis or with compensated cirrhosis and patients with decompensated cirrhosis.

The SOLAR 1 and 2 studies assessed LDV/SOF + RBV for 12 or 24 weeks in GT1 and GT4 HCV-infected patients with advanced liver disease or post-liver transplant. In these studies, the overall prevalence of NS5A resistance-associated polymorphisms (RAPs) in GT1 subjects was 25%; 20% in GT1a and 34% in GT1b. The virologic failure rates in HCV GT1 subjects were comparable for subjects with NS5A RAPs and subjects without NS5A RAPs at 3.4% (5/146) vs. 4.3% (19/443), respectively. For subjects with baseline NS5A RAPs, virologic failure rates were higher for GT1a subjects compared to GT1b subjects (5.3% vs. 1.4%, respectively). In addition, for subjects with baseline NS5A RAPS, there were no virologic failures in the 24-week duration arms.

The virologic failure rates were analyzed by liver function groups (Groups 1 and 5: Child Pugh B pre-and post-transplantation; Groups 2 and 6: Child Pugh C pre- and post-transplant; Groups 3 and 4: compensated non-cirrhotic and Child Pugh A post-transplantation). An increase in virologic failure rate in GT1 subjects with baseline NS5A RAPs is seen in Groups 2 and 6 (25%; 2/8) and GT1a subjects in Group 3 (without cirrhosis) compensated post-transplantation (13%; 3/23); however, the numbers in these subgroups are small. Importantly, virologic failure rates for Child Pugh C groups of GT1a subjects without baseline NS5A RAPS are 9% (2/23) for the 12 week duration and 25% (6/24) for 24-week duration.

After 12 weeks of treatment with HARVONI® and ribavirin (RBV), relapse rates were 7% (5/71) and 5% (10/217) with and without baseline NS5A polymorphisms, respectively, in genotype 1 subjects in the SOLAR-1 and SOLAR-2 trials. Of the 5 genotype 1 relapsers with baseline NS5A polymorphisms, 2 were pre-transplant subjects with Child Pugh C cirrhosis (1 genotype 1a with the Q30Q/H polymorphism and 1 genotype 1b with R30R/Q and Y93H polymorphisms) and 3 were post-transplantation subjects with F0-F3 fibrosis (all genotype 1a with the H58P polymorphism).

For subjects with GT4 HCV, the presence of baseline NS5A RAPs resulted in a higher percentage of virologic failures for Child Pugh B (33%; 1/3) and Child Pugh C (67%; 2/3) groups in the 12-week duration arm, although the subgroups were small. However, the applicant is not seeking an indication for GT4 subjects with Child Pugh B and C liver function.

In SOLAR 1 and 2 studies, there were 27 virologic failure subjects with GT1 (n=24) and GT4 HCV (n=3): 23 subjects relapsed and 4 subjects discontinued on treatment with a detectable viral load and were included in the FDA virologic failure analysis. Most of the virologic failures were in the 12-week duration arms (n=18) and most had GT1a HCV (n=17). Eight of the virologic failures (30%) had baseline NS5A RAPs. The remaining subjects had no pretreatment RAPs, but most developed treatment-emergent NS5A resistance substitutions at the time of relapse. Most of the virologic failures (n=10; 37%)

Reference ID: 3878352

were pretransplant CPT B, but none of the failures in this group had baseline NS5A RAPs.

Seventy-eight percent (21/27) of the virologic failures had emergent NS5A resistance substitutions, including one of the virologic failures who discontinued on treatment with detectable viral load. Fourteen virologic failures developed 1 NS5A substitution and 7 developed ≥2 substititutions. Of the virologic failures who relapsed, 91% (21/23) had emergent NS5A resistance substitutions. Emergent NS5A resistance-associated substitutions included K24K/R, M28T, Q30H/R, R30Q, M31V, H58D, and Y93H/C. The most prevalent NS5A substitutions that emerged at failure were Q30H/R or R30Q (n=9; 33%) and Y93H (n=11; 41%). The majority of treatment-emergent substitutions conferred >1,000-fold reduced susceptibility to LDV in cell culture. Three additional GT1 subjects without cirrhosis in Group 3 had a H58P polymorphism at pretreatment that was maintained or enriched at post-treatment, but according to the sponsor this substitution confers less than 2.5-fold resistance to LDV.

1. RECOMMENDATIONS

1.1. Recommendation and Conclusion on Approvability

This supplemental NDA for the fixed-dose combination of ledipasvir (LDV) and sofosbuvir (SOF) is approvable with respect to virology for the treatment of GT1 or 4 liver transplant recipients without cirrhosis or with compensated cirrhosis and GT1 patients with decompensated cirrhosis. The recommended regimen is LDV/SOF + RBV for 12 weeks.

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

There are no phase IV virology recommendations.

2. SUMMARY OF OND VIROLOGY ASSESSMENTS

2.1 Non-Clinical Virology

Sofosbuvir (SOF) is a nucleotide prodrug of 2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate that is converted to the active uridine triphosphate form (GS-461203) within the hepatocyte. In a biochemical assay, GS-461203 inhibited the RNA polymerase activity of recombinant NS5B from HCV genotypes 1b, 2a, 3a and 4a with IC_{50} values ranging from 0.7 to 2.6 µM. 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. The median EC_{50} values of sofosbuvir against chimeric replicons encoding NS5B sequences from clinical isolates were 62 nM for genotype 1a (range 29-128 nM; n=67), 102 nM for genotype 1b (range 45-170 nM; n=29), 29 nM for genotype 2 (range 14-81 nM; n=15) and 81 nM for genotype 3a (range 24-181 nM; n=106). The EC_{50} values of sofosbuvir against genotype 1a and 2a viruses were 30 and 20 nM, respectively.

Ledipasvir (LDV) inhibits HCV replication by interfering with the viral NS5A protein. Although the precise mechanism of inhibition has not been elucidated, several lines of evidence support NS5A as the target of LDV. Cell culture resistance selection studies, as well as LDV monotherapy clinical studies, identified LDV resistance substitutions in the NS5A gene. Additionally, HCV replicons encoding resistance substitutions to daclatasvir, another NS5A inhibitor, were shown to be cross-resistant to LDV. In biochemical studies, LDV does not inhibit NS3 protease, NS3 helicase, NS5B polymerase, the HCV IRES, or a broad panel of kinases.

The EC_{50} values of ledipasvir against HCV genotypes 1a and 1b replicons were 0.031 nM and 0.004 nM, respectively. In addition, LDV had EC_{50} values ranging from 0.15 to 530 nM against genotypes 2 to 6 replicons. LDV had an EC_{50} value of 21 nM against the GT2a JFH-1 replicon, which has leucine at amino acid position 31 (L31) in NS5A, and had reduced activity with an EC_{50} value of 249 nM against the GT2a J6 HCV strain, which has methionine at position 31 (M31), a common NS5A resistance substitution and polymorphism. It had less antiviral activity compared to GT1 against genotypes 4a, 5a, and 6a, with EC_{50} values of 0.39 nM, 0.15 nM and 1.1 nM, respectively. LDV had substantially lower activity against genotypes 3a and 6e with EC_{50} values of 168 nM and 264 nM, respectively.

No cytotoxicity was observed in the GT1b Rluc-2 and GT1a HRlucP replicon cells at the highest concentrations tested (CC_{50} value >44,400 nM) giving a selectivity index for LDV in these assays of >837,000 after 3 and 7 days of drug exposure. LDV did not have inhibitory activity against a related flavivirus, bovine viral diarrhea virus (BVDV), or unrelated viruses including respiratory syncytial virus, hepatitis B virus and human immunodeficiency virus. No antiviral activity was detected for LDV or SOF against a panel of flaviviruses (Yellow Fever virus, Dengue 2 virus, West Nile virus, and Benzi virus), human rhinovirus (HRV) (infectious mixture of HRV1A, HRV16 and HRV14), and influenza A and B viruses at the highest concentration tested without cytotoxicity (50 µM

Reference ID: 3878352

for HRV; 100 μM for other viruses) (except SOF had EC_{50} values of 88 μM and 73 μM for influenza B virus and Yellow Fever virus, respectively).

In cell culture, the combination of SOF and LDV exhibited additive antiviral activity. No antiviral antagonism was observed, and no significant change in cell viability was observed in combination studies of SOF and LDV. In combination studies with SOF and with LDV, no antiviral antagonism was observed with the NS3 protease inhibitors, the NS5A inhibitor daclatasvir, IFN-α, RBV, the nucleotide NS5B polymerase inhibitor R7128, the non-nucleotide NS5B polymerase inhibitor GS-9190 or HIV-1 inhibitors.

Genotypic analyses of resistant replicon RNA substitutions emerging in the NS5A gene identified Q30E and Y93H as the primary resistance substitutions selected in GT1a replicons. These substitutions resulted in approximately 1,000- and 3,000-fold increases, respectively, in the EC_{50} values of LDV relative to wild-type 1a replicon. The mutant NS5A replicons exhibited cross-resistance to another NS5A inhibitor (daclatasvir, BMS-790052), but remained sensitive to SOF and ribavirin. Genotypic analyses of resistant GT1b replicon RNA and subsequent phenotypic analyses of substitutions emerging in the NS5A gene identified Y93H as the primary resistance substitution to LDV. Y93H confers 3,310-fold resistance (EC_{50} value = 4.3 nM) to LDV in transient replicon assays. Y93H was cross-resistant to the NS5A inhibitor GS-432567, but was fully susceptible to the adenine nucleotide NS5B inhibitor 2'-C-Me-A.

Cell culture studies demonstrated no cross-resistance between SOF and LDV when tested individually against HCV substitutions resistant to other classes of HCV inhibitors. The NS5B S282T mutant replicon, which confers reduced susceptibility to SOF, was susceptible to LDV. Similarly, SOF was fully active against a panel of NS5A mutants that showed reduced susceptibility to LDV. Furthermore, double-class mutants (NS5B S282T + NS5A resistance-associated variants displayed a significant reduced replication capacity compared to wild type or single NS5A resistant variants in the replicon. In addition, LDV retained antiviral activity in cell culture against HCV mutants resistant to other classes of HCV inhibitors including NS3/4A protease inhibitors and nucleoside and non-nucleoside NS5B polymerase inhibitors. GT1 replicons encoding substitutions conferring resistance to NS5A inhibitors, NS3/4A inhibitors, or non-nucleoside NS5B inhibitors also remained fully susceptible to SOF indicating that there is no cross-resistance between SOF and HCV NS3/4A inhibitors, non-nucleotide NS5B inhibitors, or NS5A inhibitors.

2.2 Clinical Virology

In the SOLAR 1 and 2 studies, the overall prevalence of NS5A resistance-associated polymorphisms (any change at NS5A positions 24, 28, 30, 31, 32, 38, 58, 92, or 93) in GT1 subjects was 25%; 20% in GT1a and 34% in GT1b. The virologic failure rates in HCV GT1 subjects were comparable for subjects with NS5A RAPs and subjects without NS5A RAPs at 3.4% (5/146) vs. 4.3% (19/443) , respectively. For subjects with baseline NS5A RAPs, virologic failure rates were slightly higher for GT1a HCV-infected subjects compared to GT1b HCV-infected subjects (5.3% vs. 1.4%, respectively). In addition, for subjects with baseline NS5A RAPS, there were no virologic failures in the 24-week duration arms.

Reference ID: 3878352

The virologic failure rates were analyzed by liver function groups (Groups 1 and 5: Child Pugh B pre-and post-transplantation; Groups 2 and 6: Child Pugh C pre- and post-transplant; Groups 3 and 4: compensated non-cirrhotic and Child Pugh A post-transplantation). An increase in virologic failure rate in GT1 subjects with baseline NS5A RAPs is seen in Groups 2 and 6 (25%; 2/8) and GT1a subjects in Group 3 (without cirrhosis) compensated post-transplantation (13%; 3/23); however, the numbers in these subgroups are small.

The four GT1a virologic failures with baseline NS5A RAPs had only one baseline RAP; three had the H58P polymorphism and one had a mixture at Q30Q/H. The one GT1b virologic failure with baseline RAPs had 2 polymorphisms, R30R/Q and Y93H.

For subjects with GT4 HCV, the presence of baseline NS5A RAPs resulted in a higher percentage of virologic failures for Child Pugh B (33%; 1/3) and Child Pugh C (67%; 2/3) groups in the 12-week duration arm, although the subgroups were very small. However, the applicant is not seeking an indication for GT4 subjects with Child Pugh B and C liver function.

In SOLAR 1 and 2 studies, there were 27 virologic failure subjects: 23 subjects relapsed and 4 subjects discontinued on treatment with a detectable viral load and were included in the FDA virologic failure analysis. Most of the virologic failures were in the 12-week duration arms (n=18) and most were GT1a (n=17). Eight of the virologic failures (30%) had baseline NS5A RAPs. The remaining subjects had no pretreatment RAPs, but most developed treatment-emergent NS5A resistance substitutions at the time of relapse. Most of the virologic failures (n=10; 37%) were pretransplant CPT B, but none of the failures in this group had baseline NS5A RAPs.

Seventy-eight percent (21/27) of the virologic failures had emergent NS5A resistance substitutions, including one of the virologic failures who discontinued on treatment with detectable viral load. Fourteen virologic failures developed 1 NS5A substitution and 7 developed ≥2 substititutions. Of the virologic failures who relapsed, 91% (21/23) had emergent NS5A resistance substitutions. Emergent NS5A resistance-associated substitutions included K24K/R, M28T, Q30H/R, R30Q, M31V, H58D, and Y93H/C. The most prevalent NS5A substitutions that emerged at failure were Q30H/R or R30Q (n=9; 33%) and Y93H (n=11; 41%). The majority of treatment-emergent substitutions conferred >1,000-fold reduced susceptibility to LDV in cell culture. Three additional subjects without cirrhosis in Group 3 had a H58P polymorphism at pretreatment that was maintained or enriched at post-treatment, but this substitution confers less than 2.5-fold resistance to LDV.

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 ___**Jules O'Rear**_____Date _____

Reference ID: 3878352

4. VIROLOGY REVIEW

4.1 Important Milestones in Product Development

Sofosbuvir (SOF) was originally developed by Pharmasset as PSI-7977 until Pharmasset was acquired by Gilead Sciences. SOF was approved for the treatment of HCV by the FDA in December 2013 (NDA-204671). HARVONI® (LDV + SOF) was originally approved by the FDA October 10, 2014. The recommended duration of 90 mg LDV and 400 mg of SOF is 12 weeks for treatment-naïve GT1 HCV-infected patients with or without cirrhosis, treatment-experienced GT1 HCV-infected patients without cirrhosis and treatment-naïve or PI/PR-experienced GT4, 5, or 6 HCV-infected patients with or without cirrhosis. HARVONI® for 8-week duration can be considered in treatment-naïve GT1 patients without cirrhosis who have pre-treatment HCV RNA less than 6 million IU/mL. The recommended duration is 24 weeks of HARVONI or alternatively 12 weeks HARVONI + RBV for GT1 HCV-infected treatment-experienced patients with cirrhosis. The same recommendations apply to patients with HCV/HIV-1 co-infection.

4.2 Methodology

SCREENING HCV GENOTYPE
HCV genotype and subtype were determined by the central laboratory using the Siemens VERSANT® HCV Genotype INNO-LiPA 2.0 Assay, the TRUEGENE™ HCV 5'NC Genotyping Assay and NS5B sequencing.

HCV VIRAL LOAD ASSAY
For both studies, the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 was used to quantify HCV RNA. The LLOQ of the assay was 15 IU/mL.

RESISTANCE ANALYSIS
Deep sequencing of NS5A and NS5B was performed at baseline for all subjects enrolled in the Studies listed (GS-US-337-0123 and GS-US-337-0124). Resistance testing was performed for any subject meeting the criteria of the resistance analysis population. The resistance analysis population included any subject who received at least one dose of a LDV/SOF-containing regimen and met one of the following virologic failure (VF) criteria:

- Virologic breakthrough: >1 $\log_{10}$ IU/mL increase in HCV RNA over nadir or HCV RNA ≥ the lower limit of quantitation (LLOQ) after having previously had HCV RNA < LLOQ during the on treatment period, confirmed with 2 consecutive values (second confirmation value could be post-treatment) with absolute viral load values >1000 IU/mL
- Virologic Relapse: HCV RNA ≥ LLOQ during the post treatment period having achieved HCV RNA <LLOQ at the end of treatment, confirmed with 2 consecutive values or last available post treatment measurement, with HCV RNA >1000 IU/mL
- Early Discontinuation: HCV RNA >1000 IU/mL at the time of study discontinuation for any reason

Reference ID: 3878352

Resistance testing included reverse transcriptase polymerase chain reaction (RT-PCR) followed by deep sequencing of the full-length HCV NS5A and NS5B coding regions using the Illumina MiSeq deep sequencing platform. (b) (4) was the designated laboratory for all resistance analyses at baseline and VF. Deep sequencing was only attempted if the level of HCV RNA in the plasma was ≥1000 IU/mL. Pretreatment analysis with a 15% cutoff was conducted for NS5A and NS5B genes and used for the FDA analysis. For subjects with virologic failure, deep sequencing with a 1% cutoff was used for determination of pretreatment and post-treatment, NS5A and NS5B substitutions.

Phenotypic analyses of samples from subjects with virologic failure was performed in a subset of subjects using a chimeric replicon encoding the NS5A or NS5B region derived from subject plasma/serum at (b) (4)

NS5A resistance-associated polymorphisms are defined as any amino acid change from the corresponding HCV genotype specific reference at NS5A resistance-associated positions 24, 28, 30, 31, 32, 38, 58, 92, or 93. Table 1 presents the predominant amino acid residue at these positions for genotypes 1a, 1b, and 4a reference strains. Genotype 4a reference was used for subjects with all subtypes of genotype 4. The sponsor also used NS5A resistance-associated variants (RAVs) defined as specific amino acid changes in genotype 1a, 1b, and 4a that confer >2.5 fold reduced susceptibility to LDV in cell culture in at least one genotype and/or any treatment-emergent variant against LDV observed in 2 or more subjects that occurs at any positions historically associated with resistance (Table 2). RAVs include both resistance-associated polymorphisms and substitutions.

Table 1. Predominant Amino Acids Found at Resistance-Associated Positions in NS5A for HCV Genotypes 1a, 1b, and 4 (Nonclinical Report No. PC-337-2007 page 12)

Genotype	HCV Reference Sequence Name	NS5A Amino Acid Position								
		24	28	30	31	32	38	58	92	93
1a	HCV1a_H77_NC_004102	K	M	Q	L	P	S	H	A	Y
1b	HCV1b_Con1_AJ238799	Q	L	R	L	P	S	P	A	Y
4a	HCV4_ED43_GU814265	K	L	L	M	P	S	P	A	Y

Table 2. Resistance-Associated Variants of LDV in NS5A for HCV GT1a, GT1b and GT4

Genotype	HCV Reference Sequence Name	NS5A Resistance-Associated Variants (Consensus Amino Acid / Position / Variant)
1a	HCV1a_H77_NC_004102	K24G/N/R, M28A/G/T, Q30C/E/G/H/L/K/R/T, L31I/F/M/V/R, P32L, S38F, H58D, A92K/T, Y93C/F/H/N/S
1b	HCV1b_Con1_AJ238799	L31I/F/M/V, P32L, P58D, A92K, Y93C/H/N/S
4a	HCV4_ED43_ GU814265	K24G/N/R, L28M/A/G/T/V, L30E/G/H/K/R /T/S, M31V/F, P32L, S38F, P58D/L, A92K/T, Y93C/F/H/N/S

Phenotypic testing of genotype 1a and 1b HCV NS5A resistance-associated variants has categorized variants into those that have shown 2.5-100 , 100-1,000, or >1,000 fold reduced susceptibility to LDV in cell culture (Table 3).

Table 3. NS5A Resistance-Associated Variants Classified by Fold Reduction in Susceptibility to LDV

Genotype	2.5-100 Fold	100-1000 Fold	>1000 Fold[a]
1a	K24R, Q30L, Q30T, K24G, K24N, A92T, Y93F, M28T, S38F	Q30H, Q30G, Q30R, L31I, L31M, L31V, P32L,	M28A, M28G, Q30E, Q30K, H58D, Y93C, Y93H, Y93N, Y93S
1b	L31M, P32L, L31F, L31I, L31V	P58D	A92K, Y93H

a Double mutants conferring >1000 fold resistance are also utilized for tabulation and SVR calculations.

Resistance to HCV NS5B nucleoside polymerase inhibitors have previously been associated with amino acid variants at positions 96, 142, 159, 282, 289, 320 and 321 in the NS5B protein. For the purposes of this report, NS5B nucleos(t)ide analog inhibitor (NI) resistance-associated substitutions and ribavirin resistance-associated substitutions are defined as follows:

- NS5B NI resistance-associated substitutions: S96T, N142T, L159F, S282T, any S282 variant other than T, M289L/I, L320V/I/F, and V321A/I (Q355H/R has recently been identified as a sofosbuvir resistance substitution by Virology Reviewers Pat Harrington, Ph.D. and Eric Donaldson, Ph.D.)
- Ribavirin resistance-associated substitutions: genotype 1a T390I and F415Y; genotype 1b and 4 T390I

Post-baseline sequences were compared with patient-specific baseline sequences to determine if resistant substitutions had developed in NS5A, and/or NS5B during treatment. If a baseline sequence was not available, the post-baseline sequence was compared with a reference sequence. A resistant substitution was considered to have emerged if it was detected post-baseline but not at baseline or if it was detected as a mixture with other substitutions at baseline and resolved to a full mutant post-baseline.

4.3 Prior FDA Virological Reviews

IND-106739 reviews through SDN081 were done by Sr. Virology Reviewer Takashi Komatu, Ph.D. and then after SDN081 by Lisa K. Naeger, Ph.D., Sr. Virology Reviewer. Lisa Naeger was the reviewer for IND-115268 and NDA-205834 covering the fixed-dose combination (LDV/SOF) of SOF and GS-5885 (ledipasvir, LDV).

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

Reference ID: 3878352

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. 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. After a sustained virologic response has been achieved following HCV treatment, its durability has been consistently observed in long-term studies.

HCV is a small, enveloped, positive sense, single-stranded RNA virus of the Flaviviridae family. The virus contains a single, 10 kb 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 US 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 US patients with subtypes 2a, 2b and 3a most common in the US (Pawlotsky JM, et. al., 1995; Clement CG et al, 2010, Nizar N Zein, 2000) and genotype 4 is found in about 7% of US patients. In the NHANESIII study done in the US, 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].

Direct-acting antivirals (DAAs) specifically target the HCV proteins involved in the HCV life cycle. 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 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, NS5A inhibitors and NS5B polymerase inhibitors (Sarrazin and Zeuzem, 2010).

In November 2013, the FDA approved the protease inhibitor simeprevir (Olysio™) for the treatment of GT1 chronic hepatitis C virus infecton in adult patients with compensated liver disease. Simeprevir has shown cure rates of up to 80% in GT1-infected patients. The once-daily treatment must be combined with pegylated recombinant human interferon α and ribavirin or with sofosbuvir. In December 2013, the FDA approved Gilead's sofosbuvir (Sovaldi™), a nucleotide analog inhibitor of the HCV NS5B RNA polymerase enzyme. Sofosbuvir is a once-a-day treatment given in combination with ribavirin for the treatment of chronic hepatitis C virus infection in adult patients with genotype 2 or 3 virus. Sofosbuvir may also be given in combination with pegylated

Reference ID: 3878352

interferon and ribavirin for the treatment of chronic hepatitis C virus infection in treatment-naïve adult patients with GT1 and GT4 virus. In October 2014, the FDA approved Gilead's HARVONI® (LDV/SOF), an interferon-free combination therapy containing the NS5A inhibitor ledipasvir and sofosbuvir as a once-a-day treatment for GT1 infection and a 3 drug combination of AbbVie's Viekira Pak (ombitasvir/paritaprevir/ ritonaivr/dasabuvir) with or without ribavirin for GT1 infection. In November 2014, the FDA approved the combination therapy of sofosbuvir and simeprevir for use in GT1 patients. In July 2015, Bristol Myers Squibb's Daklinza™ (daclatasvir), an NS5A inhibitor was approved for GT3 infection in combination with sofosbuvir.

Recommended treatments for adults with GT1 chronic hepatitis C (CHC) virus infection without cirrhosis are treatment with LDV/SOF for 12-weeks or treatment with ombitasvir/paritaprevir/ritonaivr/dasabuvir + RBV for 12 weeks. The same recommendations apply to patients with HCV/HIV-1 co-infection. Additionally, the recommended duration for GT1 HCV-infected treatment-experienced patients with cirrhosis is 24 weeks of LDV/SOF or alternatively 12 weeks HARVONI® + RBV. Treatment with ombitasvir/paritaprevir/ritonaivr/dasabuvir + RBV for 24 weeks is recommended for GT1a subjects with cirrhosis.

In the LDV/SOF registrational trials, the overall SVR rates were 92% for GT1a-infected subjects and 82% for GT1b-infected subjects. The SVR12 rate in GT1-infected subjects with multiple baseline factors such as IL28B rs12979860 non-C/C alleles, HCV RNA >800,000 IU/mL and Metavir F3/F4 fibrosis was 71% (37/52). Additionally, physicians are prescribing SOF in combination with simeprevir both with and without RBV for 12 weeks for the treatment of HCV. In the phase 2 simeprevir study (COSMOS), overall SVR12 rates for these 12-week combinations were 93% and 96%, respectively, in patients with METAVIR scores F0-F2 and 93% in patients with GT1 HCV and advanced liver fibrosis (METAVIR scores F3 and F4). In the ombitasvir/paritaprevir/ritonaivr/ dasabuvir registrational trials, SVR12 rates were greater than 90% in both GT1a and GT1b subjects with RBV added to the ombitasvir/ paritaprevir/ritonaivr/dasabuvir regimen.

The current treatment for treatment-naïve or PI/P/R-experienced GT4, 5, or 6 HCV-infected patients with or without cirrhosis is LDV/SOF for 12-weeks. In Study 1119, the overall SVR12 rate was 93% (41/44) for GT4 HCV-infected patients and 93% (38/41) for GT5 HCV-infected patients. In ELECTRON-2 trial, the overall SVR12 for GT6 HCV-infected patients was 96% (24/25).

The current treatment for adults with GT2 is 12 weeks of SOF + RBV. The SVR12 response rates for the 12-week SOF+RBV regimen in GT2-infected subjects are 93 - 95%. Current treatment for adults with GT3 HCV infection is 24 weeks of SOF+RBV or daclatasvir + SOF (DCV/SOF) for 12 weeks. In GT3-infected subjects, response rates for SOF + RBV range from 60% in treatment-experienced cirrhotics to 93% in non-cirrhotic treatment-naïve subjects. Overall SVR12 response rate for DCV/SOF against GT3 was 89%; 96% (115/120) for subjects without cirrhosis and 63% (20/32) for subjects with cirrhosis.

Reference ID: 3878352

4.5 NON-CLINICAL VIROLOGY

Please see the NDA-204671 virology review for complete review of SOF non-clinical virology and NDA-205834 virology review for the complete review of LDV/SOF non-clinical virology.

4.6 CLINICAL STUDIES

GS-US-337-0123 (SOLAR-1) and GS-US-337-0124 (SOLAR-2)

GS-US-337-0123 (SOLAR-1) assessed LDV/SOF + RBV for 12 or 24 weeks in GT1 and 4, advanced liver disease or post-liver transplant. Subjects were enrolled at 29 sites in the United States. An identical study, Study GS-US-337-0124 (SOLAR-2), evaluated the antiviral efficacy of combination therapy with LDV/SOF as a fixed-dose combination (FDC) plus RBV for 12 or 24 weeks in subjects with genotype 1 or 4 HCV infection who had advanced liver disease and/or who had undergone liver transplantation, but subjects were enrolled at sites in Australia, France, Germany, Italy, Switzerland, Austria, Belgium, Canada, The Netherlands, Spain, United Kingdom, and New Zealand.

In both studies, subjects were enrolled into 1 of 2 cohorts based on liver transplantation status (Cohorts A or B).

In Cohort A, subjects with cirrhosis who had not undergone transplantation were enrolled into 1 of 2 following groups based on severity of hepatic impairment at their screening visit:

- **Group 1**: subjects with cirrhosis and moderate hepatic impairment (CPT B; severity of cirrhosis score of 7 to 9 [decompensated])
- **Group 2**: subjects with cirrhosis and severe hepatic impairment (CPT C; severity of cirrhosis score of 10 to 12 [decompensated])

In Cohort B, post-transplantation subjects with or without cirrhosis were enrolled into 1 of 5 groups based on severity of hepatic impairment at their screening visit.

- **Group 3**: post-transplantation subjects without cirrhosis (fibrosis stage F0-F3) and with no evidence of hepatic decompensation
- **Group 4**: post-transplantation subjects with cirrhosis and mild hepatic impairment (CPT Class A [CPT A]; severity of cirrhosis score 5 to 6 [compensated])
- **Group 5**: post-transplantation subjects with cirrhosis and moderate hepatic impairment (CPT B; severity of cirrhosis score of 7 to 9 [decompensated])
- **Group 6:** post-transplantation subjects with cirrhosis and severe hepatic impairment (CPT C; severity of cirrhosis score of 10 to 12 [decompensated])
- **Group 7:** post-transplantation subjects with fibrosing cholestatic hepatitis

Within each of the 7 treatment groups, subjects were randomized in a 1:1 ratio to receive LDV/SOF+RBV for 12 or 24 weeks. In SOLAR-1, there were 339 randomized subjects and 337 received at least 1 dose of study drug. In SOLAR-2, there were 334 randomized subjects and 333 received at least 1 dose of study drug.

Reference ID: 3878352

In SOLAR-1, among subjects with decompensated (CPT B or CPT C) cirrhosis who had not undergone a liver transplantation (Cohort A), the SVR rates were 86-88% and similar across treatment durations (Table 4). Post-transplantation subjects with stage F0-F3 fibrosis and CPT A cirrhosis (Cohort B Group 3 and 4) had the highest SVR12 rates (96% and above). Post-transplantation subjects with CPT B cirrhosis had SVR12 rates of 85%-89%. The number of subjects with CPT C cirrhosis was small, limiting interpretation of these findings, but the percentage of subjects achieving SVR12 was lower in this group compared to CPT A and B groups. There was no on-treatment virologic failure. However, there was one GT1a subject who discontinued on treatment but was not suppressed. Although SVR12 rates were similar across all groups between the 12- and 24-week treatment durations, there was a numerical trend in higher relapse rates for the shorter duration of 12 weeks. Thirteen subjects across all groups relapsed. The majority of relapses (85%, 11 of 13 subjects) occurred by post-treatment Week 8.

Table 4. GS-US-337-0123: Antiviral Response (Report PC-337-2007, page 15)

Cohort	Liver Disease Status (Group)	Study Treatment	SVR12 n/N (%)	Relapse n/N (%)[a]
A	CPT B Cirrhosis (Group 1)	LDV/SOF+RBV 12 Week	26/30 (86.7%)	3/29 (10.3%)
		LDV/SOF+RBV 24 Week	24/27 (88.9%)	1/25 (4.0%)
	CPT C Cirrhosis (Group 2)	LDV/SOF+RBV 12 Week	19/22 (86.4%)	1/20 (5.0%)
		LDV/SOF+RBV 24 Week	20/23 (87.0%)	2/22 (9.1%)
B	Stage F0-F3 Fibrosis (Group 3)	LDV/SOF+RBV 12 Week	53/55 (96.4%)	2/55 (3.6%)
		LDV/SOF+RBV 24 Week	55/56 (98.2%)	0/55
	CPT A Cirrhosis (Group 4)	LDV/SOF+RBV 12 Week	25/26 (96.2%)	0/25
		LDV/SOF+RBV 24 Week	24/25 (96.0%)	0/24
	CPT B Cirrhosis (Group 5)	LDV/SOF+RBV 12 Week	22/26 (84.6%)	1/23 (4.3%)
		LDV/SOF+RBV 24 Week	23/26 (88.5%)	0/23
	CPT C Cirrhosis (Group 6)	LDV/SOF+RBV 12 Week	3/5 (60.0%)	2/5 (40.0%)
		LDV/SOF+RBV 24 Week	3/4 (75.0%)	1/4 (25.0%)
	Fibrosing Cholestatic Hepatitis (Group 7)	LDV/SOF+RBV 12 Week	4/4 (100.0%)	0/4
		LDV/SOF+RBV 24 Week	2/2 (100.0%)	0/2

a The Full Analysis Set excluding subjects who had "other" virologic failure and subjects who had undergone transplantation prior to SVR12 with HCV RNA < LLOQ was used for the relapse analysis.

In SOLAR-2, among subjects with genotype 1 HCV infection, the SVR rates decreased with increasing severity of disease: subjects with CPT A cirrhosis had higher SVR rates than subjects with CPT B cirrhosis, and subjects with CPT B cirrhosis had higher SVR rates than subjects with CPT C cirrhosis (Table 5). The low number of subjects with GT4 HCV infection makes it difficult to interpret any trends in differences in SVR rate between treatment groups or between subjects with genotype 1 and 4 HCV infection. There was no trend in SVR12 rates across all groups between the 12- and 24-week treatment durations. There was no on-treatment virologic failure. However, there were 2 GT1a and 1 GT1b subjects who discontinued on treatment and were not suppressed. Ten subjects across all groups relapsed. All relapses occurred by post-treatment Week 8, and 9 of 10 relapses were in decompensated subjects.

Table 5. GS-US-337-0124: Antiviral Response (Report PC-337-2007, page 26)

Cohort	Liver Disease Status (Group)	Study Treatment	Genotype 1		Genotype 4	
			SVR12 n/N (%)	Relapse n/N (%)[a]	SVR12 n/N (%)	Relapse n/N (%)[a]
A	CPT B Cirrhosis (Group 1)	LDV/SOF+RBV 12 Week	20/23 (87.0%)	3/23 (13.0%)	2/3 (66.7%)	1/3 (33.3%)
		LDV/SOF+RBV 24 Week	22/23 (95.7%)	1/23 (4.3%)	2/2 (100.0%)	0/2
	CPT C Cirrhosis (Group 2)	LDV/SOF+RBV 12 Week	17/20 (85.0%)	1/18 (5.6%)	0/1	1/1 (100.0%)
		LDV/SOF+RBV 24 Week	18/23 (78.3%)	1/19 (5.3%)	1/2 (50.0%)	0/1
B	Stage F0-F3 Fibrosis (Group 3)	LDV/SOF+RBV 12 Week	42/45 (93.3%)	1/43 (2.3%)	7/7 (100.0%)	0/7
		LDV/SOF+RBV 24 Week	44/44 (100.0%)	0/44	5/5 (100.0%)	0/5
	CPT A Cirrhosis (Group 4)	LDV/SOF+RBV 12 Week	30/30 (100.0%)	0/30	3/4 (75.0%)	0/3
		LDV/SOF+RBV 24 Week	27/28 (96.4%)	0/27	5/5 (100.0%)	0/5
	CPT B Cirrhosis (Group 5)	LDV/SOF+RBV 12 Week	19/20 (95.0%)	0/19	2/2 (100.0%)	0/2
		LDV/SOF+RBV 24 Week	20/20 (100.0%)	0/20	3/3 (100.0%)	0/3
	CPT C Cirrhosis (Group 6)	LDV/SOF+RBV 12 Week	1/2 (50.0%)	0/1	0/1	1/1 (100%)
		LDV/SOF+RBV 24 Week	4/5 (80.0%)	0/4	0	0
	Fibrosing Cholestatic Hepatitis (Group 7)	LDV/SOF+RBV 12 Week	3/3 (100.0%)	0/3	0	0
		LDV/SOF+RBV 24 Week	2/2 (100.0%)	0/2	0	0

a The Full Analysis Set excluding subjects who had "other" virologic failure and subjects who had undergone transplantation prior to SVR12 with HCV RNA < LLOQ was used for the relapse analysis.

4.5 Clinical Virology

In SOLAR-1, pretreatment NS5A deep sequencing data were available for 335 of 337 subjects. One subject (Subject 0585-75239) only had nucleotide sequence data available for amino acid positions 1 to 244. Data were not available for 2 of 337 subjects due to assay failure (Subjects 0519-75114 and 5969-75524). Pretreatment NS5B deep sequencing data were obtained for 333 of 337 subjects. Full-length NS5B data were not available for 4 of 337 subjects. Two subjects only had population nucleotide sequence data available covering amino acids positions 227 to 338 (Subjects 0519-75114 and 4969-75394) and 2 subjects had assay failure (Subjects 5969-75524 and 6927-75549).

In SOLAR-2, pretreatment NS5A deep sequencing data were obtained for 332 of 333 subjects who received at least 1 dose of study drug. Assay failure occurred for 1 subject (Subject 4253-76361). Pretreatment full NS5B deep sequencing data were obtained for 324 of 333 subjects. Five subjects had population nucleotide sequence data available covering the 282 position (amino acids 227-338). Assay failure occurred for 4 subjects (Subjects 1122-76154, 4253-76232, 4253-76527 and 8631-76533).

According to the sponsor of the 34 subjects who did not achieve SVR12 in SOLAR-1, thirteen (38%) relapsed, while the other 21 subjects died, underwent transplantation, or were lost to follow-up. For subjects with GT4 infection, 3 of 5 subjects achieved SVR12 and were included in the resistance analysis. Of the 25 subjects who did not achieve SVR12 in SOLAR-2, 10 relapsed, while the other 15 subjects died, had early termination, or were lost to follow-up. In addition 7 subjects underwent transplantation and three subjects (Subjects 0676-76207, 0676-76209, and 3910-76155) were excluded from the analysis because they had advanced liver disease (Cohort A) with CPT scores of 5, 5, and 6, respectively, and were required to have a screening CPT score of 7 to 12 to be enrolled into Cohort A (Subject 0676-76207 was also lost to follow up).

CENSORING SUBJECTS FOR FDA ANALYSIS

For the FDA resistance analyses, subjects who died or experienced an AE while undetectable were removed from the analysis or who had a transplant while undetectable and subsequently died (Table 6). Thus, 11 GT1a, 11 GT1b, and 4 GT4 were removed from the FDA resistance analysis because of death or an AE while having undetectable viral load. In addition, 8 GT1a, GT1b and GT4 subjects were censored from the baseline outcome analysis because they had a transplant during treatment before SVR12 could be achieved.

Table 6. Censored Subjects from FDA Resistance Analyses

Subjects who died or experienced an AE while undetectable		
GT1a (n=11)	**GT1b (N=11)**	**GT4 (n=4)**
GS-US-337-0123-0200-75242	GS-US-337-0123-0451-75523	GS-US-337-0123-1516-75202
GS-US-337-0123-1039-75520	GS-US-337-0123-1086-75511	GS-US-337-0123-1516-75527
GS-US-337-0123-1516-75412	GS-US-337-0123-3055-75425	GS-US-337-0124-0454-76532
GS-US-337-0123-2130-75546	GS-US-337-0123-5969-75231	GS-US-337-0124-7926-76223
GS-US-337-0123-3055-75506	GS-US-337-0123-6927-75149	
GS-US-337-0123-5969-75244	GS-US-337-0123-8430-75152	
GS-US-337-0124-1067-76353	GS-US-337-0124-0557-76206	
GS-US-337-0124-1069-76201	GS-US-337-0124-0557-76544	
GS-US-337-0124-1069-76202	GS-US-337-0124-1081-76218	
GS-US-337-0124-1069-76241	GS-US-337-0124-2054-76707	
GS-US-337-0124-4472-76601	GS-US-337-0124-4472-76403	
Subjects who had a transplant during treatment before SVR12		
GT1a (n=8)	**GT1b (n=3)**	**GT4 (n=1)**
GS-US-337-0123-0585-75241	GS-US-337-0123-1039-75111	GS-US-337-0124-8632-76149
GS-US-337-0123-0585-75245	GS-US-337-0123-3055-75108	
GS-US-337-0123-1039-75213	GS-US-337-0124-0480-76236	
GS-US-337-0124-0452-76215		
GS-US-337-0124-0480-76237		
GS-US-337-0124-1119-76247		
GS-US-337-0124-1759-76125		
GS-US-337-0124-4472-76141		

An additional 3 subjects who did not have NS5A sequence at baseline were also censored from the baseline analysis: Subjects GS-US-337-0123-0519-75114 and GS-US-337-0123-5969-75524 and GS-US-337-0124-4253-76361(GT4).

PREVALENCE OF PRETREATMENT NS5A RESISTANCE-ASSOCIATED POLYMORPHISMS AND EFFECT ON TREATMENT RESPONSE

The overall prevalence of NS5A resistance-associated polymorphisms (RAPs) in SOLAR 1 and 2 subjects with HCV GT1 (any change at NS5A positions 24, 28, 30, 31, 32, 38, 58, 92, or 93) was 25%; 20% in GT1a and 34% in GT1b. The prevalence of NS5A RAPs in GT4 was 69%. [Note that consensus at NS5A position 31 in GT4 is M]. The relationship between the presence of pretreatment NS5A polymorphisms and virologic failure was assessed. Overall, virologic failure rates in HCV GT1 subjects were comparable for subjects with NS5A RAPs and subjects without NS5A RAPs at 3.4% (5/146) vs. 4.3% (19/443), respectively (Table 7; FDA analysis). For subjects with NS5A RAPs, virologic failure rates were slightly higher for GT1a subjects compared to GT1b subjects (5.3% vs. 1.4%, respectively).

Table 7. SOLAR 1 and 2

	Prevalence of NS5A Resistance-Associated Polymorphisms	Virologic Failure for NS5A Resistance-Associated Polymorphisms*	Virologic Failure for No NS5A Resistance-Associated Polymorphisms*
GT1	25% (149/600)	3.4% (5/146)	4.3% (19/443)
GT1a	20% (76/386)	5.3% (4/75)	4.3% (13/303)
GT1b	34% (73/214)	1.4% (1/71)	4.3% (6/140)
GT4	69% (25/36)	12% (3/25)	0% (0/11)

* Subjects who had a transplant during treatment before SVR12 could be achieved were censored from the baseline outcome analysis.

Virologic failure rates for GT4 subjects with NS5A RAPs was 12% (n=3) and most (n=2) failures were GT4d (Table 7 and 8; FDA analysis).

Table 8. GT4 Subtypes

	Prevalence (n=25)	Virologic Failure
GT4a	6 (24%)	0% (0/6)
GT4d	12 (48%)	17% (2/12)
GT4o	3 (12%)	0% (0/3)
GT4k	1 (4%)	0% (0/1)
GT4r	1 (4%)	0% (0/1)
GT4	2 (8%)	50% (1/2)

An examination of the effects of the presence of NS5A baseline RAPs and the 12- and 24-week durations on virologic failure rates showed that for subjects with baseline NS5A RAPS, there were no virologic failures for the 24-week duration (Table 9; FDA analysis). There were 5 GT1 virologic failures with NS5A RAPs at baseline in the 12-week arm.

There were 3 GT4 virologic failures and all were in the 12-week arm and had baseline RAPs.

Table 9. Effect of NS5A Baseline Polymorphisms and Duration in Virologic Failure Rates in SOLAR 1 and 2 (Groups 1 through 6)

	w/NS5A RAPs		No NS5A RAPs	
	12 WK	**24 WK**	**12 WK**	**24 WK**
GT1a	11% (4/38)	0% (0/35)	3% (5/146)	5% (8/150)
GT1b	3% (1/33)	0% (0/37)	7% (5/71)	1% (1/68)
GT1	7% (5/71)	0% (0/72)	5% (10/217)	4% (9/218)
GT4	21% (3/14)	0% (0/11)	0% (0/5)	0% (0/6)

When the GT1 virologic failure subjects are analyzed by liver function groups (Groups 1 and 5: Child Pugh B pre-and post-transplantation; Groups 2 and 6: Child Pugh C pre- and post-transplant; Groups 3 and 4: compensated non-cirrhotic and Child Pugh A post-transplantation), an increase in virologic failure rate based on the presence of baseline NS5A RAPs is only seen in Groups 2 and 6 and GT1a subjects in Group 3 (without cirrhosis) compensated post-transplantation (Table 10; FDA analysis). For subjects with GT4, the presence of baseline NS5A RAPs resulted in higher virologic failure rates for Child Pugh B and C groups in the 12-week duration arm, although the subgroups were very small. Note that the sponsor is not seeking an indication for GT4 subjects with Child Pugh B and C liver function.

Table 10. Effect of NS5A Baseline Polymorphisms on Virologic Failure Rates by Group in SOLAR 1 and 2

	w/NS5A RAPs		No NS5A RAPs	
GT1a	**12 WK**	**24 WK**	**12 WK**	**24 WK**
B (Grp 1 and 5)	0% (0/12)	0% (0/13)	6% (3/48)	2% (1/47)
C (Grp 2 and 6)	33% (1/3)[a]	0% (0/5)	9% (2/23)	25% (6/24)
Compensated post TX (Groups 3 and 4)	13% (3/23)[b]	0% (0/17)	0% (0/75)	1% (1/79)
GT1b				
B (Grp 1 and 5)	0% (0/9)	0% (0/10)	20% (5/25)	5% (1/20)
C (Grp 2 and 6)	20% (1/5)[c]	0% (0/9)	0% (0/12)	0% (0/13)
Compensated post TX (Groups 3 and 4)	0% (0/19)	0% (0/18)	0% (0/34)	0% (0/35)
GT1				
B (Grp 1 and 5)	0% (0/21)	0% (0/23)	11% (8/73)	3% (2/67)
C (Grp 2 and 6)	25% (2/8)[a c]	0% (0/14)	6% (2/35)	16% (6/37)
Compensated post TX (Groups 3 and 4)	7% (3/42)[b]	0% (0/35)	0% (0/109)	1% (1/114)
GT4				
B (Grp 1 and 5)	33% (1/3)	0% (0/4)	0% (0/3)	0% (0/2)
C (Grp 2 and 6)	67% (2/3)	0% (0/1)	-	-
Compensated post TX (Groups 3 and 4)	0% (0/8)	0% (0/6)	0% (0/2)	0% (0/4)

[a]30Q/H
[b]58P
[c]30R/Q and Y93H

The same analysis was done for relapsers only, removing the 4 subjects who discontinued while detectable (Table 11; FDA analysis).

Table 11. Effect of NS5A Baseline Polymorphisms on Relapse Rates by Group in SOLAR 1 and 2

	w/NS5A RAPs		No NS5A RAPs	
GT1a	**12 WK**	**24 WK**	**12 WK**	**24 WK**
B (Grp 1 and 5)	0% (0/12)	0% (0/13)	6% (3/48)	2% (1/47)
C (Grp 2 and 6)	33% (1/3)[a]	0% (0/5)	9% (2/23)	18% (4/22)
Compensated post TX (Groups 3 and 4)	13% (3/23)[b]	0% (0/17)	0% (0/75)	0% (0/78)
GT1b				
B (Grp 1 and 5)	0% (0/9)	0% (0/10)	17% (4/24)	5% (1/20)
C (Grp 2 and 6)	20% (1/5)[c]	0% (0/9)	0% (0/12)	0% (0/13)
Compensated post TX (Groups 3 and 4)	0% (0/19)	0% (0/18)	0% (0/34)	0% (0/35)

All 4 of the GT1a virologic failures with baseline NS5A RAPs had only one baseline RAP; 3 had the H58P polymorphism and 1 had a mixture at Q30Q/H. The 1 GT1b virologic failure with baseline RAPs had 2 polymorphisms, R30R/Q and Y93H. The data, although limited, do not show an effect of multiple baseline RAPs on the virologic failure (Table 12; FDA analysis). Two of the GT4 failures had the L30R polymorphism and the third failure had L28M and L30I polymorphisms. When examining the overall virologic failure rate for GT1 subjects by the presence at baseline of these key polymorphic sites (30, 58, and 93), the virologic failure rates were 8% (2/24), 5% (3/60), and 4% (1/26) for the presence of any change at positions 30, 58 and 93, respectively (Table 12; FDA analysis). For GT4, the baseline presence of L28 and L30 polymorphisms resulted in virologic failure rates of 12% (1/8) and 14% (3/22), respectively. However, as noted above, the sponsor is not seeking an indication for the GT4 subjects with Child Pugh B and C liver function.

Table 12. Virologic Failure Rates by NS5A Polymorphisms (RAPs)

NS5A RAP	Prevalence GT1a N=386	Virologic Failure GT1a	Prevalence GT1b N=214	Virologic Failure GT1b	Virologic Failure GT1	Prevalence GT4 N=36	Virologic Failure GT4
No RAPs	310	4% (13/303)	141	4% (6/140)	4% (19/443)	11 (31%)	0% (0/11)
Any RAPs	76 (20%)	5% (4/75)	73 (34%)	1% (1/71)	3% (5/146)	25 (69%)	12% (3/25)
1 RAP	67 (17%)	6% (4/66)	62 (29%)	0% (0/60)	3% (4/126)	14 (39%)	14% (2/14)
2 RAPs	7 (2%)	0% (0/7)	9 (4%)	11% (1/9)	6% (1/16)	10 (28%)	10% (1/10)
3 or more RAPs	2 (0.5%)	0% (0/2)	2 (0.9%)	0% (0/2)	0% (0/4)	1 (3%)	0/1
24	17 (4%)	0% (0/17)	2 (0.9%)	0% (0/2)	0% (0/19)	0	

28	13 (3%)	0% (0/13)	3 (1%)	0% (0/3)	0% (0/16)	8 (22%)	12% (1/8)
30	7 (2%)	17% (1/6)	18 (8%)	6% (1/18)	8% (2/24)	22 (61%)	14% (3/22)
31	6 (2%)	0% (0/6)	15 (7%)	0% (0/15)	0% (0/21)	2 (6%)	0/2
32	0		0		-	0	
38	0		0		-	0	
58	42 (11%)	7% (3/42)	18 (8%)	0% (0/18)	5% (3/60)	4 (11%)	0/4
92	0		5 (2%)	0% (o/4)	0% (0/4)	0	
93	2 (0.5%)	0% (0/2)	25 (12%)	4% (1/24)	4% (1/26)	1 (3%)	0/1

For comparison with BMS's application for daclatasvir (DCV) + SOF in the ALLY-1 and -2 trials, a separate analysis was performed looking at specific groups of NS5A RAPs (28, 30, 31 or 93) and (28, 30, 31, 58 and 93) in CPT A post-transplant and CPT B pre- and post-transplant GT1a subjects of SOLAR 1 and 2 (Table 13; FDA analysis).

Table 13. SVR12 for CPT A Post-transplantation and B Pre- and Post-Transplantation

	SOLAR-1/-2	**ALLY-1/-2**
GT1a	Child-Pugh B (Grp 1, 4 and 5) 12 Weeks+RBV	Child-Pugh A/B (Pre-transplant) or F4 (Post-transplant)
	LDV/SOF 12 Weeks +RBV	DCV/SOF 12 Weeks +/- RBV
All	97% (88/91)	90% (52/58)
NS5A RAPs @28, 30, 31, 93	100% (9/9)	43% (3/7)
No RAPs @28, 30, 31, 93	96% (79/82)	96% (49/51)
RAPs @28, 30, 31, 58, 93	100% (16/16)	64% (7/11)
No RAPs @28, 30, 31, 58, 93	96% (72/75)	96% (45/47)

Note: none had a baseline polymorphism at Y93 in these groups

TREATMENT-EMERGENT NS5A AND NS5B RESISTANCE-ASSOCIATED SUBSTITUTIONS IN VIROLOGIC FAILURES

Overall, there were 27 virologic failure subjects: 17 GT1a subjects, 7 GT1b subjects and 3 GT4 subjects (Table 14; FDA analysis). Note that four of the virologic failures discontinued on treatment with a detectable viral load and were not relapsers, but were included in the FDA virologic failure analysis (Subjects 75312, 76607, 76242, and 76207). Most of the virologic failures (n=18) were in the 12-week duration arms. Eight of the virologic failures (30%) had baseline NS5A RAPs. The remaining subjects had no pretreatment RAPs, but most developed treatment-emergent NS5A resistance substitutions at the time of relapse.

Seventy-eight percent (21/27) of the virologic failures had emergent NS5A resistance substitutions (Table 14 and 15; FDA analysis), including one of the virologic failures who discontinued on treatment with detectable viral load (Subject 75312). Fourteen virologic failures developed 1 NS5A resistance substitution and 7 developed ≥2 NS5A resistance substitutions. Of the virologic failures who relapsed, 91% (21/23) had emergent NS5A resistance substitutions. Emergent NS5A resistance-associated substitutions included K24K/R, M28T, Q30H/R, R30Q, M31V, H58D, and Y93H/C. The most prevalent NS5A substitutions that emerged at failure were Q30H/R or R30Q (33%; n=9) and Y93H (41%; n=11) (Table 15; FDA analysis). The majority of treatment-emergent RAVs conferred >1,000-fold reduced susceptibility to LDV in cell culture. Three additional subjects without cirrhosis in Group 3 had a H58P polymorphism, which confers less than 2.5-fold resistance to LDV, at pretreatment that was maintained or enriched at post-treatment.

In addition, four virologic failures had an emergent NS5B substitution, E237G (Table 14 and 15).

Table 14. Virologic Failures and Emergent Substitutions (n=27)

PID	GT	Arm	Group	Baseline NS5A RAPs	Emergent NS5A Resistance Substitutions	Baseline NS5B Poly	Emergent NS5B Substitutions
75312*	1a	24	Group 3		**H58H/D Y93Y/C**	F415F/Y	
75127	1a	12	Group 1		**Q30R H58D**		K69K/R A73A/T **E237E/G** A487A/T
75608	1a	12	Group 6		**Y93H**		82M 206R
75251	1a	24	Group 2		**M28T Q30H**		198A 238T
75343	1a	12	Group 3	H58P			
75134	1a	24	Group 1		**Y93C/H**		
75601	1a	12	Group 6		**Q30R**	F415Y	
75606	1a	24	Group 6		**Q30R**	F415F/Y	**E237G**
75224	1a	24	Group 2		**Y93C**		
75379	1a	12	Group 3	H58H/P	**H58P**		
76216	1a	12	Group 2	Q30Q/H	**Q30R**		
76226	1a	24	Group 2		**Q30Q/R L31L/V**	F415F/Y	F415Y **E237G**
76104	1a	12	Group 1		**Y93C**		
76121	1a	12	Group 1		**M28T Q30R**	F415F/Y	
76320	1a	12	Group 3	H58P	**K24K/R Q30Q/R Y93Y/H**		
76607*	1a	24	Group 6	none	none		
76242*	1a	24	Group 2	none	none		
75235	1b	12	Group 2	R30R/Q Y93H	**R30Q**		
75139	1b	12	Group 1		**Y93H**		

75126	1b	12	Group 1		**Y93H**		
75519	1b	12	Group 5		**Y93H/N**		
76207*	1b	12	Group 1	none	none	L159F	
76126	1b	12	Group 1		**L31M Y93H**		
76136	1b	24	Group 1		**Y93H**	L159F	
76608	4	12	Group 6	L30R			**E237G**
76152	4	12	Group 1	L30R	**M31V**		
76232	4	12	Group 2	L28M L30I			

*DC while detectable; not relapser

Table 15. Proportion of Virologic Failures (VF) with Emergent NS5A and NS5B Substitutions

	Proportion of VF with Emergent NS5A Substitutions	**Proportion of VF with Emergent NS5B Substitutions**
Total	78% (21/27)*	4/27 (15%)
GT1a	14/17	
GT1b	6/7	
GT4	1/3	
NS5A 24	1 (4%)	
NS5A 28	2 (7%)	
NS5A 30	9 (33%)	
NS5A 31	3 (11%)	
NS5A 58	3 (11%)	
NS5A 93	11 (41%)	
NS5B E237G		4/27 (15%)

*For relapsers only: 91% (21/23)

The applicant identified a total of 20 subjects with GT1 infection experienced virologic relapse in Studies GS-US-337-0123 and GS-US-337-0124; 14 with genotype 1a infection and 6 with genotype 1b infection (Appendix A). The FDA analysis includes 4 additional subjects who discontinued while detectable. The applicant stated that pretreatment NS5A RAPs conferring >100-fold resistance to LDV were observed in 5 of 20 subjects who relapsed and these pretreatment RAPs were maintained or enriched at the time of failure. The applicant used a cutoff of <15% for baseline NS5A polymorphisms. The Division of Antiviral Products' virologists have decided that a cutoff of 15% and above for baseline polymorphisms is appropriate given the current available assays in use and what we have seen clinically thus far. With a cutoff of 15%, there are only 2 GT1 relapse subjects (Subjects 76216 and 75235) both in Group 2) who have baseline NS5A RAPs with >100 fold resistance.

Most of the virologic failures (37%; n=10) were in Group 1 (pretransplant CPT B), but none of the GT1 failures in Group 1 had baseline NS5A RAPs. The combined Groups 1 and 5 and combined Groups 2 and 6 each had comparable number of virologic failures; 41% (n=11) and 44% (n=12) (Table 16; FDA analysis). Of the 12 virologic failures in Groups 2 and 6, 4 of them had baseline NS5A RAPs (1 GT1a, 1 GT1b and 2 GT4).

Table 16. Virologic Failures (both GT1 and 4) by Group

Group	Number of Virologic Failures (%) N=27
Group 1 pretransplant CPT B	10 (37%)
Group 2 pretransplant CPT C	7 (26%)
Group 3 post-transplant F0-F3	4 (15%)
Group 4 post-transplant CPT A	0 (0%)
Group 5 Post-transplant CPT B	1 (4%)
Group 6 Post-transplant CPT C	5 (19%)
Groups 1 and 5 CPT B	11 (41%)
Groups 2 and 6 CPT C	12 (44%)
Groups 3 and 4 F0-F3/CPT A	4 (15%)

5. CONCLUSION

The SOLAR 1 and 2 studies assessed LDV/SOF + RBV for 12 or 24 weeks in GT1 and GT4 HCV-infected patients with advanced liver disease or post-liver transplant. In these studies, the overall prevalence of NS5A RAPs (any change at NS5A positions 24, 28, 30, 31, 32, 38, 58, 92, or 93) in GT1 subjects was 25%; 20% in GT1a and 34% in GT1b. The virologic failure rates in HCV GT1 subjects were comparable for subjects with NS5A RAPs and subjects without NS5A RAPs at 3.4% (5/146) vs. 4.3% (19/443), respectively. For subjects with baseline NS5A RAPs, virologic failure rates were slightly higher for GT1a subjects compared to GT1b subjects (5.3% vs. 1.4%, respectively). In addition, for subjects with baseline NS5A RAPS, there were no virologic failures in the 24-week duration arms.

The virologic failure rates were analyzed by liver function groups (Groups 1 and 5: Child Pugh B pre-and post-transplantation; Groups 2 and 6: Child Pugh C pre- and post-transplant; Groups 3 and 4: compensated non-cirrhotic and Child Pugh A post-transplantation). An increase in virologic failure rate in GT1 subjects with baseline NS5A RAPs is seen in Groups 2 and 6 (25%; 2/8) and GT1a subjects in Group 3 (without cirrhosis) compensated post-transplantation (13%; 3/23); however, the numbers in these subgroups are small.

The four GT1a virologic failures with baseline NS5A RAPs had only one baseline RAP; three had the H58P polymorphism and one had a mixture at Q30Q/H. The one GT1b virologic failure with baseline RAPs had 2 polymorphisms, R30R/Q and Y93H.

Reference ID: 3878352

In the SOLAR-1 and SOLAR-2 trials, after 12 weeks of treatment with HARVONI® and RBV, relapse rates were 7% (5/71) and 5% (10/217) in genotype 1 subjects with and without baseline NS5A polymorphisms at resistance-associated positions, respectively. Of the 5 genotype 1 relapsers with baseline NS5A polymorphisms, 2 were pre-transplant subjects with Child Pugh C cirrhosis (1 genotype 1a with the Q30Q/H polymorphism and 1 genotype 1b with R30R/Q and Y93H polymorphisms) and 3 were post-transplantation subjects with F0-F3 fibrosis (all genotype 1a with the H58P polymorphism).

For subjects with GT4 HCV, the presence of baseline NS5A RAPs resulted in a higher percentage of virologic failures for Child Pugh B (33%; 1/3) and Child Pugh C (67%; 2/3) groups in the 12-week duration arm, although the subgroups were very small. However, the applicant is not seeking an indication for GT4 subjects with Child Pugh B and C liver function.

In SOLAR 1 and 2 studies, there were 27 virologic failure subjects with GT1 (n=24) or GT4 (n=3): 23 subjects relapsed and 4 subjects discontinued on treatment with a detectable viral load and were included in the FDA virologic failure analysis. Most of the virologic failures were in the 12-week duration arms (n=18) and most were GT1a (n=17). Eight of the virologic failures (30%) had baseline NS5A RAPs. The remaining subjects had no pretreatment RAPs, but most developed treatment-emergent NS5A RAPs at the time of relapse. Most of the virologic failures (n=10; 37%) were pretransplant CPT B, but none of them had baseline NS5A RAPs.

Seventy-eight percent (21/27) of the virologic failures had emergent NS5A resistance substitutions, including one of the virologic failures who discontinued on treatment with detectable viral load. Fourteen virologic failures developed 1 NS5A substitution and 7 developed ≥2 substititutions. Of the virologic failures who relapsed, 91% (21/23) had emergent NS5A resistance substitutions. Emergent NS5A resistance-associated substitutions included K24K/R, M28T, Q30H/R, R30Q, M31V, H58D, and Y93H/C. The most prevalent NS5A substitutions that emerged at failure were Q30H/R or R30Q (n=9; 33%) and Y93H (n=11; 41%;). The majority of treatment-emergent substitutions conferred >1000-fold reduced susceptibility to LDV in cell culture. Three additional GT1 subjects without cirrhosis in Group 3 had a H58P polymorphism at pretreatment that was maintained or enriched at post-treatment, but this substitution confers less than 2.5-fold resistance to LDV.

6.1 SPONSOR-PROPOSED PACKAGE INSERT

12.1 Mechanism of Action

HARVONI is a fixed-dose combination of ledipasvir and sofosbuvir which are direct-acting antiviral agents against the hepatitis C virus *[see Microbiology (12.4)]*.

12.4 Microbiology

Mechanism of Action

Ledipasvir is an inhibitor of the HCV NS5A protein, which is required for viral replication. Resistance selection in cell culture and cross-resistance studies indicate ledipasvir targets NS5A as its mode of action.

Sofosbuvir is an inhibitor of the HCV NS5B RNA-dependent RNA polymerase, which is required 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 genotypes 1b and 4a with IC_{50} values of 3.3 and 2.7 microM, respectively. GS-461203 is neither 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 ledipasvir against full-length replicons from genotypes 1a and 1b were 0.031 nM and 0.004 nM, respectively. The median EC_{50} values of ledipasvir against chimeric replicons encoding NS5A sequences from clinical isolates were 0.018 nM for genotype 1a (range 0.009–0.085 nM; N=30) and 0.006 nM for genotype 1b (range 0.004–0.007 nM; N=3). Ledipasvir has less antiviral activity compared to genotype 1 against genotypes 4a, 5a, and 6a, with EC_{50} values of 0.39 nM, 0.15 nM, and 1.1 nM, respectively, and substantially lower activity against genotype 6e with an EC_{50} value of 264 nM.

In HCV replicon assays, the EC_{50} values of sofosbuvir against full-length replicons from genotypes 1a, 1b, and 4a, and chimeric 1b replicons encoding NS5B from genotypes 5a or 6a ranged from 14–110 nM. The median EC_{50} value of sofosbuvir against chimeric replicons encoding NS5B sequences from clinical isolates was 62 nM for genotype 1a (range 29–128 nM; N=67) and 102 nM for genotype 1b (range 45–170 nM; N=29). In replication competent virus assays, the EC_{50} value of sofosbuvir against genotype 1a was 30 nM. Evaluation of sofosbuvir in combination with ledipasvir showed no antagonistic effect in reducing HCV RNA levels in replicon cells.

Resistance

In Cell Culture

HCV replicons with reduced susceptibility to ledipasvir have been selected in cell culture for genotypes 1a and 1b. Reduced susceptibility to ledipasvir was associated with the primary NS5A amino acid substitution Y93H in both genotypes 1a and 1b. Additionally, a Q30E substitution emerged in genotype 1a replicons. Site-directed mutagenesis of the Y93H in both genotypes 1a and 1b, as well as the Q30E

Reference ID: 3878352

substitution in genotype 1a, conferred high levels of reduced susceptibility to ledipasvir (fold change in EC_{50} greater than 1000-fold).

HCV replicons with reduced susceptibility to sofosbuvir have been selected in cell culture for multiple genotypes including 1b, 4a, 5a, and 6a. Reduced susceptibility to sofosbuvir was associated with the NS5B substitution S282T in all replicon genotypes examined. An M289L substitution developed along with the S282T substitution in genotype 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.

In Clinical Trials
Genotype 1
In a pooled analysis of subjects who received HARVONI in Phase 3 trials (ION-3, ION-1 and ION-2), 37 subjects (29 with genotype 1a HCV and 8 with genotype 1b HCV) qualified for resistance analysis due to virologic failure (35 with virologic relapse, 2 with breakthrough on-treatment due to documented non-adherence). Post-baseline NS5A and NS5B deep nucleotide sequence analysis data (assay cutoff of 1%) were available for 37/37 and 36/37 subjects' viruses, respectively.

Of the 29 genotype 1a virologic failure subjects, 55% (16/29) of subjects had virus with emergent NS5A resistance-associated substitutions K24R, M28T/V, Q30R/H/K/L, L31M, or Y93H/N at failure. Five of these 16 subjects' viruses also had baseline NS5A polymorphisms at resistance-associated amino acid positions. The most common substitutions detected at failure were Q30R, Y93H or N, and L31M.

Of the 8 genotype 1b virologic failure subjects, 88% (7/8) had virus with emergent NS5A resistance-associated substitutions L31V/M/I or Y93H at failure. Virus from three of these 7 subjects also had baseline NS5A polymorphisms at resistance-associated positions. The most common substitution detected at failure was Y93H.

At failure, 38% (14/37) of virologic failure subjects' viruses had 2 or more NS5A substitutions at resistance-associated positions.

In the SOLAR-1 and SOLAR-2 trials (liver transplant recipients or subjects with decompensated liver disease), treatment-emergent NS5A resistance-associated substitutions K24R, M28T, Q30R/H/K, L31V, H58D and/or Y93H/C were detected in (b) (4) genotype 1a virologic failure subjects, and L31M and/or Y93H/N were detected in 6/ (b) (4) genotype 1b virologic failure subjects.

In phenotypic analyses, post-baseline isolates from subjects who harbored NS5A resistance-associated substitutions at failure showed 20- to >243-fold reduced susceptibility to ledipasvir.

Treatment-emergent NS5B substitutions L159 (n=1) and V321 (n=2) previously associated with sofosbuvir failure were detected in the Phase 3 trials (ION-3, ION-1 and ION-2). In addition, treatment-emergent NS5B substitutions at highly conserved positions D61G (n=3), A112T (n=2), E237G (n=2), and S473T (n=1) were detected at low frequency by next generation sequencing in treatment failure subjects infected

Reference ID: 3878352

with HCV genotype 1a. The D61G substitution was previously described in subjects infected with HCV genotype 1a in a liver pre-transplant trial. The E237G substitution was detected in 3 subjects infected with HCV GT1a in the SOLAR-1 and SOLAR-2 trials.The clinical significance of these substitutions is currently unknown. The sofosbuvir-associated resistance substitution S282T in NS5B was not detected in any failure isolate from the Phase 3 trials. NS5B substitutions S282T, L320V/I, and V321I in combination with NS5A substitutions L31M, Y93H, and Q30L were detected in one subject at failure following 8 weeks of treatment with HARVONI in a Phase 2 trial.

Genotype 4, 5 or 6
Resistance analysis was performed for 6 relapse subjects infected with HCV genotype 4 (Study 1119 and ION-4, N=3), genotype 5 (Study 1119, N=2) or genotype 6 (ELECTRON-2; N=1) and treated with HARVONI for 12 weeks. All the relapse subjects with NS5A sequencing data (5 of 6) had pretreatment NS5A resistance-associated polymorphisms (single or combinations at positions 24, 28, 30, 31 and 58). NS5A resistance substitutions (Y93C or L28V) emerged in two of the genotype 4 relapse subjects post-treatment who also had NS5A polymorphisms pretreatment that were retained post-treatment. Two of the relapsers with genotype 4 HCV infection had an NS5B V321I substitution pretreatment, which was retained post-treatment. Three of the relapse subjects (1 each for genotype 4, 5, and 6) had virus with emergent sofosbuvir resistance-associated substitution S282T at relapse; the genotype 5 relapse subject also had emergent nucleotide inhibitor substitution M289I.

Persistence of Resistance-Associated Substitutions
No data are available on the persistence of ledipasvir or sofosbuvir resistance-associated substitutions. NS5A resistance-associated substitutions for other NS5A inhibitors have been found to persist for >1 year in some patients. The long-term clinical impact of the emergence or persistence of virus containing ledipasvir or sofosbuvir resistance-associated substitutions is unknown.

Effect of Baseline HCV Polymorphisms on Treatment Response
Genotype 1
Analyses were conducted to explore the association between pre-existing baseline NS5A polymorphisms at resistance-associated positions and relapse rates. In the pooled analysis of the Phase 3 trials, 23% (370/1589) of subjects' virus had baseline NS5A polymorphisms at resistance-associated positions (any change from reference at NS5A amino acid positions 24, 28, 30, 31, 58, 92, or 93) identified by population or deep nucleotide sequence analysis.

In treatment-naïve subjects whose virus had baseline NS5A polymorphisms at resistance-associated positions in Studies ION-1 and ION-3, relapse rates were 6% (3/48) after 8 weeks and 1% (1/113) after 12 weeks of treatment with HARVONI. Relapse rates among subjects without baseline NS5A polymorphisms at resistance-associated positions were 5% (8/167) after 8 weeks and 1% (3/306) after 12 weeks treatment with HARVONI.

Reference ID: 3878352

In treatment-experienced subjects in Study ION-2 whose virus had baseline NS5A polymorphisms at resistance-associated positions, relapse rates were 22% (5/23) after 12 weeks and 0% (0/19) after 24 weeks of treatment with HARVONI. In another study in treatment-experienced subjects (SIRIUS), 0/15 (0%) subjects with NS5A polymorphisms at resistance-associated positions relapsed after 12 weeks of treatment with HARVONI+RBV compared to 2/15 (13%) subjects treated with 24 weeks of HARVONI. In the SOLAR-1 and SOLAR-2 trials (liver transplant recipients or subjects with decompensated liver disease), after 12 weeks of treatment with HARVONI and RBV, relapse rates were 7% (5/7 (b) (4)) and (b) (4)% ((b) (4)) in genotype 1 subjects with and without baseline NS5A polymorphisms at resistance-associated positions, respectively.

(b) (4)

SVR was achieved in all 24 subjects (N=20 with L159F+C316N; N=1 with L159F; and N=3 with N142T) who had baseline polymorphisms associated with resistance to sofosbuvir and/or other NS5B nucleoside inhibitors. The NS5B S282T substitution associated with resistance to sofosbuvir was not detected in the baseline NS5B sequence of any subject in Phase 3 trials by population or deep nucleotide sequence analysis.

Genotype 4, 5 or 6
Phylogenetic analysis of HCV sequences from genotype 4-infected subjects in Study 1119 (N=44) and ION-4 (N=8) identified 7 HCV genotype 4 subtypes (4a, 4b, 4d, 4f, 4m, 4o and 4r). Most subjects were infected with subtype 4a (N=32; 62%) or 4d (N=11; 21%); 1 to 3 subjects were infected with each of the other genotype 4 subtypes. There were 3 subjects with subtype 4r, 2 of whom experienced virologic relapse, and both had a combination of (b) (4) pretreatment NS5A resistance-associated polymorphisms (L28M/V+L30R (b) (4)).

Phylogenetic analysis of HCV sequences from genotype 5-infected subjects in Study 1119 showed almost all were subtype 5a (N=39) with one subject not having a subtype identified at screening or by analysis.

Phylogenetic analysis of HCV sequences from genotype 6-infected subjects in ELECTRON-2 identified 7 HCV genotype 6 subtypes (6a, 6e, 6l, 6m, 6p, 6q and 6r). Thirty-two percent of the subjects had subtype 6a and 24% had subtype 6e. One to three subjects were infected with the other subtypes 6l, 6m, 6p, 6q, or 6r. The one subject who did not achieve SVR12 had subtype 6l.

Although the data are limited, baseline HCV NS5A resistance-associated polymorphisms are not expected to impact the likelihood of achieving SVR when HARVONI is used as recommended to treat HCV genotype 4, 5, or 6-infected patients, based on the low virologic failure rate observed in Study 1119 and ELECTRON-2. The specific baseline polymorphisms observed in subjects with

virologic failure were L28M/V, L30R, (b) (4) and P58T for genotype 4; L31M for genotype 5; and Q24K, F28V, R30A and T58P for genotype 6.

Relapse occurred in 2 of 3 genotype 4 subjects who had baseline NS5B V321I, a polymorphism at a position associated with treatment failure to sofosbuvir and other nucleoside inhibitors; these two subjects also had baseline NS5A resistance-associated polymorphisms. For genotype 5 and 6, SVR12 was achieved in subjects who had baseline NS5B polymorphisms at positions associated with resistance to nucleoside inhibitors (N=1 with N142T in genotype 5; N=1 with M289I in genotype 5; N=15 with M289L/I in genotype 6). The sofosbuvir resistance-associated substitution S282T was not detected in the baseline NS5B sequence of any subject with genotype 4, 5 or 6 HCV in clinical trials by population or deep nucleotide sequence analysis.

Cross Resistance
Based on resistance patterns observed in cell culture replicon studies and HCV-infected subjects, cross-resistance between ledipasvir and other NS5A inhibitors is expected. Both sofosbuvir and ledipasvir were fully active against substitutions associated with resistance to other classes of direct-acting antivirals with different mechanisms of action, such as NS5B non-nucleoside inhibitors and NS3 protease inhibitors. The efficacy of ledipasvir/sofosbuvir has not been established in patients who have previously failed treatment with other regimens that include an NS5A inhibitor.

6.2 FDA-NEGOTIATED PACKAGE INSERT

12.4 Microbiology

Mechanism of Action
Ledipasvir is an inhibitor of the HCV NS5A protein, which is required for viral replication. Resistance selection in cell culture and cross-resistance studies indicate ledipasvir targets NS5A as its mode of action.

Sofosbuvir is an inhibitor of the HCV NS5B RNA-dependent RNA polymerase, which is required 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 genotypes 1b and 4a with IC_{50} values of 3.3 and 2.7 microM, respectively. GS-461203 is neither 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 ledipasvir against full-length replicons from genotypes 1a and 1b were 0.031 nM and 0.004 nM, respectively. The median EC_{50} values of ledipasvir against chimeric replicons encoding NS5A sequences from clinical isolates were 0.018 nM for genotype 1a (range 0.009–0.085 nM; N=30) and 0.006 nM for genotype 1b (range 0.004–0.007 nM; N=3). Ledipasvir has less antiviral activity compared to genotype 1 against genotypes 4a, 5a, and 6a, with EC_{50} values of 0.39 nM, 0.15 nM, and 1.1 nM, respectively, and substantially lower activity against genotype 6e with an EC_{50} value of 264 nM.

Reference ID: 3878352

In HCV replicon assays, the EC_{50} values of sofosbuvir against full-length replicons from genotypes 1a, 1b, and 4a, and chimeric 1b replicons encoding NS5B from genotypes 5a or 6a ranged from 14–110 nM. The median EC_{50} value of sofosbuvir against chimeric replicons encoding NS5B sequences from clinical isolates was 62 nM for genotype 1a (range 29–128 nM; N=67) and 102 nM for genotype 1b (range 45–170 nM; N=29). In replication competent virus assays, the EC_{50} value of sofosbuvir against genotype 1a was 30 nM. Evaluation of sofosbuvir in combination with ledipasvir showed no antagonistic effect in reducing HCV RNA levels in replicon cells.

Resistance

In Cell Culture

HCV replicons with reduced susceptibility to ledipasvir have been selected in cell culture for genotypes 1a and 1b. Reduced susceptibility to ledipasvir was associated with the primary NS5A amino acid substitution Y93H in both genotypes 1a and 1b. Additionally, a Q30E substitution emerged in genotype 1a replicons. Site-directed mutagenesis of the Y93H in both genotypes 1a and 1b, as well as the Q30E substitution in genotype 1a, conferred high levels of reduced susceptibility to ledipasvir (fold change in EC_{50} greater than 1000-fold).

HCV replicons with reduced susceptibility to sofosbuvir have been selected in cell culture for multiple genotypes including 1b, 4a, 5a, and 6a. Reduced susceptibility to sofosbuvir was associated with the NS5B substitution S282T in all replicon genotypes examined. An M289L substitution developed along with the S282T substitution in genotype 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.

In Clinical Trials

Genotype 1

In a pooled analysis of subjects who received HARVONI in Phase 3 trials (ION-3, ION-1 and ION-2), 37 subjects (29 with genotype 1a HCV and 8 with genotype 1b HCV) qualified for resistance analysis due to virologic failure (35 with virologic relapse, 2 with breakthrough on-treatment due to documented non-adherence). Post-baseline NS5A and NS5B deep nucleotide sequence analysis data (assay sensitivity of 1%) were available for 37/37 and 36/37 subjects' viruses, respectively.

Of the 29 genotype 1a virologic failure subjects, 55% (16/29) of subjects had virus with emergent NS5A resistance-associated substitutions K24R, M28T/V, Q30R/H/K/L, L31M, or Y93H/N at failure. Five of these 16 subjects' viruses also had baseline NS5A polymorphisms at resistance-associated amino acid positions. The most common substitutions detected at failure were Q30R, Y93H or N, and L31M.

Of the 8 genotype 1b virologic failure subjects, 88% (7/8) had virus with emergent NS5A resistance-associated substitutions L31V/M/I or Y93H at failure. Virus from three of these 7 subjects also had baseline NS5A polymorphisms at resistance-associated positions. The most common substitution detected at failure was Y93H.

At failure, 38% (14/37) of virologic failure subjects' viruses had 2 or more NS5A substitutions at resistance-associated positions.

Reference ID: 3878352

In the SOLAR-1 and SOLAR-2 trials (liver transplant recipients or subjects with decompensated liver disease), there were 24 virologic failures with genotype 1 (20 relapsers and 4 subjects who discontinued treatment while detectable). Treatment-emergent NS5A resistance-associated substitutions K24R, M28T, Q30R/H/K, L31V, H58D/P and/or Y93H/C were detected in 14/17 (82%) genotype 1a virologic failure subjects, and R30Q, L31M and/or Y93H/N were detected in 6/7 (86%) genotype 1b virologic failure subjects.

In phenotypic analyses, post-baseline isolates from subjects who harbored NS5A resistance-associated substitutions at failure showed 20- to >243-fold reduced susceptibility to ledipasvir.

Treatment-emergent NS5B substitutions L159 (n=1) and V321 (n=2) previously associated with sofosbuvir failure were detected in the Phase 3 trials (ION-3, ION-1 and ION-2). In addition, treatment-emergent NS5B substitutions at highly conserved positions D61G (n=3), A112T (n=2), E237G (n=2), and S473T (n=1) were detected at low frequency by next generation sequencing in treatment failure subjects infected with HCV genotype 1a. The D61G substitution was previously described in subjects infected with HCV genotype 1a in a liver pre-transplant trial. The E237G substitution was detected in 3 subjects infected with HCV GT1a in the SOLAR-1 and SOLAR-2 trials. The clinical significance of these substitutions is currently unknown. The sofosbuvir-associated resistance substitution S282T in NS5B was not detected in any failure isolate from the Phase 3 trials. NS5B substitutions S282T, L320V/I, and V321I in combination with NS5A substitutions L31M, Y93H, and Q30L were detected in one subject at failure following 8 weeks of treatment with HARVONI in a Phase 2 trial.

Genotype 4, 5 or 6
Resistance analysis was performed for 6 relapse subjects infected with HCV genotype 4 (Study 1119 and ION-4, N=3), genotype 5 (Study 1119, N=2) or genotype 6 (ELECTRON-2; N=1) and treated with HARVONI for 12 weeks. All the relapse subjects with NS5A sequencing data (5 of 6) had pretreatment NS5A resistance-associated polymorphisms (single or combinations at positions 24, 28, 30, 31 and 58). NS5A resistance substitutions (Y93C or L28V) emerged in two of the genotype 4 relapse subjects post-treatment who also had NS5A polymorphisms pretreatment that were retained post-treatment. Two of the relapsers with genotype 4 HCV infection had an NS5B V321I substitution pretreatment, which was retained post-treatment. Three of the relapse subjects (1 each for genotype 4, 5, and 6) had virus with emergent sofosbuvir resistance-associated substitution S282T at relapse; the genotype 5 relapse subject also had emergent nucleotide inhibitor substitution M289I.

Persistence of Resistance-Associated Substitutions
No data are available on the persistence of ledipasvir or sofosbuvir resistance-associated substitutions. NS5A resistance-associated substitutions for other NS5A inhibitors have been found to persist for >1 year in some patients. The long-term clinical impact of the emergence or persistence of virus containing ledipasvir or sofosbuvir resistance-associated substitutions is unknown.

Effect of Baseline HCV Polymorphisms on Treatment Response

Genotype 1

Analyses were conducted to explore the association between pre-existing baseline NS5A polymorphisms at resistance-associated positions and relapse rates. In the pooled analysis of the Phase 3 trials, 23% (370/1589) of subjects' virus had baseline NS5A polymorphisms at resistance-associated positions (any change from reference at NS5A amino acid positions 24, 28, 30, 31, 58, 92, or 93) identified by population or analysis of deep nucleotide sequences with a 15% sensitivity threshold.

In treatment-naïve subjects whose virus had baseline NS5A polymorphisms at resistance-associated positions in Studies ION-1 and ION-3, relapse rates were 6% (3/48) after 8 weeks and 1% (1/113) after 12 weeks of treatment with HARVONI. Relapse rates among subjects without baseline NS5A polymorphisms at resistance-associated positions were 5% (8/167) after 8 weeks and 1% (3/306) after 12 weeks treatment with HARVONI.

In treatment-experienced subjects in Study ION-2 whose virus had baseline NS5A polymorphisms at resistance-associated positions, relapse rates were 22% (5/23) after 12 weeks and 0% (0/19) after 24 weeks of treatment with HARVONI. In another study in treatment-experienced subjects (SIRIUS), 0/15 (0%) subjects with NS5A polymorphisms at resistance-associated positions relapsed after 12 weeks of treatment with HARVONI+RBV compared to 2/15 (13%) subjects treated with 24 weeks of HARVONI.

SVR was achieved in all 24 subjects (N=20 with L159F+C316N; N=1 with L159F; and N=3 with N142T) who had baseline NS5B polymorphisms associated with resistance to sofosbuvir and/or other NS5B nucleoside inhibitors. The NS5B S282T substitution associated with resistance to sofosbuvir was not detected in the baseline NS5B sequence of any subject in Phase 3 trials by population or deep nucleotide sequence analysis.

In the SOLAR-1 and SOLAR-2 trials (liver transplant recipients or subjects with decompensated liver disease), after 12 weeks of treatment with HARVONI and RBV, relapse rates were 7% (5/71) and 5% (10/217) in genotype 1 subjects with and without baseline NS5A polymorphisms at resistance-associated positions, respectively.

In the Phase 3 trials and SOLAR trials, the specific baseline NS5A resistance-associated polymorphisms observed among subjects with relapse were M28T/V, Q30H, Q30R, L31M, H58D/P, Y93H, and Y93N in genotype 1a, and L28M, L31M, A92T, and Y93H in genotype 1b. Subjects with multiple NS5A polymorphisms at resistance-associated positions appeared to have higher relapse rates.

Genotype 4, 5 or 6

Phylogenetic analysis of HCV sequences from genotype 4-infected subjects in Study 1119 (N=44) and ION-4 (N=8) identified 7 HCV genotype 4 subtypes (4a, 4b, 4d, 4f, 4m, 4o and 4r). Most subjects were infected with subtype 4a (N=32; 62%) or 4d (N=11; 21%); 1 to 3 subjects were infected with each of the other genotype 4

Reference ID: 3878352

subtypes. There were 3 subjects with subtype 4r, 2 of whom experienced virologic relapse, and both had a combination of 2 pretreatment NS5A resistance-associated polymorphisms (L28M/V+L30R).

Phylogenetic analysis of HCV sequences from genotype 5-infected subjects in Study 1119 showed almost all were subtype 5a (N=39) with one subject not having a subtype identified at screening or by analysis.

Phylogenetic analysis of HCV sequences from genotype 6-infected subjects in ELECTRON-2 identified 7 HCV genotype 6 subtypes (6a, 6e, 6l, 6m, 6p, 6q and 6r). Thirty-two percent of the subjects had subtype 6a and 24% had subtype 6e. One to three subjects were infected with the other subtypes 6l, 6m, 6p, 6q, or 6r. The one subject who did not achieve SVR12 had subtype 6l.

Although the data are limited, baseline HCV NS5A resistance-associated polymorphisms are not expected to impact the likelihood of achieving SVR when HARVONI is used as recommended to treat HCV genotype 4, 5, or 6-infected patients, based on the low virologic failure rate observed in Study 1119 and ELECTRON-2. The specific baseline polymorphisms observed in subjects with virologic failure were L28M/V, L30R, and P58T for genotype 4; L31M for genotype 5; and Q24K, F28V, R30A and T58P for genotype 6.

Relapse occurred in 2 of 3 genotype 4 subjects who had baseline NS5B V321I, a polymorphism at a position associated with treatment failure to sofosbuvir and other nucleoside inhibitors; these two subjects also had baseline NS5A resistance-associated polymorphisms. For genotype 5 and 6, SVR12 was achieved in subjects who had baseline NS5B polymorphisms at positions associated with resistance to sofosbuvir and other nucleoside inhibitors (N=1 with N142T in genotype 5; N=1 with M289I in genotype 5; N=15 with M289L/I in genotype 6). The sofosbuvir resistance-associated substitution S282T was not detected in the baseline NS5B sequence of any subject with genotype 4, 5 or 6 HCV in clinical trials by population or deep nucleotide sequence analysis.

Cross Resistance

Based on resistance patterns observed in cell culture replicon studies and HCV-infected subjects, cross-resistance between ledipasvir and other NS5A inhibitors is expected. Both sofosbuvir and ledipasvir were fully active against substitutions associated with resistance to other classes of direct-acting antivirals with different mechanisms of action, such as NS5B non-nucleoside inhibitors and NS3 protease inhibitors. The efficacy of ledipasvir/sofosbuvir has not been established in patients who have previously failed treatment with other regimens that include an NS5A inhibitor.

APPENDIX A. GS-US-337-0123 and GS-US-337-0124: Sequence Analysis for Subjects with Genotype 1 HCV Infection and Virologic Failure: Applicant Analysis

(Summary of Clinical Pharmacology Studies pages 40-42)

Subject Number	Genotype	Treatment	NS5A RAPs		NS5B NI RAVs		NS5B RBV RAVs	
			Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
1516-75127	1a	Cohort A, CPT B (Group 1) – LDV/SOF+RBV 12W	**H58D (3.27%)**	**Q30R (98.3%)** **H58D (> 99%)**	None	E237G (23.3%)	None	None
5969-75343	1a	Cohort B, F0–F3 Fibrosis (Group 3) – LDV/SOF+RBV 12W	H58P (> 99%)	H58P (> 99%)	None	None	None	None
8430-75379	1a	Cohort B, F0–F3 Fibrosis (Group 3) – LDV/SOF+RBV 12W	H58P (65.51%)	H58P (> 99%)	None	None	None	None
1759-76320	1a	Cohort B, F0–F3 Fibrosis (Group 3) – LDV/SOF+RBV 12W	M28V (2.4%) H58P (> 99%)	**K24R (63.9%)** **Q30R (63.1%)** H58P (> 99%) **Y93H (36.6%)**	None	None	None	None
0465-76216	1a	Cohort A, CPT C (Group 2) – LDV/SOF+RBV 12W	**Q30H (15.7%)** **Y93C (1.2%)** **Y93H (14.0%)**	**Q30R (> 99%)**	None	None	None	None
6927-75601	1a	Cohort B, CPT C (Group 6) – LDV/SOF+RBV 12W	**Q30H (2.71%)** **Q30R (3.1%)**	**Q30R (> 99%)**	None	None	F415Y (> 99%)	F415Y (> 99%)
1249-75235	1b	Cohort A, CPT C (Group 2) – LDV/SOF+RBV 12W	R30Q (56.13%) **Y93H (> 99%)**	R30Q (> 99%) **Y93H (> 99%)**	None	NA	None	NA
1036-76126	1b	Cohort A, CPT B (Group 1) – LDV/SOF+RBV 12W	L31M (13.4%)	**L31M (> 99%)** **Y93H (> 99%)**	None	NA	None	NA
6927-75134	1a	Cohort A, CPT B (Group 1) – LDV/SOF+RBV 24W	None	**Y93H (15.44%)** **Y93C (84.15%)**	None	None	None	None

Subject Number	Genotype	Treatment	NS5A RAPs		NS5B NI RAVs		NS5B RBV RAVs	
			Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
8429-75224	1a	Cohort A, CPT C (Group 2) – LDV/SOF+RBV 24W	None	**Y93C (> 99%)**	None	NA	None	NA
2738-75251	1a	Cohort A, CPT C (Group 2) – LDV/SOF+RBV 24W	None	**M28T (> 99%)** **Q30H (> 99%)**	None	None	None	None
2130-75608	1a	Cohort B, CPT C (Group 6) – LDV/SOF+RBV 12W	None	**Y93C (1.53%)** **Y93H (97.56%)**	None	None	None	F415Y (2.87%)
7275-75606	1a	Cohort B, CPT C (Group 6) – LDV/SOF+RBV 24W	None	**Q30R (> 99%)**	None	E237G[a] (> 99%)	F415Y (22.42%)	None
0557-76104	1a	Cohort A, CPT B (Group 1) – LDV/SOF+RBV 12W	None	**Y93C (97.8%)**	None	None	None	None
1069-76121	1a	Cohort A, CPT B (Group 1) – LDV/SOF+RBV 12W	None	**M28T (> 99%)** **Q30R (> 99%)**	None	None	F415Y (42.0%)	None
0477-76226	1a	Cohort A, CPT C (Group 2) – LDV/SOF+RBV 24W	None	**K24T (1.7%)** **M28T (8.6%)** **Q30K (4.6%)** **Q30H (7.1%)** **Q30R (52.9%)** **L31V (82.0%)** **Y93H (1.7%)** **Y93C (2.3%)**	None	E237G[a] (2.0%)	F415Y (47.4%)	F415Y (> 99%)
5627-75126	1b	Cohort A, CPT B (Group 1) – LDV/SOF+RBV 12W	None	**Y93H (> 99%)**	None	None	None	None
2130-75139	1b	Cohort A, CPT B (Group 1) – LDV/SOF+RBV 12W	None	**Y93H (> 99%)**	None	None	None	None
8224-75519	1b	Cohort B, CPT B (Group 5) – LDV/SOF+RBV 12W	None	**Y93N (56.94%)** **Y93H (62.73%)**	None	None	T3901 (13.03%)	None

Subject Number	Genotype	Treatment	NS5A RAPs		NS5B NI RAVs		NS5B RBV RAVs	
			Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
6437-76136	1b	Cohort A, CPT B (Group 1) – LDV/SOF+RBV 24W	None	**Y93H** (> 99%)	L159F (> 99%)	NA	None	NA

NA = not applicable; W = week
a E237G, a conserved site substitution in NS5B. E237G showed a small reduction in susceptibility to SOF (1.3-fold change) in a genotype 1a replicon assay. The clinical significance of E237G is unknown.
Bold text denotes RAVs.
Source: m5.3.5.4, PC-337-2007

GS-US-337-0123 and GS-US-337-0124: Sequence Analysis for Subjects with Genotype 4 HCV Infection and Virologic Failure
(Summary of Clinical Pharmacology Studies page 44)

Subject Number	Genotype	Treatment	NS5A RAPs		NS5B NI RAVs		NS5B RBV RAVs	
			Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
0480-76608	4d	Cohort B, CPT C (Group 6) – LDV/SOF+RBV 12W	**L30R** (> 99%)	**L30R** (> 99%)	None	E237G[a] (> 99%)	None	None
3922-76152	4d	Cohort A, CPT B (Group 1) – LDV/SOF+RBV 12W	**L30R** (> 99%)	**L30H (4.1%)** **L30R (95.7%)** **M31V (87.3%)** **P58L (3.2%)** **Y93C (7.2%)**	None	None	None	None
4253-76232[b]	4	Cohort A, CPT C (Group 2) – LDV/SOF+RBV 12W	**L28M** (> 99%) L30I (98.9%)	Assay failure	Assay failure	No RAVs	Assay failure	NA

NA = not applicable; W = week
a E237G, a conserved site substitution in NS5B. E237G showed a small reduction in susceptibility to SOF (1.3-fold change) in a genotype 1a replicon assay. The clinical significance of E237G is unknown.
b Subject had only NS5B population sequence data available covering amino acids 227–338 at posttreatment.
Bolded text denotes RAVs.
Source: m5.3.5.4, PC-337-2007

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