CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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MICROBIOLOGY / VIROLOGY REVIEW(S)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 205834 **SDN** 002 **DATE REVIEWED:** 06/27/2014

Reviewer: Eric F. Donaldson, Ph.D.
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Sponsor: Gilead Sciences, Inc.
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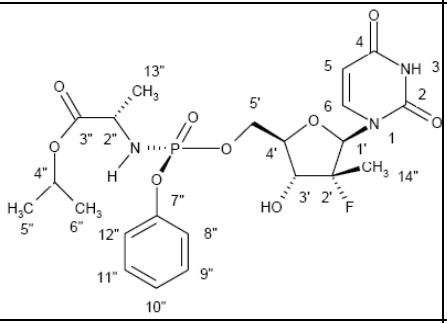
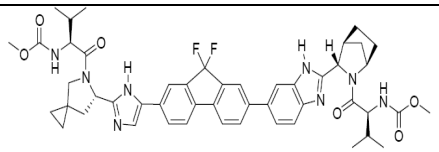
002	02/10/2014	02/10/2014	02/10/2014

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	529.46	889.00 Da

Drug category: Antiviral

Indication: Fixed-dose combination of ledipasvir, a hepatitis C virus (HCV) NS5A inhibitor and sofosbuvir, an HCV uridine nucleotide analog NS5B polymerase inhibitor, which is indicated for the treatment of chronic hepatitis C virus genotype 1 infection

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
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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; GT, genotype; HCV, hepatitis C virus; HSA, human serum albumin; IC_{50}, inhibitory concentration at 50%; IFN, recombinant human interferon α; mt, mitochondria; NGS, next generation sequencing; NAPI, nucleos(t)ide analog polymerase inhibitor; NNAPI, non-nucleoside analog polymerase inhibitor; NRTIs, nucleoside reverse transcriptase inhibitors; PBL, peripheral blood lymphocytes; PDVF, protocol defined virologic failure; PEG, pegylated human interferon; PR, protease; P/R, pegylated interferon/ribavirin; RAV, resistance-associated variant; RBV, ribavirin; SDM, site-directed mutants; SOF, sofosbuvir; SVR, sustained virologic response; WT, wild-type.

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EXECUTIVE SUMMARY

This review focused on the next generation sequencing (NGS) data provided in support of NDA 205834 for the fixed-dose combination (FDC) of ledipasvir (LDV) and sofosbuvir (SOF; LDV/SOF) indicated for the treatment of hepatitis C virus (HCV) genotype (GT) 1 infection. Overall, assessment of the NGS data by the Division of Antiviral Products (DAVP) indicated that the data and analysis provided by the sponsor, Gilead Sciences (GSI), was acceptable and this NDA is approvable with respect to virology.

SOF (NDA 204671; approved December 2013) 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 hepatocytes. It is an inhibitor of the NS5B RNA dependent RNA polymerase. In HCV replicon assays, the EC_{50} values of sofosbuvir against full-length replicons from genotype 1a, 1b, 2a, 3a and 4a, and chimeric 1b replicons encoding NS5B from genotype 2b, 5a or 6a ranged from 0.014 to 0.11 µM. The median EC_{50} value of sofosbuvir against chimeric replicons encoding NS5B sequences from clinical isolates was 0.062 µM for genotype 1a (range 0.029-0.128 µM; N=67), 0.102 µM for genotype 1b (range 0.045-0.170 µM; N=29), 0.029 µM for genotype 2 (range 0.014-0.081 µM; N=15) and 0.081 µM for genotype 3a (range 0.024-0.181 µM; N=106). In infectivity assays, the EC_{50} values of sofosbuvir against genotype 1a and 2a viruses were 0.03 µM and 0.02 µM, respectively.

LDV is a new molecular entity that inhibits HCV replication by interfering with the viral NS5A protein. It has antiviral activity against HCV genotype 1a and 1b replicons, with EC_{50} values of 0.031 nM and 0.004 nM, respectively. In addition, LDV has EC_{50} values ranging from 0.15 to 530 nM against genotypes 2 to 6 replicons. LDV has an EC_{50} value of 21 nM against the GT2a JFH-1 replicon with L31 in NS5A, but has a reduced activity with an EC_{50} value of 249 nM against the GT2a J6 HCV strain with M31, a common resistance-associated substitution in GT 1. LDV has 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 has substantially lower activity against genotypes 3a and 6e with EC_{50} values of 168 nM and 264 nM, respectively.

Data from three phase 3 studies, including Study GS-US-337-0102 (ION-1; treatment-naïve subjects), Study GS-US-337-0108 (ION-3; treatment-naïve non-cirrhotic subjects), and Study GS-US-337-0109 (ION-2; treatment-experienced subjects) and two phase 2 studies, including Study P7977-0532 (ELECTRON) and Study GS-US-337-0118 (LONESTAR) were submitted for resistance analyses. Cell culture selection experiments were performed using the HCV GT1a and GT1b replicon systems to identify resistance-associated substitutions that emerged in NS5A in response to LDV. These experiments, along with phenotypic assessments, showed that Q30E and Y93H were associated with resistance to LDV in the HCV GT1a replicon and Y93H was the predominant resistance-associated substitution in the GT1b replicon (see the review of Clinical Virology Reviewer Lisa Naeger, Ph.D. for complete details). In the phase 2 and phase 3 clinical trials, additional resistance-associated substitutions were identified and phenotyped by the sponsor. According to their analyses, NS5A resistance-associated substitutions Y93H, Y93N, Y93C, M28A, or H58D that emerge in

HCV GT1a and A92K or Y93H that emerges in HCV GT1b confer >1,000-fold reductions in susceptibility to LDV in cell culture. The L31M, L31I, L31V, Q30H, Q30R, Q30G, or P32L substitutions that emerge in HCV GT1a and the P58D substitution that emerges in HCV GT1b are in the 100- to 1,000-fold resistance category. The K24R, K24G, K24N, M28T, Q30L, Q30T, S38F, A92T, or Y93F substitutions that emerge in GT1a and the L31M, L31V, L31I, or P32L substitutions that emerge in GT1b are in the <100-fold resistance category. Based on these results, the LDV resistance analysis focused on, but was not limited to, these NS5A positions. NS5A polymorphisms at amino acid positions K24, M28, Q30, L31, P32L, H58, A92, and Y93 were analyzed in the FDA virology resistance analysis. Substitutions or mixtures of substitutions at these NS5A positions were detected at baseline in 23% (370/1615) of the subjects in the phase 3 studies (ION-1, ION-2, and ION-3).

For the virology analyses, relapse rates were used as the measure of efficacy outcome for the three phase 3 studies and the two phase 2 studies. The overall relapse rate was 2.7% in all the studies submitted. In GT1a subjects, the relapse rate was 3% (41/1378). In GT1b subjects, the relapse rate was 1.7% (7/411). When the effect of individual baseline NS5A polymorphisms on relapse rates was examined, the highest relapse rates were seen in subjects with baseline polymorphisms at positions Q30, L31, and Y93 where relapse rates were 6.6% (5/76), 10% (5/50), and 15% (8/54), respectively. Relapse rates for subjects with one baseline NS5A resistance-associated polymorphism were 3.6%, but were higher for subjects with 2 or 3 baseline NS5A resistance-associated polymorphisms with relapse rates of 9.5% and 9%, respectively.

There were a total of 50 subjects (GT1a=42 and GT1b=8) who failed treatment with the FDC of LDV/SOF and who comprised the resistance analysis population that was analyzed by next generation sequencing. The most common substitutions associated with resistance to LDV (as determined comparing three variant detection algorithms and only counting those detected by two) were at positions Y93 (n=19; GT1a=15 and GT1b=4), Q30 (n=14; GT1a=14 and GT1b=0), M28 (n=10; GT1a=9 and GT1b=1), L31 (n=6; GT1a=6 and GT1b=0), and H58 (n=3, GT1a=3 and GT1b=0).

For SOF resistance, there were several substitutions associated with resistance that had been identified in the review of SOF (NDA 204671), including positions S62 (n=23; GT1a=23 and GT1b=0), D61 (n=9; GT1a=9 and GT1b=0), E440 (n=8; GT1a=1 and GT1b=7), V321 (n=3; GT1a=2 and GT1b=1), L159 (n=1; GT1a=1 and GT1b=0), S282 (n=1; GT1a=1 and GT1b=0), and L320 (n=1; GT1a=1 and GT1b=0) that emerged in these studies. In addition, two additional amino acid positions had substitutions that were treatment emergent, including A112 (n=3, GT1a=3 and GT1b=0) and E237 (n=2; GT1a=2 and GT1b=0). Of note, in this dataset, substitutions at positions HCV GT1a NS5B_S62 and HCV GT1b NS5B_E440 appeared to be polymorphic and not associated with resistance as compared to the SOF dataset that was used to identify these positions (SOF NDA 204671, original review and addendum). However, the D61G substitution was treatment emergent and detected in the NS5B HCV protein of several subjects infected with HCV GT1a who failed treatment with the LDV/SOF FDC. This same substitution was detected and associated with treatment failure among subjects infected with HCV GT1a in the Liver Pre-Transplant Study P7977-2025 (reviewed in SOF NDA 204671 addendum). Additional substitutions that should be phenotypically evaluated for SOF resistance include, NS5B_A112T, NS5B_E237G, and NS5B_S473T.

BACKGROUND AND SUMMARY

Sofosbuvir is a nucleotide prodrug of 2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate that is converted to the active uridine triphosphate form (GS-461203) within the hepatocyte. In HCV replicon assays, the EC_{50} values of sofosbuvir against full-length replicons from genotype 1a, 1b, 2a, 3a and 4a, and chimeric 1b replicons encoding NS5B from genotype 2b, 5a or 6a ranged from 0.014 to 0.11 μM. The median EC_{50} values of sofosbuvir against chimeric replicons encoding NS5B sequences from clinical isolates were 0.062 μM for genotype 1a (range 0.029-0.128 μM; N=67), 0.102 μM for genotype 1b (range 0.045-0.170 μM; N=29), 0.029 μM for genotype 2 (range 0.014-0.081 μM; N=15) and 0.081 μM for genotype 3a (range 0.024-0.181 μM;

N=106). In infectivity assays, the EC_{50} values of sofosbuvir against genotype 1a and 2a viruses were 0.03 μM and 0.02 μM, respectively.

Ledipasvir inhibits HCV replication by interfering with the viral NS5A protein. It has antiviral activity against HCV genotypes 1a and 1b replicons, with EC_{50} values of 0.031 nM and 0.004 nM, respectively. In addition, LDV has EC_{50} values ranging from 0.15 to 530 nM against genotypes 2 to 6 replicons. LDV has an EC_{50} value of 21 nM against the GT2a JFH-1 replicon with L31 in NS5A, but has a reduced activity with an EC_{50} value of 249 nM against the GT2a J6 HCV strain expressing M31, a common resistance substitution. LDV has 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 has substantially lower activity against genotypes 3a and 6e with EC_{50} values of 168 nM and 264 nM, respectively.

Data from three phase 3 studies, including Study GS-US-337-0102 (ION-1; treatment-naïve subjects), Study GS-US-337-0108 (ION-3; treatment-naïve non-cirrhotic subjects), and Study GS-US-337-0109 (ION-2; treatment-experienced subjects), and two phase 2 studies, including Study P7977-0532 (ELECTRON) and Study GS-US-337-0118 (LONESTAR) were submitted for resistance analyses. The sponsor provided next generation sequencing (NGS) data that were used in the resistance analysis of the five clinical trials (Table 1).

Table 1. Phase 2 and 3 LDV/SOF and LDV + SOF studies analyzed for resistance by NGS (Table 1, page 15, Integrated Phase 2&3 Virology Study Report).

Study	Phase	Population	Regimen
GS-US-337-0118 (LONESTAR)	2	GT1, Treatment-naïve without cirrhosis or IFN+RBV+protease inhibitor-experienced without or with cirrhosis	SOF/LDV ± RBV for 8 or 12 weeks treatment duration
P7977-0523 (ELECTRON arms 12, 13, 16,17, 20, and 21)	2	GT1, Treatment-naïve, null responders, null responders with compensated cirrhosis, hemophiliacs	SOF+LDV or SOF/LDV ± RBV for 6 or 12 weeks treatment duration
GS-US-337-0102 (ION-1)	3	GT1, Treatment-naïve	SOF/LDV ± RBV for 12 or 24 weeks treatment duration
GS-US-337-0109 (ION-2)	3	GT1, IFN+RBV Treatment-experienced and IFN+RBV+ protease inhibitor-experienced	SOF/LDV ± RBV for 12 or 24 weeks treatment duration
GS-US-337-0108 (ION-3)	3	GT1, Treatment-naïve non-cirrhotic	SOF/LDV ± RBV for 8 or 12 weeks treatment duration

The sponsor provided the NGS data on a hard drive and the dataset included: 1) frequency tables showing amino acid variation that occurred at each position of 3 viral proteins (NS3/4A, NS5A, and NS5B) for each failure sample that was successfully sequenced using Illumina; 2) raw sequence data in fastq format for all samples that were deep sequenced; 3) summary resistance data for each study; and 4) cross study comparisons of resistance data.

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

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

Rationale for Requesting and Analyzing NGS Data

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

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

Providing accurate resistance information is imperative for protecting public health to prevent emergence of novel resistant and cross-resistant viral variants that have the potential to infect others and cause major outbreaks of disease that cannot be controlled by approved drugs. In addition, the resistance information provides important guidance for health care professionals who oversee the use of these therapeutics and is included in the drug product information approved by DAVP.

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

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

NGS Data Analysis Pipeline

DAVP worked with the Office of Scientific Computing within CDER to acquire the resources to analyze NGS data for review purposes. The CLC Genomics Workbench was installed for use on the High Performance Computer at CDRH and was used to establish an analysis pipeline for independently analyzing NGS data. CLC Genomics was used to evaluate each of the sequence runs, trim and filter the sequences prior to mapping, and to map the sequences to HCV GT1a and GT1b reference sequences. Two independent variant detection algorithms were used to call variants from each mapping, and the variant tables were exported from CLC Genomics Workbench and combined to generate frequency tables and resistance summary tables (Figure 1).

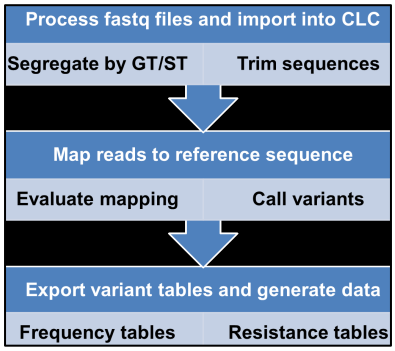

Figure 1. An overview of the NGS analysis Pipeline using CLC Genomics Workbench.

NGS Analysis Parameters and Overview of Data Analysis

Each step of the analysis process is briefly described below. For a more detailed description, please see the SOF NDA review (NDA 20467 SDN 004).

1. **Processing fastq files with CLC Genomics Workbench**. Data were received on a portable hard drive, which included fastq files for each subject and timepoint that was sequenced using the Illumina platform. The sequences were uploaded via the CLC Genomics interface, using the Illumina specific criteria. Failed reads were removed, read names were discarded, and Quality scores were calculated using the NCBI/Sanger (Illumina Pipeline 1.8) option.
2. **Segregating sequences by HCV genotype and trimming the sequence reads prior to mapping.** The fastq files were separated by genotype and subtype and the NS5A and NS5B genes for HCV GT1a (H77) and GT1b (Con1) were imported and annotated as coding sequences to be used as reference sequences for mapping. The individual reads from each fastq file were subjected to trimming using the default parameters for CLC Genomics Workbench.
3. **Mapping reads to the appropriate reference sequence for each HCV genotype/subtype.** The reads from each fastq file were aligned to the appropriate reference sequence to generate a mapping for each timepoint. The mapping contained the target of interest (the NS5A and NS5B gene sequences) and was used to generate a consensus sequence for each sequence run. The consensus sequences were conceptually translated to amino acid sequences to compare changes that occurred at the amino acid level. In general, the mappings were assessed to determine the depth of coverage at each nucleotide position and to evaluate read directionality (ratio of forward to reverse reads) to identify regions of bias.

NGS Analysis Pipeline Output

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

    1. **Probabilistic Variant Detection (PVD75)** – calls variants from a read mapping using a probabilistic model (combines a Bayesian model and a Maximum Likelihood approach to calculate prior and error probabilities). Parameters are calculated on the mapped reads without considering the reference

sequence. The variant probability parameter was reduced from a default value of 90 to 75 to increase the number of variant calls, given that false calls would likely be filtered during data analysis.

2. **Quality-based Variant Detection (QbVD)** - based on the Neighborhood Quality Standard algorithm, it uses a combination of quality filters and user-specified thresholds for coverage and frequency to call variants covered by aligned reads.

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

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

5. **Generating resistance analysis tables.** An ETL/Kettle script was used to convert the frequency tables into resistance analysis tables, allowing the resistance tables to be populated using different frequency thresholds. For example, the frequency tables generated from CLC Genomics Workbench output or submitted by the sponsor contained all variants with a frequency greater than or equal to 1%, and this tool allowed resistance analysis tables to be generated showing variants at different levels of sensitivity (5%, 15%, 25%, etc.) as defined by the user.

6. **Conducting independent resistance analysis.** The frequency tables and resistance analysis tables were then analyzed to identify substitutions that occurred above a defined frequency threshold of 10%, using the following criteria:

   a. **SUBS10 criteria** – Identified all substitutions that were not detected at baseline (<0.01 frequency) but were detected at a frequency of 0.10 or greater at later timepoints or detected at baseline at a frequency of 0.10 and not detected at later timepoints.

NGS Data Comparison

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

CLINICAL STUDIES

The sponsor submitted NGS data for three phase 3 and two phase 2 clinical trials that were provided to support the NDA for LDV/SOF. This portion of the virology NDA review focused exclusively on the independent analysis of NGS data from these clinical trials. The primary review of the pivotal phase 3 and phase 2 clinical trials and overall conclusions drawn from phase 2 and phase 3 resistance data can be found in the review of Senior Clinical Virology Reviewer Lisa Naeger, Ph.D. (NDA 205834 SDN 000).

The NGS data submitted for the five clinical trials included:

1. Raw sequence data in fastq format
2. Frequency tables showing the frequency of amino acid substitutions at each timepoint
3. Resistance analysis tables populated at a 15% cutoff
4. Summary of resistance associated substitutions within and between studies

There were a total of 1,733 NGS files for the five clinical trials for 1,813 subjects, with study P7977-0523 (ION-3) containing the most subjects sequenced (n=647) and the most NGS files (n=670) (Table 2).

Table 2. NGS data files submitted for the three pivotal phase 3 and two phase 2 trials for LDV/SOF FDC.

Clinical Trial	Trial Name	No. Subjects	Virologic Failures	NGS files Baseline	NGS files Follow up	NGS files Total
GS-US-337-0118	Lonestar	100	2	150	16	166
P7977-0523	Electron	92	11	89*	27	116*
GS-US-337-0102	Ion-1	534	2	326*	2	328*
GS-US-337-0109	Ion-2	440	12	440	13	453
GS-US-337-0108	Ion-3	647	23	647	23	670
Totals		**1813**	**50**	**1652**	**81**	**1733**

* = Not all subjects had a baseline sequence determined by NGS

Of the 1,813 subjects who were treated with LDV/SOF (some in combination with ribavirin) in these trials, only 50 subjects were determined to be treatment failures (Table 2). A total of 42 of the treatment failures were infected with HCV GT1a and 8 subjects were infected with HCV GT1b.

DAVP performed an independent analysis of the NGS data using the NGS Analysis Pipeline described above to analyze the raw sequence data (fastq sequences) submitted by the sponsor for the 50 subjects from the five clinical trials. Briefly, the reads for each sample were aligned to the appropriate HCV reference sequence for the NS5A or NS5B polymerase gene. Variants were called using two algorithms, PVD75 and QbVD, and the variant tables were combined to generate frequency tables showing all amino acid substitutions that occurred above a frequency ≥1% for a given position within a given sample for each subject. Resistance analysis tables were generated for each frequency table for each variant detection algorithm using a threshold of 0.05, and the three resistance analysis tables were compared to determine how closely the three algorithms agreed with one another. To further assess the overall population-based results generated using the resistance analysis tables, DAVP used the SUBS10 filtering threshold to look at individual changes occurring at each position in the frequency tables.

Using this approach, the resistance analysis tables were used to compare algorithms and identify population based positions of interest and the SUBS10 threshold for analyzing the frequency tables provided the treatment emergent substitutions of each individual subject that met this threshold. This provided a more robust prediction of substitutions that were likely to be associated with resistance. DAVP considered all substitutions to be potential emerging resistance-associated substitutions if they met these criteria: 1) met the

SUBS10 threshold, 2) were detected by 2-of-3 variant detection methods (QbVD, PVD75, or variants calls made by the sponsor), and 3) occurred in 2 or more subjects.

For NS5A, polymorphisms and substitutions at positions K24, M28, Q30, L31, P32, H58, A92, and Y93 were analyzed at baseline and time of failure, respectively, and additional treatment-emergent substitutions at additional sites were searched for using the SUBS10 criteria. For NS5B, polymorphisms and substitutions at positions D61, S62, L159, E202, S282, C316, L320, V321, and E440 were analyzed at baseline and time of failure, respectively, and additional treatment-emergent substitutions at additional sites were searched for using the SUBS10 criteria.

REVIEW OF PHASE 2 TRIALS

GS-US-337-0118 (LONESTAR)

Study GS-US-337-0118 (LONESTAR) evaluated the efficacy of LDV and SOF in a fixed dose combination (LDV/SOF FDC) administered for 8 or 12 weeks ± RBV in subjects infected with HCV GT1. Treatment-naïve subjects without cirrhosis were treated with LDV/SOF±RBV for eight weeks, or for 12 weeks with LDV/SOF without ribavirin. Subjects who failed previous treatment with a PI-containing standard of care regimen (PI+PEG+RBV) without or with cirrhosis were treated with LDV/SOF±RBV for 12 weeks:

- LDV/SOF: SOF 400 mg + LDV 90 mg daily fixed dose combination (FDC) for eight weeks or 12 weeks in treatment-naïve subjects without cirrhosis for 12 weeks in PI-experienced subjects without or with cirrhosis
- LDV/SOF+RBV: SOF 400 mg + LDV 90 mg FDC + RBV 1,000 or 1,200 mg daily for eight weeks in treatment-naïve subjects without cirrhosis for 12 weeks in PI-experienced subjects without or with cirrhosis

The sponsor reported that 58 of 100 subjects treated in this clinical trial received LDV/SOF while 42 received LDV/SOF+RBV. All 100 subjects were included in the efficacy and resistance analyses. Overall, 55/58 (95%) of the subjects receiving LDV/SOF achieved a sustained virologic response for 12 weeks after the end of treatment (SVR12) and 42/42 (100%) of subjects receiving LDV/SOF+RBV achieved SVR12 (Table 3).

Table 3. Summary of baseline characteristics and viral response in GS-US-337-0118 (LONESTAR) (Table 8, page 23, Integrated Phase 2/3 Virology Study Report).

	SVR12 n/total number of subjects (% of total)	
	SOF/LDV (n=58)	SOF/LDV+RBV (n=42)
Treatment-naïve, non-cirrhotic, eight weeks	19[a]/20 (95)	21/21 (100)
Treatment-naïve, non-cirrhotic, 12 weeks	18[b]/19 (95)	-
PI-experienced, non-cirrhotic, 12 weeks	8/8 (100)	10/10 (100)
PI-experienced, cirrhotic, 12 weeks	10[a]/11 (91)	11/11 (100)

a One subject relapsed.
b One subject achieved SVR8 but withdrew consent prior to the SVR12 visit.

GS-US-334-0118 Baseline Sequence Data

The sponsor reported that baseline samples from all subjects enrolled in LONESTAR (N=100) were sequenced in the NS5A, NS5B, and NS3/4A coding regions of the virus. The NS3/4A gene was sequenced to evaluate HCV protease resistance in the viruses of subjects who failed previous treatment with boceprevir and telaprevir, but given that these substitutions did not impact the efficacy of LDV/SOF, these data were not evaluated in this review. NGS was performed on the NS5A and NS3/4A regions of all baseline samples. NS5B

sequences were obtained by population sequencing from all baseline samples and by deep sequencing from 23 of 100 baseline samples. Sequencing results were compared to the appropriate reference sequence based on genotype 1 subtype to identify the prevalence of amino acid variants occurring at baseline. The viral targets of ledipasvir and sofosbuvir, NS5A and NS5B, respectively, were examined to determine if any baseline polymorphisms might be correlated with treatment outcome.

Conclusions reached by the sponsor for baseline sequence NS5A analysis were that 11/100 subjects had NS5A resistance-associated amino acid polymorphisms at baseline. The variants observed in this study occurred at genotype 1a NS5A positions K24, M28, Q30, L31, H58 and Y93 and at genotype 1b NS5A position Y93. Two subjects whose virus had baseline NS5A resistance-associated polymorphisms relapsed; the other nine subjects whose virus had baseline NS5A resistance-associated polymorphisms achieved SVR12. The sponsor reported that among the 9 subjects who achieved SVR12 and whose virus had baseline NS5A resistance-associated polymorphisms, 4/9, 2/9, and 1/9 subjects' viruses had at least one NS5A resistance-associated polymorphism that conferred >1,000-fold, 500-fold to 1,000-fold, or 100-fold to 500-fold reduced susceptibility to LDV, respectively (Table 4).

Table 4. GS-US-337-0118 (LONESTAR) baseline NS5A resistance-associated polymorphisms and treatment outcomes (Table 12, page 26, Integrated Phase 2/3 Virology Study Report). RAV, resistance-associated variant.

Subject	Viral Genotype	Duration (Weeks)	Regimen	RAV (%)[a]	LDV EC_{50} Fold-Change	SVR12
2760-2504	1a	8	SOF/LDV	L31M (25.5%)	554	No
2760-2521	1a	8	SOF/LDV	L31M (91.5%) Q30H (3.3%) H58D (1.2%)	554 183 1127	Yes
2760-2529	1a	8	SOF/LDV	K24R (1.1%)	4	Yes
2760-2537	1a	8	SOF/LDV+RBV	K24R (70.0%)	4	Yes
2760-2561	1b	8	SOF/LDV+RBV	Y93H (43.5%)	1319	Yes
2760-2506	1a	12	SOF/LDV	Y93C (14.1%)	1602	Yes
2760-2519	1a	12	SOF/LDV	Q30R (1.3%)	632	Yes
2760-2524	1a	12	SOF/LDV	Q30H (95.0%)	183	Yes
2760-2635	1a	12	SOF/LDV	Q30H (>99%) Y93H (>99%)	183 1677	No
2760-2666	1a	12	SOF/LDV+RBV	K24G (50.1%) M28T (53.8%) Q30R (96.7%)	43 61 632	Yes
2760-2671	1a	12	SOF/LDV+RBV	Q30L (98.8%) Y93H (>99%)	4 1677	Yes

a Percentage of deep sequences with the indicated variant is shown
Data Source: iVSR Virology Listing 2

Both subjects who relapsed in this clinical trial had virus with NS5A resistance-associated polymorphisms at baseline.

In addition, the sponsor sequenced the NS5B gene and examined it for resistance-associated polymorphisms reported to confer reduced susceptibility to nucleoside/nucleotide analog polymerase inhibitors (NAPI), non-nucleoside analog polymerase inhibitors (NNAPI) or ribavirin (RBV). They reported that no baseline resistance-associated polymorphisms that confer resistance to NAPIs were detected; however, 13 subjects had virus with NNAPI resistance-associated polymorphisms at baseline. The sponsor reported that all subjects having virus with baseline NS5B NNAPI polymorphisms achieved SVR12, regardless of viral genotype, treatment duration or inclusion of ribavirin in the treatment regimen (Table 5).

Table 5. GS-US-337-0118 (LONESTAR) baseline NS5B resistance-associated polymorphisms and treatment outcome (Table 13, page 27, Integrated Phase 2/3 Virology Study Report). RAV, resistance-associated variant.

Subject	Viral Genotype	Duration (Weeks)	Ribavirin	RAV (%)[a]	SVR12
2760-2565	1b	8	-	L419I (37.4%) C445F (5.9%) V494A (>99%)	Yes
2760-2568	1b	8	-	Y452H	Yes
2760-2572	1b	8	-	S556G	Yes
2760-2532	1c	8	-	Y452H	Yes
2760-2528	1a	8	+	S556G	Yes
2760-2508	1a	12	-	M423I (>99%)	Yes
2760-2519	1a	12	-	R422K (>99%)	Yes
2760-2571	1b	12	-	V499A	Yes
2760-2632	1a	12	-	L419I	Yes
2760-2741	1a	12	-	M423I	Yes
2760-2666	1a	12	+	Y448H (18.0%)	Yes
2760-2705	1b	12	+	L419I S556G	Yes
2760-2747	1b	12	+	V499A	Yes

a Where deep sequences are available, the percentage of sequences with the indicated variant is shown; otherwise the variant detected by population sequencing is shown

Baseline ribavirin resistance-associated polymorphisms in NS5B did not appear to have an impact on treatment outcome, although F415Y was present at baseline in the virus from one subject infected with HCV GT1a who relapsed. This subject had previously failed a regimen that contained ribavirin. Five additional subjects whose virus had F415Y at baseline achieved SVR12.

GS-US-334-0118 Resistance Analyses in Subjects Experiencing Virologic Failure

The sponsor reported that no subject experienced on-treatment virologic failure in study GS-US-334-0118 (LONESTAR); however, three subjects did not achieve SVR12. Of these subjects, one reached SVR8 and withdrew consent prior to the post-treatment week 12 visit. Two subjects infected with HCV GT1a and the IL28B C/T genotype experienced post-treatment relapse. These two subjects comprised the resistance analysis population for this study.

Deep sequencing of NS5A and NS5B using the Illumina platform (described in Methods) with a 1% assay cut-off was performed at both baseline and virologic failure time points for the two subjects in the resistance analysis population.

The sponsor reported the following resistance-associated polymorphisms and emergent substitutions for subject 2760-2504 from the treatment-naïve, non-cirrhotic 8 week LDV/SOF cohort (Figure 2):

- NS5A M31 at baseline in 25.5% of sequences examined and no baseline NS5B resistance-associated polymorphisms
- NS5A M31 increased its penetrance to >99% after relapse
- NS5A Y93H substitution emerged in 96.7% of sequences examined after relapse
- NS5A L30 polymorphism was also observed (4.5%) at baseline
- NS5B S282T substitution was detected in 91.2% of sequences following relapse; however, this frequency dropped to 8% by five days later
- NS5B L320I was detected in 2.7% of relapse sequences, and an L320V variant was also detected at a 6.1%; however, 5 days later L320I or L320V variants were no longer detected
- NS5A RAVs Y93H, L31M and Q30L variants persisted at approximately the same penetrance as at the time of relapse
- A new L31V substitution emerged at low level (4.7%) at 5 days post relapse

The sponsor reported that this subject was subsequently retreated with LDV/SOF+RBV for 24 weeks. Despite the presence of these NS5A and NS5B resistance-associated substitutions at the time of retreatment, the subject achieved SVR12.

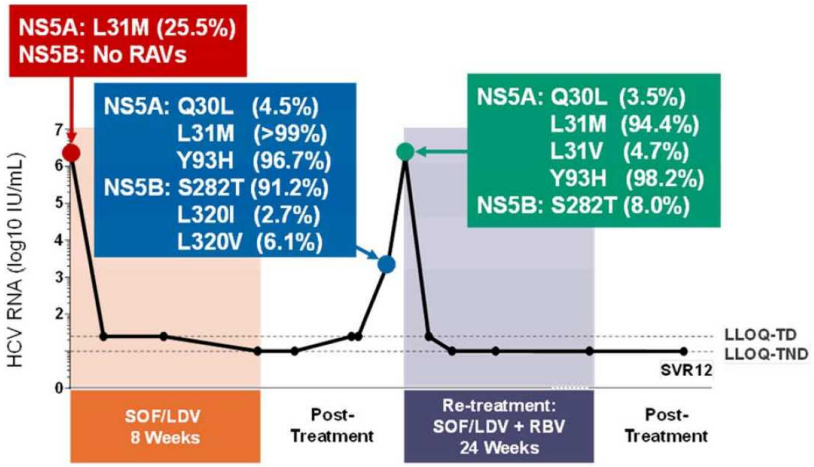

Figure 2. GS-US-337-0118 (LONESTAR) resistance analysis and treatment response in subject 2760-2504 infected with HCV GT1a (Figure 2, page 30, Integrated Phase 2/3 Virology Study Report).

The sponsor reported the following resistance-associated polymorphisms and emergent substitutions for subject 2760-2635, who was a treatment-experienced, cirrhotic who received LDV/SOF for 12 weeks (Figure 3):

- NS5A H30 and H93 polymorphisms at near-complete penetrance (>99%) at baseline
- No NS5B resistance-associated polymorphisms were detected at baseline
- H30 and H93 persisted at greater than 99% penetrance after relapse
- NS5B resistance-associated substitutions did not emerge

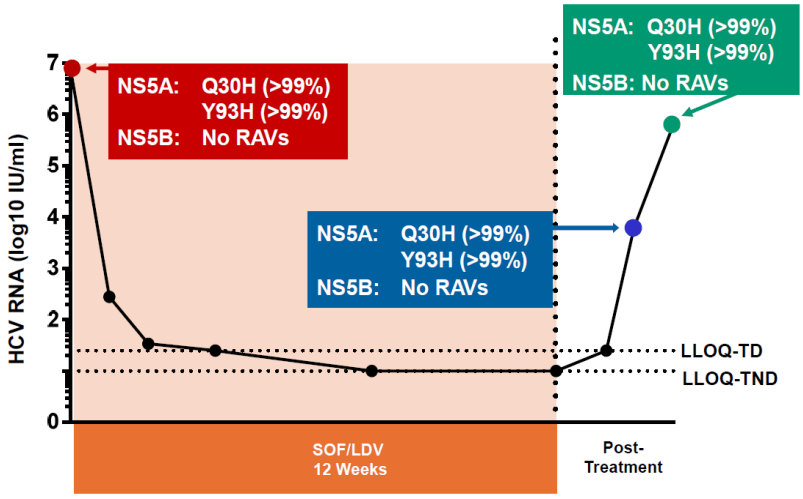

Figure 3. GS-US-337-0118 (LONESTAR) resistance analysis and treatment response in subject 2760-2635 infected with HCV GT1a (Figure 3, page 31, Integrated Phase 2/3 Virology Study Report).

The NGS data were analyzed for these subjects as described above. Overall, the results obtained by DAVP were comparable to the results reported by the sponsor. However, there were two differences. First, the L31V substitution that reportedly emerged at low level (4.7%) at 5 days post relapse in the virus of subject 2760-

2504 was not detected by the two algorithms used by DAVP. Second, NS5B substitution D66G emerged and was detected at ~20% frequency in all timepoints after relapse as detected by all three algorithms (Table 6).

Table 6. Potential resistance-associated polymorphisms and emergent substitutions identified by DAVP among relapsers in GS-US-337-0118 (DAVP analysis). For the complete DAVP dataset see Appendix 1.

USUBJID	GT/ST	TXCAT	REG	GENE	SUBS	STAT	VDAG
GS-US-337-0118-2760-2504	1a	Naïve	SOF/LDV 8	NS5A	Q30L	E	2
					L31M	B and E	3
					Y93H	E	3
					I121V	B and E	3
				NS5B	S282T	E	3
					L320V/I	E	2
					V321I	E	2
GS-US-337-0118-2760-2635	1a	PI Failure	SOF/LDV 12	NS5A	Q30H	B	3
					Y93H	B	3
				NS5B	D66G	E	3

USUBJID, unique subject identification code; **GT/ST**, HCV genotype and subtype; **TXCAT**, prior treatment category; **REG**, regimen; **GENE**, viral gene sequenced; **SUBS**, substitutions detected at 0.10 or greater frequency at failure timepoint but not detected at baseline; **STAT**, status of substitution; **VDAG**, number of variant detection algorithms that detected the substitution out of a possible of three; **E**, emergent substitution; **B**, baseline polymorphism.

For subject 2760-2504's virus, M31 was present at baseline at 25.5% which increased to >90% at timepoints after relapse. Y93H also emerged and was detected at >90% after relapse. S282T was present at >90% at the timepoint closest to relapse and L320I and L320V were present at 2.7 and 6.1%, respectively. The L31M and Y93H likely prevented the activity of LDV, while S282T and substitutions at L320 likely prevented SOF activity. For subject 2760-2635, H30 and H93 polymorphisms were present at baseline at >99% and persisted at this level throughout follow-up testing. The sponsor reported no NS5B polymorphisms; however, D66G emerged and was detected at 23.2% at FU-2 and 20.8% at FU-4. This substitution has not previously been associated with SOF treatment failure. H30 and H93 likely prevented the activity of LDV, while D66G may have knocked out SOF activity.

GS-US-337-0118 Phenotype Analyses

The sponsor performed population phenotypic analyses for the two treatment failures from GS-US-337-0118 (LONESTAR). Virus from subjects 2760-2504 and 2760-2635 was extracted from baseline and relapse samples for evaluation of phenotypic resistance to LDV and SOF. The viral NS5A and NS5B regions from subject samples were separately inserted into HCV replicons to determine susceptibility to LDV and SOF, respectively (Table 7).

Table 7. GS-US-337-0118 (LONESTAR) phenotypic resistance analysis of treatment failures (Table 16, page 32, Integrated Phase 2/3 Virology Study Report).

Subject Number	GT	Treatment	Relevant RAVs (%)				Drug Susceptibility (EC_{50} Fold-Change from Reference)					
			NS5A		NS5B		LDV		SOF		RBV	
			Baseline	Relapse	Baseline	Relapse	Baseline	Relapse	Baseline	Relapse	Baseline	Relapse
2760-2504	1a	SOF/LDV 8 Weeks (Tx-Naïve)	L31M (25.5%)	Q30L (4.5%) L31M (>99%) Y93H (96.7%)	(none)	S282T (91.2%) L320I (2.7%) L320V (6.1%)	2.4	>791	0.45	1.3	0.31	0.36
2760-2504	1a	SOF/LDV 8 Weeks (Tx-Naïve)			(none)	S282T (clonal)			0.45	11.3	0.31	0.17
2760-2635	1a	SOF/LDV 12 Weeks (PI Failures)	Q30H (>99%) Y93H (>99%)	Q30H (>99%) Y93H (>99%)	F415Y (65.7%)	(none)	126	113	1.0	0.87	1.2	0.72

For subject 2760-2504, the viral NS5A sequence identified at relapse was >791-fold (comparing EC_{50} values to the wild type sequence HCV GT1a NS5A sequence) less susceptible to LDV than the wild type NS5A sequence for HCV GT1a. The baseline NS5A sequence for this subject was 2.4-fold less susceptible to LDV, but fully susceptible to SOF.

According to the sponsor, baseline and relapse samples from subject 2760-2635, which had both H30 and H93 polymorphisms, showed a high level of resistance to LDV at baseline (126-fold reduction in susceptibility based on EC_{50} value) and relapse (113-fold reduction in susceptibility based in EC_{50} value). In contrast, both the baseline (1.0 fold-shift) and relapse (0.9 fold-shift) samples from subject 2760-2635 showed no shift in susceptibility to sofosbuvir.

Note: DAVP uses phenotypic assessments to confirm resistance associations; however, lack of a phenotype does not exclude a role in resistance. Modest and no shifts in susceptibility have been seen for known resistance substitutions of other nucleoside polymerase inhibitors.

GS-US-334-0118 Resistance Analyses Conclusions
The sponsor concluded that of 100 subjects in this study, no viral breakthrough was observed and only two subjects experienced viral relapse. The viral relapse was associated with resistance to LDV in both subjects and to SOF in one of two subjects. No genotypic or phenotypic resistance to SOF was detected in the remaining subject.

DAVP concluded that for subject 2760-2504, resistance-associated polymorphisms NS5A_M31, which was present at baseline and also emerged to high frequency at relapse and NS5A_Y93H, which emerged during treatment, blocked the activity of LDV. In addition, emergent resistance-associated substitutions NS5B_S282T and substitutions at position NS5B_L320 likely knocked out SOF activity.

DAVP concluded that for subject 2760-2635, baseline resistance-associated polymorphisms NS5A_H30 and NS5A_H93 inhibited the activity of LDV, while D66G, which emerged and was detected at 23.2% at FU-2 and 20.8% at FU-4, may have played a role in reducing susceptibility to SOF.

REVIEW OF P7977-0523 (FUSION)
Study P7977-0523 (ELECTRON) evaluated the efficacy of sofosbuvir in 22 treatment arms, the arms varying by viral genotype to be treated, subject population, treatment duration and addition of other drugs to combine with sofosbuvir in the treatment regimen. Six treatment groups combined sofosbuvir and ledipasvir, with or without ribavirin, for treatment of subjects infected with genotype 1 HCV. Sofosbuvir and ledipasvir were administered as individual drugs (SOF + LDV) in two groups reported in this study, and administered as a fixed dose combination (LDV/SOF) in four groups. The virology analyses of these six treatment groups are presented in this discussion.

According to the sponsor, a total of 92 genotype 1-infected subjects were treated with LDV/SOF, LDV+SOF+RBV or LDV/SOF+RBV in this study. Of the 57 who received LDV+SOF+RBV or LDV/SOF+RBV for 12 weeks, 57/57 achieved SVR. In prior null responders with cirrhosis, 10/10 (100% of subjects achieved SVR12 following LDV/SOF+RBV while 70% (7/10) achieved SVR12 following treatment with LDV/SOF. Treatment duration of six weeks reduced the SVR12 rate to 68% (17/25) in treatment-naïve subjects (Table 8).

Table 8. Summary of viral response data in P7977-0523 (ELECTRON) (Table 17, page 36, Integrated Phase 2/3 Virology Study Report).

Population	Group	Treatment	Duration (weeks)	SVR12 n/total number of subjects (% of total)
Null responders	12	SOF + LDV + RBV	12	9/9 (100)
Treatment-naïve	13	SOF + LDV + RBV	12[a]	25/25 (100)
Null responders, compensated cirrhosis	16	SOF/LDV	12	7[b]/10 (70)
Null responders, compensated cirrhosis	17	SOF/LDV + RBV	12	9/9 (100)
Hemophiliacs	20	SOF/LDV + RBV	12	14/14 (100)[c]
Treatment-naïve	21	SOF/LDV + RBV	6	17[d]/25 (68)

a One subject with SAE's unrelated to study drugs discontinued study drugs on study day 62 but achieved SVR12.
b Three subjects relapsed.
c SVR4 is reported for Group 20 pending results from follow-up week 12 visits.
d Eight subjects relapsed.

P7977-0523 Baseline Sequence Data

All baseline sequences from subjects enrolled in P7977-0523 (ELECTRON) were examined to determine the presence of viral resistance-associated polymorphisms at baseline. Only one subject infected with HCV GT1a with NS5A_H30 present at high frequency (>99%) at baseline failed to achieve SVR12 (Table 9).

Table 9. P7977-0523 (ELECTRON) baseline NS5A resistance-associated polymorphisms and treatment outcome (Table 21, page 39, Integrated Phase 2/3 Virology Study Report).

Subject	Viral Genotype	Duration (Weeks)	Ribavirin	RAV (%)[a]	LDV EC_{50} Fold-Change	SVR12
1030-5241	1a	12	+	L31M (20.6%)	554	Yes
1031-5215	1a	12	+	Q30H	183	Yes
1031-5234	1a	12	+	M28T	61	Yes
1069-5503	1a	12	+	M28T (98.9%)	61	Yes
5868-5514	1a	12	+	L31M (>99%)	554	Yes
1069-5586	1a	12	+	K24R (1.1%) L31M (1.1%)	4 554	Yes[b]
1069-5589	1a	12	+	M28T (88.8%)	61	Yes
1030-5202	1b	12	+	L31M	5	Yes
1069-5505	1b	12	+	L31M (1.4%) L31I (29.1%)	5 13	Yes
1069-5513	1a	12	-	Q30H (>99%)	183	No[c]
5868-5519	1a	12	-	M28T (>99%) Q30R (>99%)	61 632	Yes
1069-5502	1b	12	-	Y93H (51.3%)	1319	Yes
1069-5540	1a	6	+	H58D (3.9%)	1127	Yes
5868-5546	1a	6	+	H58D (9.7%)	1127	Yes

a Where deep sequencing results were available, the percent of sequences bearing the indicated substitution is shown.
b SVR4
c Subject 1069-5513 relapsed.

The sponsor reported that additional NS5A baseline polymorphisms were noted, but these were not associated with treatment failure. Moreover, there were no baseline NS5B polymorphisms that were associated with treatment failure, as all subjects with differences at baseline achieved SVR12 (Table 10).

Table 10. P7977-0523 (ELECTRON) baseline NS5B resistance-associated polymorphisms and treatment outcome (Table 22, page 40, Integrated Phase 2/3 Virology Study Report).

Subject	Viral Genotype	Duration (Weeks)	Ribavirin	RAV (%)[a]	SVR12
1069-5518	1a	12	+	M423A	Yes
1030-5202	1b	12	+	C316N[b]	Yes
1030-5210	1b	12	+	C316N, V499A	Yes
1030-5222	1b	12	+	C316N	Yes
1069-5577	1b	12	+	S556G (52.6%)	Yes[c]
1069-5578	1b	12	+	L419I (34.2%) S556G (9.2%)	Yes[c]
5868-5516	1b	12	-	S556G	Yes
1069-5533	1b	6	+	V499A	Yes

a Where deep sequences were available, the percentage of sequences bearing the indicated substitution is shown
b Virus also had the NS5B NI RAV L159F
c SVR4 result, pending SVR12 visit results

P7977-0523 Resistance Analyses in Subjects Experiencing Virologic Failure

The sponsor reported that no subject experienced on-treatment virologic failure in P7977-0523 (ELECTRON). However, 11 subjects with undetectable virus at the end of treatment subsequently failed to achieve SVR12 due to virologic relapse and these subjects were included in the resistance analysis population. All 11 of these subjects were infected with HCV GT1a, 8/11 were in the LDV/SOF+RBV 6 week treatment arm, and 3/11 subjects were in Group 16, a prior null responder arm treated with LDV/SOF without ribavirin for 12 weeks (all cirrhotic). Deep sequencing of NS5A and NS5B was obtained at baseline and virologic failure time points for 11/11 and 10/11 subjects in the resistance analysis population, respectively. Sequencing of the NS5B region was unsuccessful in 1/11 subjects at relapse. Resistance-associated substitutions for the 11 treatment failures are shown in Table 11.

Table 11. Summary of substitutions found by the sponsor among subjects who experienced relapse in P7977-0523 (Table 24, pages 44-45, Integrated Phase 2/3 Virology Study Report).

Subject Number	GT	Treatment	Relevant RAVs				Drug Susceptibility (EC50 Fold-Change from Reference)					
			NS5A		NS5B		LDV		SOF		RBV	
			Baseline	Relapse	Baseline	Relapse	Baseline	Relapse	Baseline	Relapse	Baseline	Relapse
1069-5504	1a	Arm 16: SOF/LDV 12 Weeks (GT1 Prior Null)	(none)	Q30R (1.15%) Q30E (1.16%) Y93H (68.49%) Y93C (29.81%)	(none)	(none)	0.58	>42	0.78	0.81	0.83	0.91
1069-5506[a]	1a	Arm 16: SOF/LDV 12 Weeks (GT1 Prior Null)	(none)	K24R (1.10%) Y93H (>99%)	(none)	no data	0.59	>42	0.76	0.73	0.82	0.91
1069-5513	1a	Arm 16: SOF/LDV 12 Weeks (GT1 Prior Null)	Q30H (>99%)	M28A (>99%) Q30H (>99%)	(none)	(none)	16.6	>42	0.71	no data[b]	0.79	no data[b]
1069-5534	1a	Arm 21: SOF/LDV + RBV 6 Weeks (GT1 Tx-Naïve)	(none)	(none)	(none)	(none)	0.97	1.2	0.64	0.67	1.1	1.0
5868-5537[c]	1a	Arm 21: SOF/LDV + RBV 6 Weeks (GT1 Tx-Naïve)	(none)	Q30R (30.68%)	(none)	(none)	0.74	0.78	0.76	0.79	0.61	0.76
1069-5541	1a	Arm 21: SOF/LDV + RBV 6 Weeks (GT1 Tx-Naïve)	(none)	M28T (7.98%)	(none)	(none)	0.87	0.86	0.76	0.67	0.73	0.79
1069-5545	1a	Arm 21: SOF/LDV + RBV 6 Weeks (GT1 Tx-Naïve)	(none)	(none)	(none)	(none)	0.57	2.8	0.76	no data[b]	1.1	no data[b]
1069-5547	1a	Arm 21: SOF/LDV + RBV 6 Weeks (GT1 Tx-Naïve)	(none)	L31M (>99%)	(none)	(none)	0.60	>42	0.75	0.81	0.78	0.79
1069-5548	1a	Arm 21: SOF/LDV + RBV 6 Weeks (GT1 Tx-Naïve)	(none)	(none)	(none)	T390I (2.01%)	0.62	0.87	0.93	0.79	0.94	0.84
1069-5551	1a	Arm 21: SOF/LDV + RBV 6 Weeks (GT1 Tx-Naïve)	(none)	(none)	(none)	(none)	0.70	0.89	0.76	0.74	0.78	0.86
1069-5555	1a	Arm 21: SOF/LDV + RBV 6 Weeks (GT1 Tx-Naïve)	(none)	(none)	(none)	(none)	1.3	1.4	0.69	0.71	0.83	0.73

a Relapse genotype is from 8 week followup sample; relapse phenotype is from 12 week followup sample.
b Assay failed.
c Relapse genotype is from 12 week followup sample; relapse phenotype is from 8 week followup sample.

The NGS data were independently analyzed for these subjects by DAVP. Overall, the results obtained were comparable to the results reported by the sponsor (Table 12). However, the sponsor stated that there were no NS5B substitutions at baseline or time of relapse that were associated with resistance. By contrast, DAVP identified two potential resistance-associated substitutions: 1) S62N that arose in 8 subjects who relapsed and 2) D61G that emerged in 6 subjects who failed treatment (Table 12). Of note, D61G and S62N were identified as potential resistance-associated substitutions among subjects infected with HCV GT1a in the liver pre-transplant study P7977-2025 and were identified as sites of interest for post marketing follow-up for SOF (see review of NDA204671 SDN 004).

Table 12. Resistance-associated substitutions identified by DAVP among relapsers in P7977-0523 (DAVP analysis).

STUDYID	NS5A POS	No. Subjects	SUBS	Ratio
P7977-0523	28*	3	M28V/V28M	2-1
	30*	3	Q30R/H	2-1
	58*	2	H58R/P	1-1
	93*	2	Y93H/C	1-1
	31*	1	L31M	
	62	1	E62D	
	NS5B POS	**No. Subjects**	**SUBS**	**Ratio**
	62	8	S62N/N62S	7-1
	61*	6	D61G/G61D	5-1

NS5A and **NS5B POS**, the amino acid position of the indicated gene; **SUBS**, substitutions detected at 0.10 or greater frequency at failure timepoint but not detected at baseline; **Ratio**, ratio of different substitutions at a position in the order of most to least; *, resistance-associated.

P7977-0523 Phenotype Analyses

The sponsor performed phenotypic analysis of the NS5A and NS5B sequences derived from the viruses from subjects who failed treatment. Briefly, viral RNA was extracted from baseline and relapse samples for evaluation of phenotypic resistance to ledipasvir and sofosbuvir. The viral NS5A region from subject samples and the NS5B region from subject samples were separately inserted into replicons to determine susceptibility to ledipasvir and sofosbuvir, respectively. The sponsor reported that the results from testing baseline samples from subjects only showed resistance to LDV if high levels of LDV specific resistance-associated polymorphisms were present (Table 11). No baseline samples showed reduced susceptibility to SOF or RBV. Similarly, relapse samples showed reduced susceptibility to LDV only if high levels of NS5A RAVs were present, and no baselines samples showed reduced susceptibility to SOF or RBV (Table 11).

P7977-0523 Resistance Analyses Conclusions

The sponsor concluded that of the 92 subjects in this study, no virologic breakthroughs were observed and a total of 11 subjects experienced viral relapse. Viral resistance to LDV was detected in 6/11 subjects, but was not detected in the remaining 5/11 subjects at relapse. No genotypic or phenotypic resistance to SOF was detected in the any of these 11 subjects.

In contrast, DAVP identified treatment-emergent NS5B substitutions S62N (emerged in 8 subjects) and D61G (emerged in 6 subjects) in the virus from subjects who relapsed in this trial. Of note, D61G and S62N were identified as resistance-associated substitutions among subjects infected with HCV GT1a in the liver pre-transplant study P7977-2025 and were identified as sites of interest for post marketing follow-up for SOF.

REVIEW OF PHASE 3 CLINICAL TRIALS

REVIEW OF GS-US-337-0102 (ION-1)

Study GS-US-337-0102 (ION-1) evaluated the efficacy of SOF with LDV in a fixed dose combination (LDV/SOF FDC) administered for 12 or 24 weeks ± RBV in treatment-naïve subjects with chronic genotype 1 hepatitis C virus (HCV) infection.

The sponsor reported that out of 534 treated subjects described here, 214 subjects received LDV/SOF for 12 weeks, with 217 receiving LDV/SOF+RBV for 12 weeks. An additional 52 subjects received 24 weeks of LDV/SOF, while 51 received 24 weeks of LDV/SOF+RBV. All 534 subjects were included in the efficacy and resistance analyses. Overall, 209/214 (97.7%) of subjects receiving 12 weeks of LDV/SOF achieved SVR12 and 211/217 (97.2%) of subjects receiving 12 weeks of LDV/SOF+RBV achieved SVR12 (Table 13). Additionally, all 52 subjects receiving 24 weeks of LDV/SOF achieved SVR12, and 50/51 (98) subjects receiving 24 weeks of LDV/SOF+RBV achieved SVR12 (Table 13).

Table 13. GS-US-337-0102 (ION-1) summary of viral response data (Table 18, page 39, Integrated Phase 2/3 Virology Study Report).

	SVR12 n/total number of subjects (% of total)	
	SOF/LDV (n=266)	SOF/LDV+RBV (n=268)
Treatment-naïve, 12 weeks	209[a]/214 (97.7)	211[b]/217 (97.2)
Treatment-naïve, 24 weeks	52/52 (100)	50[c]/51 (98.0)

a One subject relapsed, 2 subjects had early discontinuation, and 2 achieved SVR 4 but lost to follow-up for the 12 weeks post treatment visit

b Four subjects had early discontinuation, and 2 achieved SVR 4 but lost to follow-up for the 12 weeks post treatment visit

c One subject had early discontinuation

GS-US-337-0102 Baseline Sequence Data

The sponsor sequenced all baseline samples from subjects enrolled in the ION-1 study to determine the presence of viral NS5A polymorphisms at baseline. In addition, the sponsor reported that a subset of subjects also had the viral NS5B gene sequenced to determine the presence of NS5B polymorphisms at baseline. The

viral NS5A region was examined for baseline polymorphisms reported to confer reduced susceptibility to ledipasvir. Overall, 78/532 (14.7%) subjects (GT1a, n=51/372 (13.7%), GT1b n=24/157 (15.3%), other GT n=3/3 (100%)) were identified as having baseline NS5A polymorphisms.

GS-US-337-0102 Resistance Analyses in Subjects Experiencing Virologic Failure

The sponsor reported that no subject had on treatment virologic failure in the 12 week LDV/SOF or LDV/SOF+RBV arms or the 24-week LDV/SOF or LDV/SOF+RBV groups in Part A. In the LDV/SOF+RBV 12-week group and 24-week group in Part A, no subject relapsed. In the LDV/SOF 12-week group, 1/214 subject (Subject 1603-71276, a cirrhotic, GT1a, IL28 CT) experienced viral relapse. According to the sponsor, sequencing revealed this subject's virus had the M31 NS5A polymorphism (>99% of viral population) at baseline but remained suppressed while on treatment. At week 4 post-treatment, the subject had detectable HCV RNA and M31 retained at >99%. No additional NS5A substitutions emerged at Week 4 post-treatment. The sponsor also reported that no NS5B NAPI substitutions were detected at any timepoint tested. Moreover, phenotypic analysis of NS5A and NS5B genes showed a reduced susceptibility to LDV, but no phenotypic change to SOF or RBV (Table 15).

The sponsor reported that subject 5663-71589, a noncirrhotic subject with GT1b chronic HCV infection and IL28 CT allele was randomized to the LDV/SOF 24 week treatment arm and completed 12 weeks treatment with 88.1% adherence. This subject was suppressed at Week 6, but at Week 8, had detected HCV RNA, with emergence of an NS5A Y93H substitution. At Week 12, the subject discontinued treatment due to documented noncompliance. Y93H was also detected 4 weeks post-treatment (>99%). The sponsor stated that no NS5B NAPI substitutions were detected at any of these visits. Phenotypic analysis of the NS5A and NS5B genes showed high levels of resistance to LDV (>208), but no resistance to SOF or RBV (Table 15).

DAVP's analyses of the HCV viral protein sequences derived from these two subjects was largely in agreement with the conclusions reached by the sponsor (Table 14). For subject 5663-71589, Y93H likely reduced susceptibility to LDV and no clear resistance-associated substitutions were detected in the NS5B protein (Table 14). For subject 1603-71276, M31 likely reduced susceptibility to LDV and no clear resistance-associated substitutions were detected in the NS5B protein (Table 14).

Table 14. Potential resistance-associated substitutions identified by DAVP among relapsers in GS-US-337-0102 (DAVP analysis).

USUBJID	GT/ST	TXCAT	REG	GENE	SUBS	STAT	VDAG
GS-US-337-0102-5663-71589	1b	Cirr, Non-CC	SOF/LDV 24	**NS5A**	Y93H	E	3
				NS5B	K355T	E	3
GS-US-337-0102-1603-71276	1a	Non-CC	SOF/LDV 12	**NS5A**	L31M	B	3
					L255I	E	2
					D392N	E	2
				NS5B	M36L	E	2

USUBJID, unique subject identification code; **GT/ST**, HCV genotype and subtype; **TXCAT**, prior treatment category; **REG**, regimen; **GENE**, viral gene sequenced; **SUBS**, substitutions detected at 0.10 or greater frequency at failure timepoint but not detected at baseline; **STAT**, status of substitution; **VDAG**, number of variant detection algorithms that detected the substitution out of a possible of three; **E**, emergent substitution; **B**, baseline polymorphism.

GS-US-337-0102 Phenotype Analyses

Virus derived from subjects who failed treatment was extracted from baseline and relapse samples for evaluation of phenotypic resistance to ledipasvir and sofosbuvir. The viral NS5A and NS5B regions from subject samples were separately inserted into replicons to determine susceptibility to ledipasvir and sofosbuvir, respectively (Table 15).

The sponsor reported that the results from testing the baseline sample from Subject 1603-71276 showed resistance to LDV, but not SOF or RBV at both baseline and failure timepoints, consistent with the presence of the NS5A_M31 polymorphism in this subject's virus at both timepoints. Subject 5663-71589 only showed resistance to LDV at the failure timepoint, consistent with the emergence of NS5A_Y93H. No reduced

susceptibility to LDV was observed at baseline, nor was any reduced susceptibility observed at any timepoint for SOF or RBV.

Table 15. GS-US-337-0102 (ION-1) phenotypic resistance analysis in subjects with virologic failure (Table 32, page 56, Integrated Phase 2/3 Virology Study Report).

Subject ID	GT	Treatment	Relevant RAVs				Drug Susceptibility (EC_{50} Fold-Change from Reference)					
			NS5A		NS5B		LDV[c]		SOF[d]		RBV[d]	
			BL	Post BL	BL	Post BL	BL	Post BL	BL	Post BL	BL	Post BL
1603-71276[a]	1a	12 WKS SOF/LDV	L31M (>99%)	L31M(>99%)	None	No change from baseline	>42	>42	0.84	0.9	0.79	0.99
5663-71589[b]	1b	24 WKS SOF/LDV	None	Y93H (>99%)	None	None	0.67	>208	0.88	0.72	1.1	0.79

a Post-BL time point is week 4 post-treatment
b Post-BL time point is week 8 of treatment
c Fold-shift from reference as tested in a NS5A replicon system
d Fold-shift from reference as tested in a NS5B replicon system
BL fold-shifts are relative to reference
Post BL fold-shifts are relative to baseline

GS-US-337-0102 Resistance Analyses Conclusions

The sponsor concluded that the presence of NS5A polymorphisms did not affect treatment response to LDV/SOF in HCV-infected subjects irrespective of treatment duration and regimen. In the two virologic failure subjects, no genotypic or phenotypic resistance to SOF was detected while a single NS5A LDV resistance-associated substitution was detected at the time of virologic failure in both subjects, one of whom had this polymorphism at baseline.

The independent assessment of the NGS data performed by DAVP for the two treatment failures from this clinical trial support the sponsor's conclusion.

REVIEW OF GS-US-337-0109 (ION-2)

Study GS-US-337-0109 (ION-2) evaluated the efficacy of LDV with SOF in a fixed dose combination (LDV/SOF FDC) administered for 12 or 24 weeks ± RBV in treatment-experienced subjects with chronic genotype 1 HCV infection. The sponsor reported that out of 440 treated subjects, 109 received LDV/SOF for 12 weeks, with 111 receiving LDV/SOF+RBV for 12 weeks. An additional 109 subjects received 24 weeks of LDV/SOF, while 111 received 24 weeks of LDV/SOF+RBV. All of these subjects were included in the efficacy and resistance analyses. Overall, 102/109 (93.6%) of subjects receiving 12 weeks of LDV/SOF achieved SVR12 and 107/111 (96.4%) of subjects receiving 12 weeks of LDV/SOF+RBV achieved SVR12 (Table 16).

Table 16. GS-US-337-0109 (ION-2) summary of viral response data (Table 33, page 59, Integrated Phase 2/3 Virology Study Report).

	SVR12 n/total number of subjects (% of total)	
	SOF/LDV (n=218)	SOF/LDV+RBV (n=222)
Treatment-experienced, 12 weeks	102[a]/109 (93.6)	107[b]/111 (96.4)
Treatment-experienced, 24 weeks	108[c]/109 (99.1)	110[d]/111 (99.1)

a Seven subjects relapsed
b Four subjects relapse
c One subject achieved SVR 4 but lost to follow-up for the 12 weeks post treatment visit
d Subject experienced on-treatment rebound in the setting of non-compliance

GS-US-337-0109 Baseline Sequence Data

The sponsor reported that the NS5A sequence was successfully obtained for the viruses from 439/440 enrolled subjects treated with LDV/SOF±RBV for 12 or 24 weeks (346 GT1a and 93 GT1b). Likewise, NS5B sequence was successfully obtained for 438/440 subjects' viruses (345 GT1a and 93 GT1b). NS3 sequence was obtained for 433/440 enrolled subjects' viruses (342 GT1a; 91 GT1b). Of note, 203 of these subjects' viruses

with NS3 sequence were previous PEG+RBV treatment failures (157 GT1a; 46 GT1b), and an additional 228 subjects (184 GT1a and 44 GT1b) were previously treated with PEG+RBV+PI unsuccessfully.

The sponsor reported that overall, 62/439 (14.1%) subjects' viruses with successful NS5A sequencing (GT1a, n=49/346 [14.2%], GT1b n=13/93 [14.0%]) were identified as having baseline NS5A polymorphisms associated with treatment failure. Of these, 55/62 (88.7%) subjects' viruses with baseline NS5A polymorphisms achieved SVR12 following 12 or 24 weeks treatment with LDV/SOF±RBV. In the 12-week LDV/SOF treatment group, 13/17 (76.5%) subjects' viruses with baseline NS5A polymorphisms achieved SVR12 (4 relapsed), and 15/17 (88.2%) subjects in the LDV/SOF+RBV arm achieved SVR12 (2 relapsed). In the 24-week treatment arm, 13/13 subjects with NS5A polymorphisms in their viruses and treated with LDV/SOF achieved SVR12 despite the presence of >100-fold LDV resistance NS5A polymorphisms in 6/13 subjects' viruses, and 14/15 (93.3%) subjects achieved SVR12 treated with LDV/SOF+RBV with the lone exception occurring in the setting of non-compliance. Of these 62 subjects, 38 (61.3%) subjects' viruses had at least 1 NS5A polymorphism conferring >100-fold reduced susceptibility to LDV and 38 (61.3%) subjects had virus with NS5A polymorphisms at a frequency >10%. Therefore, the sponsor concluded that the presence of baseline NS5A polymorphisms, even high-level resistance-associated polymorphisms, did not preclude subjects from achieving SVR12 in this study. However, a lower response rate (9/13, 69%) was observed among the 13 treatment-experienced subjects whose virus had baseline NS5A polymorphisms associated with >100-fold resistance to LDV and who were treated with LDV/SOF for 12 weeks (Table 17).

NS5B deep sequencing was successfully obtained for 438 subjects' viruses, including 218 subjects in the 12-week LDV/SOF and LDV/SOF+RBV groups (GT1a, n=172; GT1b n=46), and 220 subjects in the 24-week LDV/SOF and LDV/SOF+RBV arms (GT1a, n=173; GT1b n=47). Subjects were analyzed for NAPI, NNAPI, and RBV resistance-associated polymorphisms.

The sponsor reported that the NS5B S282T polymorphism was not detected in any subject's virus. Four viruses out of 438 sequenced were observed to have NAPI resistance-associated polymorphisms at baseline (all 4 GT1b). Of these 4 subjects, 3 subjects' viruses had both F159+N316 in NS5B and 1 subject's had T142. All 4 subjects achieved SVR12. In addition, the sponsor reported that of the 83 GT1a subjects with baseline RBV polymorphisms, 79 (95.2%) achieved SVR12, with 3 relapsers (n=2 12-week LDV/SOF, n=1 LDV/SOF+RBV), and one virologic rebound in the setting of non-compliance (24-week LDV/SOF+RBV). Additionally, 61/438 (13.9%) subjects were found to have NS5B NNAPI resistance-associated polymorphisms at baseline (GT1a, n=23; GT1b n=38). Of these 61 subjects, 59 (96.7%) achieved SVR12 with one relapse subject in the 12-week LDV/SOF treatment arm, and one virologic rebound in the setting of non-compliance in the 24-week LDV/SOF+RBV.

GS-US-337-0109 Resistance Analyses in Subjects Experiencing Virologic Failure
Subjects who did not achieve SVR12 are summarized in Table 17, with resistance-associated polymorphisms detected at baseline and emergent resistance-associated substitutions at virologic failure, as well as phenotypic analysis of the isolates derived from these subjects.

The sponsor reported that in the LDV/SOF 12-week treatment arm, 7 of 109 subjects (6.4%) did not achieve SVR12 (all 7 relapsed). Four of these 7 subjects' viruses had NS5A resistance-associated polymorphisms at baseline (R/H30, N/H93, M31) which confer high levels of resistance to LDV (>500). The other 3 subjects' viruses had no NS5A resistance-associated polymorphisms at baseline. All baseline resistance-associated polymorphisms were maintained at the virologic failure time point with the exception of T28 (1.03%) in subject 79303 and R30 (1.43%) in subject 79214 that were no longer detectable at the 1% cutoff. Interestingly, no new NS5A resistance-associated substitutions emerged at relapse in the viruses from all 4 subjects. The 3 subjects' viruses with no resistance-associated polymorphisms at baseline had high levels of LDV resistance variants detected (H93, V/M31, H30) at virologic failure. The sponsor also stated that no S282T or other NAPI resistance-associated substitutions were detected in any subject's virus at relapse. In the LDV/SOF+RBV 12-week group, 4 of 111 subjects (3.6%) did not achieve SVR12 due to relapse. Two of the 4 subjects' viruses

had NS5A resistance-associated polymorphisms at baseline (M31, H93). The other two subjects' viruses did not have any detected at baseline. The 2 subjects' viruses with baseline resistance-associated polymorphisms maintained them at virologic failure. The 2 subjects whose virus had no resistance-associated polymorphisms at baseline had high levels of LDV resistance-associated substitutions detected (Q30R/K and M28T) at virologic failure. No NAPI resistance-associated substitutions were detected in these subjects. In the LDV/SOF 24-week group, 1 of 109 subjects (0.9%) did not achieve SVR12. The sponsor reported that this subject (7864-79383) experienced on treatment virologic failure (rebound) due to documented noncompliance. The subject's virus had R30 and R24 polymorphisms at baseline and these were still detected at higher frequencies at the virologic failure timepoint. No NAPI resistance-associated polymorphisms were detected for this subject. For all virologic failure timepoints, the phenotypic analysis of NS5A and NS5B genes showed reduced susceptibility to LDV, but no change in susceptibility to SOF (Table 17).

Table 17. GS-US-337-0109 (ION-2) phenotypic resistance analysis in subjects with virologic failure (Table 39, page 71, Integrated Phase 2/3 Virology Study Report).

				Relevant RAVs				Drug Susceptibility (Fold-Change from WT)[c]					
				NS5A		NS5B		LDV[a]		SOF[b]		RBV[b]	
Subject ID	GT	Treatment	Prior Treatment	BL	Post BL	BL	Post BL	BL	Post BL	BL	Post BL	BL	Post BL
79034[d]	1a	SOF/LDV + RBV 12 Weeks	PI+Peg-IFN+RBV	L31M(>99%)	Q30H(>99%); L31M(>99%)	None	None	>42	>42	0.86	0.93	0.84	0.85
79041[d]	1a	SOF/LDV + RBV 12 Weeks	Peg-IFN+RBV	None	M28T(>99%); Q30R(>99%)	None	None	0.68	30	0.88	0.77	0.79	0.8
79063[d]	1a	SOF/LDV + RBV 12 Weeks	PI+Peg-IFN+RBV	None	Q30K(>99%)	None	None	0.61	24	0.75	0.75	0.82	0.86
79070[d]	1a	SOF/LDV + RBV 12 Weeks	Peg-IFN+RBV	Y93H(1.2%)	Q30L(76.43%); Q30R(22.94%); Y93H(>99%)	F415Y (96.98%)	F415Y(>99%)	1.3	>42	0.85	0.87	0.83	0.83
79003[e]	1b	SOF/LDV 12 Weeks	PI+Peg-IFN+RBV	None	L31M(96.81%); Y93H(>99%)	C316H (>99%)	C316H (>99%)	0.65	143	1.4	0.97	1	0.89
79051[d]	1b	SOF/LDV 12 Weeks	Peg-IFN+RBV	None	L31V(>99%)	None	None	0.76	109	1.1	1.2	1.1	1.1
79062[e]	1a	SOF/LDV 12 Weeks	PI+Peg-IFN+RBV	None	Q30H(9.80%); Y93H(93.93%)	F415Y (>99%)	F415Y (>99%)	0.72	20	0.55	0.59	0.87	0.92
79214[d]	1a	SOF/LDV 12 Weeks	PI+Peg-IFN+RBV	Q30R(1.43%); Y93N(97.60%)	Y93N(>99%)	None	None	>42	>42	0.66	0.6	1	1
79303[d]	1a	SOF/LDV 12 Weeks	PI+Peg-IFN+RBV	M28T(1.03%); Q30R(>99%); L31M(>99%)	Q30R(>99%); L31M(>99%)	None	None	>42	>42	0.77	0.88	0.85	0.92
79378[d]	1a	SOF/LDV 12 Weeks	Peg-IFN+RBV	Q30H (98.76%); Y93H (98.07%)	Q30H(98.92%); Y93H(>99%)	F415Y (3.94%)	None	>42	>42	0.99	0.81	0.94	0.84
79179[d]	1b	SOF/LDV 12 Weeks	Peg-IFN+RBV	Y93H (59.82%)	Y93H(>99%)	None	None	21	>243	0.86	0.88	1.1	1.1
79383[f]	1a	SOF/LDV + RBV 24 Weeks	Peg-IFN+RBV	K24R (1.06%); Q30R (2.61%)	K24R(79.95%); Q30R(98.37%)	F415Y(17.21 %)	None	0.38	>42	0.41	0.85	0.85	1.3

BL = baseline; GT = genotype
a Fold changes from reference as tested in a NS5A replicon system
b Fold changes from reference as tested in a NS5B replicon system
c Fold changes were calculated relative to reference.
d Post-baseline timepoint is post-treatment Week 4
e Post-baseline timepoint is post-treatment Week 12
f Post-baseline timepoint is Week 2 of treatment

The independent assessment of the NGS data by DAVP resulted in similar findings and overall conclusions (Table 18). Substitutions at NS5A positions Q30, Y93, and M28 were most frequently associated with LDV/SOF treatment failure (Table 18).

Table 18. Resistance-associated substitutions identified by DAVP among virologic failures in GS-US-337-0109 (DAVP analysis). For the complete DAVP dataset see Appendix 2.

STUDYID	GT/ST	NS5A POS	No. Subjects	SUBS	Ratio
GS-US-337-0109	1a	30*	5	Q30R/Q30H/Q30L/R	3-1-1
		93*	4	Y93H/Y93N	3-1
		28*	3	M28V/M28T	2-1
		31*	1	M31L	
	1b	28*	1	L28M	
		92*	1	A92T	
		93*	1	Y93H	
		NS5A POS	**No. Subjects**	**SUBS**	**Ratio**
	1a	62	4	S62N/N62S	3-1
		61*	1	D61G	
	1b	440	3	E440G	

NS5A and **NS5B POS**, the amino acid position of he indicated gene; **SUBS**, substitutions detected at 0.10 or greater frequency at failure timepoint but not detected at baseline; **Ratio**, ratio of different substitutions at a position in the order of most to least; *, resistance-associated.

GS-US-337-0109 Phenotype Analyses

Viral RNA derived from subjects who failed treatment was extracted from baseline and relapse samples for evaluation of phenotypic resistance to LDV and SOF. The viral NS5A and NS5B regions from subject samples were separately inserted into replicons to determine cell culture susceptibility to ledipasvir and sofosbuvir respectively (see Table 17).

Results from testing baseline samples from subjects only showed resistance to LDV if high levels of LDV specific resistance-associated polymorphisms were present. No baseline samples showed reduced susceptibility to SOF or RBV. Similarly, relapse samples showed reduced susceptibility to LDV only if high levels of NS5A resistance-associated polymorphisms were present, and no baselines samples showed reduced susceptibility to SOF or RBV. The sponsor reported that this was consistent with no SOF signature resistance-associated substitutions being detected.

GS-US-337-0109 Resistance Analyses Conclusions

The sponsor concluded that overall, an SVR12 rate of 100% (24/24) was achieved in subjects harboring virus with baseline NS5A polymorphisms corresponding to 2.5-fold to 100-fold LDV resistance irrespective of treatment duration and with or without RBV added to the LDV/SOF regimen. A lower response rate was observed among the treatment-experienced subjects whose virus had baseline NS5A polymorphisms associated with >100-fold resistance to LDV and who were treated with LDV/SOF for 12 weeks. However, all 13 subjects whose virus had baseline NS5A polymorphisms including 6 subjects harboring >100-fold LDV resistance achieved SVR12 after 24 weeks of treatment with LDV/SOF. The sponsor further concluded that among the 12 subjects with virologic failure, single class resistance to LDV was detected, while all subjects showed no detectable resistance to SOF. Additionally, the presence of NS5B NAPI, NNAPI or RBV polymorphisms did not affect treatment response. NS3 PI substitutions were present at much higher rates at baseline in subjects that had previously been treated with PEG+RBV+PI regimens; however the presence of NS3 PI substitutions showed no effect on overall SVR rates.

DAVP concluded that baseline polymorphisms and treatment-emergent substitutions at NS5A positions Q30, Y93, and M28 accounted for the majority of resistance to LDV. Two resistance-associated substitutions to SOF were detected at positions S62 (n=4) and D61 (n=1) and these may have played a role in resistance to SOF.

REVIEW OF GS-US-337-0108 (ION-3)

Study GS-US-337-0108 (ION-3) evaluated the efficacy of SOF with LDV in a fixed dose combination (LDV/SOF FDC) administered for 12 weeks or LDV/SOF FDC ± RBV for 8 weeks in treatment-naïve subjects with chronic genotype 1 hepatitis C virus infection.

The sponsor reported that HCV consensus sequences were generated for all successfully deep sequenced samples. Out of 647 treated subjects described here, 216 subjects received LDV/SOF for 12 weeks. An additional 215 subjects received 8 weeks of LDV/SOF, while 216 received 8 weeks of LDV/SOF+RBV. All subjects were included in the efficacy and resistance analyses. Overall, 206/216 (95.4%) of subjects receiving 12 weeks of LDV/SOF achieved SVR12 (Table 19).

Table 19. GS-US-337-0108 (ION-3) summary of viral response data (Table 40, page 76, Integrated Phase 2/3 Virology Study Report).

	SVR12 n/total number of subjects (% of total)	
	SOF/LDV (n=431)	SOF/LDV+RBV (n=216)
Treatment-naïve, 12 weeks	206[a]/216 (95.4)	NA
Treatment-naïve, 8 weeks	202[b]/215 (94.0)	201[c]/216 (93.1)

a Three subjects relapsed, 5 had early discontinuation, and 2 achieved SVR 4 but lost to follow-up for the 12 weeks post treatment visit

b Eleven subjects relapsed, and 2 achieved SVR 4 but lost to follow-up for the 12 weeks post treatment visit

c Nine subjects relapsed, 5 had early discontinuation and one achieved SVR 4 but lost to follow-up for the 12 weeks post treatment visit

Additionally, 202/215 (94%) of subjects receiving 8 weeks of LDV/SOF achieved SVR12, and 201/216 (93.1%) subjects receiving 8 weeks of LDV/SOF+RBV achieved SVR12 (Table 19). Of the 38 subjects who did not achieve SVR12, 23 subjects relapsed, 10 had no post-treatment data, and 5 achieved SVR4 but were lost to follow up without post-treatment Week 12 data.

GS-US-337-0108 Baseline Sequence Data

At baseline, NS5A sequence was successfully obtained for 647/647 enrolled subjects treated with LDV/SOF±RBV for 8 or 12 weeks (515 GT1a and 130 GT1b, 1 GT1a/1b and 1 GT1c). NS5B sequence was successfully obtained for 643/647 subjects (511 GT1a and 130 GT1b, 1 GT1a/1b and 1 GT1c). NS5B sequence was not available from 4 subjects due to assay failure.

The sponsor reported that the viral NS5A region was examined for baseline resistance-associated polymorphisms reported to confer reduced susceptibility to ledipasvir. Overall, 116/647 (17.9%) subjects' viruses with successful NS5A sequencing were identified as having baseline NS5A resistance-associated polymorphisms. Of these, 104/116 (89.7%) subjects whose virus had baseline NS5A resistance-associated polymorphisms achieved SVR12 following 8 or 12 weeks treatment with LDV/SOF±RBV. In the 8-week LDV/SOF treatment group, 34/38 (89.5%) subjects whose virus had baseline NS5A resistance-associated polymorphisms achieved SVR12 (4 relapsed), and 32/38 (84.2%) subjects in the 8-week LDV/SOF+RBV arm achieved SVR12 (5 relapsed and 1 early discontinuation). In the 12-week treatment arm, 38/40 (95%) subjects treated with LDV/SOF achieved SVR12 (1 relapsed and 1 lost to follow up after achieving SVR4). Of these 116 subjects, 80 (69.0%) subjects' viruses had at least 1 NS5A resistance-associated polymorphisms conferring >100-fold reduced susceptibility to LDV and 69/80 (86.3%) subjects achieved SVR12. In addition, 65/116 (56.0%) subjects had NS5A resistance-associated polymorphisms at a frequency of >10%.

NS5B deep sequencing was successfully obtained for 643 subjects (GT1a=511, GT1b = 130, other genotypes: n=2). Nine out of 643 successfully sequenced subjects were observed to have NAPI resistance-associated

polymorphisms at baseline. One GT1a subject whose virus had G282 achieved SVR12. Of the other 8 subjects, 6 GT1b subjects' viruses had F159+N316 (n=6), one subjected infected with HCV GT1b had F159 (n=1), and one infected with GT1a had T142 (n=1) in NS5B. All 7 subjects whose viruses had F159 or F159+N316 achieved SVR12. An additional 9 subjects with H316 (n=1), Y316 (n=2), or N316 (n=6) also achieved SVR12 (Table 20). NS5B NNAPI resistance-associated polymorphisms were observed in 118/643 (18.4%) subjects' viruses with NS5B sequence, and overall 114/118 (96.6%) of these subjects achieved SVR12. Additionally, 19/643 (3%) of subjects were observed to have baseline RBV associated RAVs with 16/19 achieving SVR12.

Table 20. GS-US-337-0108 (ION-3) prevalence of baseline NS5B T142, F159, N316, and S320 polymorphisms in subjects infected with HCV GT1a and 1b (Table 45, page 82, Integrated Phase 2/3 Virology Study Report).

NS5B RAV or Treatment-Emergent Variant	GT	SOF/LDV 8 Weeks n/N	SOF/LDV+ RBV 8 Weeks n/N	SOF/LDV 12 Weeks n/N	Total subjects with RAVs n/N (%)	SVR12 for subjects with RAVs n/N (%)
S282G	GT 1a	0/171	1/170	0/170	1/511(0.2)	1/1(100)
	GT 1b	0/43	0/44	0/43	0/130(0)	
L159F (NI) + C316N (NNI)	GT 1a	0/171	0/170	0/170	0/511(0)	6/6(100)
	GT 1b	1/43	3/44	2/43	6/130(4.6)	
N142T (NI)	GT 1a	0/171	1/170	0/170	1/511(0.2)	0[a]/1(0)
	GT 1b	0/43	0/44	0/43	0/130(0)	
L159F (NI)	GT 1a	0/171	0/170	0/170	0/511(0)	1/1(100)
	GT 1b	0/43	1/44	0/43	1/130(0.8)	
C316H/N/Y (NNI)	GT 1a	1[b]/171	1[b]/170	0/170	2/511(0.4)	9/9(100)
	GT 1b	4[c]/43	1[d]/44	2[e]/43	7/130(5.4)	

a One GT1a Subject 4238-73209 was lost to follow-up after Day 1
b One GT 1a subject had C316Y
c Four GT 1b subjects had C316N
d One GT 1b subject had C316H
e Two GT 1b had C316N
n/N = Number of subjects with RAVs / Number of subjects successfully sequenced

GS-US-337-0108 Resistance Analyses in Subjects Experiencing Virologic Failure

The sponsor stated that no on-treatment virologic breakthroughs were observed in any of 647 subjects in this study. A total of 23 subjects experienced viral relapse. NS5A and NS5B were deep sequenced from these subjects at baseline and virologic failure timepoints (Table 21).

According to the sponsor's analysis, 11 of 215 subjects (5.1%) experienced viral relapse in the LDV/SOF 8-week treatment group. Four of these 11 subjects had NS5A resistance-associated polymorphisms at baseline (T/A28, Y/H30, N/H93, and M31) which confer high levels of resistance to LDV. All baseline resistance-associated polymorphisms were enriched at the virologic failure time point with the exception of A28 (6.09%) in subject 73274 and H30 (1.16%) in subject 73313 that were no longer detectable at the 1% cutoff. No new NS5A resistance-associated substitutions emerged in 3/4 subjects at relapse. The other 7 subjects had virus with no NS5A resistance-associated polymorphisms at baseline. Four of 7 subjects whose virus had no baseline NS5A polymorphisms had high levels of LDV resistance-associated substitutions detected (Y93H and Q30R) at virologic failure. In contrast, no NS5A resistance-associated polymorphisms were detected by NGS in 3/7 subjects' viruses. No S282T or other NAPI resistance-associated substitutions were detected in any subject at relapse.

Table 21. Summary of substitutions found by the sponsor among subjects who experienced relapse in GS-US-337-0108 (Table 46, pages 84, Integrated Phase 2/3 Virology Study Report).

Subject Number	GT	Treatment	Relevant RAVs			
			NS5A		NS5B	
			BL	Post BL	BL	Post BL
73033[a]	1a	SOF/LDV 8 Weeks	L31M(19.25%);	Q30R(>99%); L31M(>99%);	None	None
73114[b]	1a	SOF/LDV 8 Weeks	None	None	None	None
73227[a]	1a	SOF/LDV 8 Weeks	Y93N(15.37%);	Y93N(>99%);	None	None
73274[a]	1a	SOF/LDV 8 Weeks	M28T(93.52%); M28A(6.09%);	M28T(>99%);	None	None
73300[a]	1b	SOF/LDV 8 Weeks	None	Y93H(>99%);	None	None
73313[b]	1a	SOF/LDV 8 Weeks	Q30Y(2.04%); Q30H(1.16%); Y93H(3.60%);	Q30Y(>99%); - Y93H(>99%);	F415Y(95.43%);	F415Y(>99%);
73453[a]	1a	SOF/LDV 8 Weeks	None	Y93H(>99%);	F415Y(84.99%);	F415Y(>99%);
73490[b]	1a	SOF/LDV 8 Weeks	None	Q30R(>99%);	None	None
73514[a]	1a	SOF/LDV 8 Weeks	None	None	None	None
73538[a]	1a	SOF/LDV 8 Weeks	None	Q30R(>99%);	None	None
73408[a]	1a	SOF/LDV 8 Weeks	None	None	None	
73049[b]	1b	SOF/LDV+RBV 8 Weeks	None	None	None	None
73185[a]	1a	SOF/LDV+RBV 8 Weeks	Q30R(71.06%); Q30H(28.84%); Y93H(24.58%);	Q30R(>99%); L31P(1.13%);	None	None
73277[a]	1a	SOF/LDV+RBV 8 Weeks	L31M(1.12%);	L31M(>99%);	None	None
73564[a]	1a	SOF/LDV+RBV 8 Weeks	None	S38F(>99%); Y93H(>99%);	None	None
73610[a]	1a	SOF/LDV+RBV 8 Weeks	None	None	None	None
73335[b]	1b	SOF/LDV+RBV 8 Weeks	Y93H(63.83%);	Y93H(>99%);	None	None
73385[a]	1a	SOF/LDV+RBV 8 Weeks	Y93N(>99%);	Y93N(>99%);	None	None
73416[b]	1a	SOF/LDV+RBV 8 Weeks	Y93C(8.65%);	None	None	None
73445[a]	1a	SOF/LDV+RBV 8 Weeks	None	None	None	None
73124[a]	1b	SOF/LDV 12 Weeks	None	L31I(>99%); Y93H(>99%);	None	None
73230[a]	1a	SOF/LDV 12 Weeks	None	None	None	V321A(1.10%);
73078[a]	1a	SOF/LDV 12 Weeks	Y93F(10.81%); Y93N(1.71%);	Y93N(>99%);	None	L159F(2.45%);

BL = baseline; GT = genotype
a Post-baseline timepoint is 4 weeks post-treatment
b Post-baseline timepoint is 12 weeks post-treatment

In the LDV/SOF+RBV 8-week group, 9 of 216 subjects (4.2%) did not achieve SVR12 due to relapse. A total of 5 of 9 subjects' viruses had NS5A resistance-associated polymorphisms at baseline (M31, H/N/C93 and R/H30). NS5A resistance-associated polymorphisms were retained in the virus of 4/5 subjects, but no longer detected in 1/5 subject's virus at relapse. The other 4 subjects didn't have any detected NS5A resistance-associated polymorphisms in their virus at baseline. Of these 4 subjects, NS5A resistance-associated polymorphisms (F38, H93) emerged in 1 of 4 subjects' virus, but no NS5A resistance-associated substitutions emerged in 3 of 4 subjects' viruses. No S282T or other NAPI polymorphisms were detected in any of these 9 subjects' viruses. In the LDV/SOF 12-week group, 3 of 216 subjects (1.4%) relapsed. Of these 3 subjects, one had virus with NS5A resistance-associated polymorphisms at baseline. In the subject whose virus had baseline NS5A resistance-associated polymorphisms, the frequency of NS5A polymorphisms increased at relapse and 2.5% of F159 in NS5B was detected by deep sequencing. Two of 3 subjects' viruses did not have baseline NS5A resistance-associated polymorphisms. New NS5A resistance-associated substitutions (L31I and Y93H) emerged in one subject's virus. In contrast, no NS5A resistance-associated substitutions were detected in the remaining subject's virus, but NS5B V321A was detected at 1.1% at relapse.

The analysis performed by DAVP largely agreed with the analyses performed by and conclusions drawn by the sponsor for study GS-US-337-0108. Treatment failure was predominantly associated with NS5A positions H93, Q30, and M28 and NS5B positions S62, D61, A112, and E237 (Table 22).

Table 22. Resistance-associated substitutions identified by DAVP among virologic failures in GS-US-337-0108 (DAVP analysis).

STUDYID	GT/ST	NS5A POS	No. Subjects	SUBS	Ratio
GS-US-337-0108	1a	93*	8	Y93H/Y93N/Y93C/Y93F/N/H93Y	3-2-1-1-1
		30*	4	Q30R/Q30H/R/Q30H/Y	2-1-1
		28*	3	M28V/M28T	2-1
		31*	2	L31M	
		38	1	S38F	
		58*	1	H58P	
	1b	93*	2	Y93H	
	GT/ST	**NS5B POS**	**No. Subjects**	**SUBS**	**Ratio**
	1a	62	11	S62N/S62G/C/N/T	10-1
		61*	2	D61G	
		112*	2	A112T	
		237*	2	E237G	
		540	2	P540A/A540S	1-1
		159*	1	L159F	
		321*	1	V321A	
	1b	440	4	E440G	

NS5A and **NS5B POS**, the amino acid position of the indicated gene; **SUBS**, substitutions detected at 0.10 or greater frequency at failure timepoint but not detected at baseline; **Ratio**, ratio of different substitutions at a position in the order of most to least; *, resistance-associated.

GS-US-337-0108 Phenotype Analyses

Virus derived from subjects who failed treatment was extracted from baseline and relapse samples for evaluation of phenotypic resistance to LDV and SOF, looking at the NS5A region and NS5B regions from subject samples, which were separately inserted into replicons to determine susceptibility to these drugs.

The sponsor stated that the results from baseline samples only showed resistance to LDV if high frequencies of LDV specific polymorphisms were present (Table 23). No baseline samples showed reduced susceptibility to SOF or RBV. Similarly, relapse samples showed reduced susceptibility to LDV only if high frequencies of NS5A polymorphisms were present, and no post-baseline samples showed reduced susceptibility to SOF or RBV. The sponsor concluded that this was consistent with no SOF signature resistance substitutions being detected.

Table 23. GS-US-337-0108 (ION-3) phenotypic resistance analysis in subjects with virologic failure (Table 39, page 71, Integrated Phase 2/3 Virology Study Report).

			Relevant RAVs				Drug Susceptibility (Fold-Change from Reference)					
			NS5A		NS5B		LDV		SOF		RBV	
Subject ID	GT	Treatment	BL	Post BL	BL	Post BL	BL	Post BL	BL	Post BL	BL	Post BL
73033[a]	1a	SOF/LDV 8 Weeks	L31M(19.25%)	Q30R(>99%) L31M(>99%)	None	None	7.4	>45	0.96	1.1	0.71	0.65
73049[b]	1b	SOF/LDV + RBV 8 Weeks	None	None	None	None	1.3	1.5	0.84	1.0	1.1	0.90
73078[a]	1a	SOF/LDV 12 Weeks	Y93F(10.81%) Y93N(1.71%)	Y93N(>99%)	None	L159F(2.45%)	0.83	>42	0.97	1.1	0.76	0.75
73114[b]	1a	SOF/LDV 8 Weeks	None	None	None	None	1.3	1.6	0.88	1.1	1.0	0.92
73124[a]	1b	SOF/LDV 12 Weeks	None	L31I(>99%) Y93H(>99%)	None	None	1.4	>212	1.1	1.2	0.91	1.4
73185[a]	1a	SOF/LDV + RBV 8 Weeks	Q30R(71.06%) Q30H(28.84%) Y93H(24.58%)	Q30R(>99%) L31P(1.13%)	None	None	>42	>42	0.73	0.76	0.76	0.70
73227[a]	1a	SOF/LDV 8 Weeks	Y93N(15.37%)	Y93N(>99%)	None	None	0.83	>42	0.74	0.75	0.70	0.82
73230[a]	1a	SOF/LDV 12 Weeks	None	None	None	V321A(1.10%)	0.53	0.54	0.77	0.77	0.84	0.86
73274[a]	1a	SOF/LDV 8 Weeks	M28T(93.52%) M28A(6.09%)	M28T(>99%)	None	None	17.6	20	0.87	0.88	0.98	0.86
73277[a]	1a	SOF/LDV + RBV 8 Weeks	L31M(1.12%)	L31M(>99%)	None	None	0.94	>58	0.74	0.65	1.1	1.1
73300[a]	1b	SOF/LDV 8 Weeks	None	Y93H(>99%)	None	None	0.61	>243	0.97	0.95	0.86	0.55

Table 23 (continued). GS-US-337-0108 (ION-3) phenotypic resistance analysis in subjects with virologic failure (Table 39, page 71, Integrated Phase 2/3 Virology Study Report).

Subject ID	GT	Treatment	Relevant RAVs				Drug Susceptibility (Fold-Change from Reference)					
			NS5A		NS5B		LDV		SOF		RBV	
			BL	Post BL	BL	Post BL	BL	Post BL	BL	Post BL	BL	Post BL
73313[b]	1a	SOF/LDV 8 Weeks	Q30Y(2.04%) Q30H(1.16%) Y93H(3.60%)	Q30Y(>99%) Y93H(>99%)	F415Y(95.43%)	F415Y(>99%)	0.83	>52	1.0	2.3	1.0	1.3
73335[b]	1b	SOF/LDV + RBV 8 Weeks	Y93H(63.83%)	Y93H(>99%)	None	None	2.1	>192	1.1	1.1	1.2	1.1
73385[a]	1a	SOF/LDV + RBV 8 Weeks	Y93N(>99%)	Y93N(>99%)	None	None	>58	NA[c]	0.94	0.82	1.3	1.3
73408[a]	1a	SOF/LDV 8 Weeks	None	None	None	None	0.63	NA[c]	0.70	0.77	1.3	1.5
73416[b]	1a	SOF/LDV + RBV 8 Weeks	Y93C(8.65%)	None	None	None	0.71	0.63	1.0	0.98	0.78	0.63
73445[a]	1a	SOF/LDV + RBV 8 Weeks	None	None	None	None	2.3	2.2	0.73	0.82	0.63	0.86
73453[a]	1a	SOF/LDV 8 Weeks	None	Y93H(>99%)	F415Y(84.99%)	F415Y(>99%)	1.1	>42	0.65	0.69	0.84	0.79
73490[b]	1a	SOF/LDV 8 Weeks	None	Q30R(>99%)	None	None	0.83	>52	0.62	0.62	0.90	0.96
73514[a]	1a	SOF/LDV 8 Weeks	None	None	None	None	1.3	1.0	1.2	1.7	1.3	1.1
73538[a]	1a	SOF/LDV 8 Weeks	None	Q30R(>99%)	None	None	0.80	>58	0.66	0.77	1.3	1.3
73564[a]	1a	SOF/LDV + RBV 8 Weeks	None	S38F(>99%) Y93H(>99%)	None	None	0.49	>42	0.76	0.82	0.66	0.60
73610[a]	1a	SOF/LDV + RBV 8 Weeks	None	None	None	None	0.68	0.60	0.83	0.71	0.94	0.78

a Post-baseline time point is post treatment Week 4
b Post-baseline time point is post treatment Week 12
c Data not available due to assay failure

GS-US-337-0108 Resistance Analyses Conclusions

The sponsor reported that overall, SVR12 rates of 84% to 95% were achieved in the presence of baseline NS5A resistance-associated polymorphisms across the 3 arms of the ION-3 study. The presence of NS5B NAPI, NNAPI or RBV resistance-associated polymorphisms did not affect treatment response. No phenotypic resistance to SOF was detected in any subject, while virologic failure was associated with single class LDV resistance in 16/23 relapse subjects. Low levels of NS5B_ A321 and NS5B_F159 were detected in a single subject infected with HCV GT1a at relapse.

DAVP concluded that treatment failure was predominantly associated with NS5A positions H93, Q30, and M28 and NS5B positions S62, D61, A112, and E237. NS5B substitutions A112T and E237G were novel substitutions that emerged in this trial.

COMBINED RESISTANCE ANALYSIS

The sponsor reported that in the pooled phase 3 analysis, 23% (370/1615) of subjects' virus had baseline NS5A resistance-associated polymorphisms (any change at NS5A amino acid positions K24, M28, Q30, L31, H58, A92 or Y93) identified by population or deep sequencing. Relapse rates for subjects whose virus had one baseline NS5A polymorphism was 3.6%. Relapse rates were higher for subjects whose virus had 2 or more baseline NS5A resistance-associated polymorphisms; 9.5% and 9% for subjects whose virus had 2 and 3 baseline NS5A resistance polymorphisms, respectively.

The sponsor also reported that SVR12 was achieved in all 24 subjects (N=20 with F159F+N316; N=1 with F159; and N=3 with T142) who had baseline polymorphisms associated with resistance to NS5B nucleoside analog polymerase inhibitors. The sofosbuvir resistance-associated substitution S282T was not detected in the baseline NS5B sequence of any subject's virus in phase 3 trials by population or deep sequencing.

In addition to analyzing all of the data for each clinical trial independently, DAVP also performed a meta-analysis that combined all GTs from all three phase 3 studies and both phase 2 studies to determine if there

were novel substitutions present in any of the clinical trials. The combined analysis determined the predominant and low frequency substitutions that were treatment emergent and identified the substitutions that contributed to LDV/SOF resistance.

Results of the combined resistance analysis indicated that polymorphisms/substitutions at NS5A positions Y93, Q30, M28, L31, H58, and K24 and NS5B substitutions at positions D61, V321, L159, S282, and L320 played a role in failure among subjects infected with HCV GT1a. In addition, substitutions at NS5B positions E202 and E440 are potential resistance-associated sites. In subjects infected with HCV GT1b, polymorphisms/substitutions at NS5A positions Y93, L28, and A92 and substitutions at NS5B position I321 played a role in failure (Table 24).

Table 24. Resistance-associated substitutions for LDV/SOF FDC.

STUDYID	GT/ST	NS5A POS	No. Subjects	SUBS	Ratio	Conservation	Substitutions	Baseline	Conclusion	Fail%	SVR%
All 5 Trials	1a	**93**	15	Y93H/Y93N/Y93F/N/Y93H/C/Y93C/H93Y	8-3-1-1-1-1	97.73%	H, N, F, C, Y	Low	RAP	0	2.1
		30	14	Q30R/Q30H/Q30H/Y/Q30H/R/Q30L/Q30L/R	7-3-1-1-1-1	96.92%	R, H, Y, L	Low	RAP	0	3.3
		28	9	M28V/M28T/V28M	6-2-1	92.14%	V,T	Equal	RAP	7.9	8.6
		31	6	L31M/M31L	5-1	97.49%	M	Low	RAP	0	2.3
		58	3	H58P/H58R	2-1	91.33%	P, R	Equal	RAP	13.2	7.2
		121	2	I121V/I121M	1-1	77.39%	I/V	Equal	Not likely	28.9	23
		349	2	S349T/S349P	1-1	85.92%	P/S	Equal	Not likely	7.9	12.1
		392	2	D392N/N392D	1-1	58.66%	D/N/S	Equal	Not likely	47.4	44.4
		24	1	K24R		98.22%	R	Low	RAP	0	1.8
		62	1	E62D		93.44%	D	Equal	Not likely	7.9	4.4
		NS5B POS	**No. Subjects**	**SUBS**	**Ratio**	**Conservation**	**Substitutions**	**Baseline**	**Conclusion**	**Fail%**	**SVR%**
		62	23	S62N/N62S/S62G/C/N/T	20-2-1	63.74%	N/S	Equal	Not likely	44.6	33.3
		61	9	D61G/G61D	8-1	99.62%	G	None	RAS	0	0
		112	3	A112T		98.43%	T	Low	RAS	0	1.2
		540	3	P540A/A540S/A540P	1-1-1	94.10%	S, P	H-SVR	Not likely	1.5	5.5
		237	2	E237G		99.73%	G	Low	Potenital RAS	0	0.3
		321	2	V321A/V321I	1-1	100%	A/I	Low	Not likely	0	0.1
		473	2	S473T		99.30%	T	H-FAIL	Potenital RAS	7.7	1.1
		159	1	L159F		99.89%	F	None	RAS	0	0
		202	1	E202D		98.59%	D	Low	Potenital RAS	0	1.3
		282	1	S282T		100%	T	None	RAS	0	0
		320	1	L320V/I		100%	V/I	None	RAS	0	0
		440	1	E440D		99.51%	D	Low	Potenital RAS	0	0.6
	1b	**NS5A POS**	**No. Subjects**	**SUBS**	**Ratio**	**Conservation**	**Substitutions**	**Baseline**	**Conclusion**	**Fail%**	**SVR%**
		93	4	Y93H		91.04%	H	H-FAIL	RAP	28.6	6
		28	1	L28M		98.11%	M	Low	RAP	0	0.25
		92	1	A92T		95.05%	T	H-FAIL	RAP	14.3	4.5
		NS5B POS	**No. Subjects**	**SUBS**	**Ratio**	**Conservation**	**Substitutions**	**Baseline**	**Conclusion**	**Fail%**	**SVR%**
		440	7	E440G		91.23%	G	H-SVR	Not likely	0	7.4
		321	1	I321V		97.59%	V	H-FAIL	RAS	14.3	1.3

GT/ST – genotype and subtype of HCV
SUBS10 – Substitutions detected if present at 0.10 or greater frequency at failure timepoint but not detected at baseline.
Ratio – ratio of different subs itutions at a position in the order or most to least
Conservation – percent conservation reported by sponsor
Substitutions – common variants at that position
Baseline – comparison of subjects who had he substitution at baseline
None – not present at baseline in any subjects
Low – less than 3.5% frequency in any subject population
H-FAIL – higher frequency at baseline in subjects who failed treatment
H-SVR - higher frequency at baseline in subjects who achieved SVR12
Equal - present at baseline at similar frequencies for subjects that achieved SVR12 and those who failed
RAS – potentially a resistance-associated substitu ion
RAP – resistance associated polymorphism
Fail% – percent of subjects who failed who had a polymorphism at this position of their virus at baseline
SVR% – percent of subjects who achieved SVR12 who had a polymorphism at this position of their virus at baseline

Combined Resistance Analysis Conclusions

Overall, the LDV/SOF fixed dose combination had high SVR rates greater than 90% and resulted in very few virologic failures. Most of the subjects who failed treatment relapsed after treatment had been completed. In those subjects, most had NS5A polymorphisms at baseline that likely inhibited the LDV component of the FDC, resulting in a functional monotherapy with SOF. In other cases, NS5A resistance-associated substitutions emerged over the course of treatment. Polymorphisms/substitutions at Y93 were the predominant polymorphisms/substitutions associated with failure in both HCV GT1 subtypes. Treatment-emergent substitutions associated with SOF failure were also detected, including substitutions at V321 (n=3), L159 (n=1), S282 (n=1), and V320 (n=1). In addition, NS5B_D61G (n=8) was associated with treatment failure, and this substitution was previously described in the liver pre-transplant study (P7977-2025). NS5B_A112T, NS5B_E237G, and NS5B_S473T were novel treatment-emergent substitutions that may play a role in SOF resistance. Taken together the results of this independent analysis confirm that LDV/SOF was highly effective

against HCV GT1, and has a high resistance to barrier. However, additional substitutions should be evaluated to determine if these impact the efficacy of LDV/SOF FDC. These include: NS5B_A112T, NS5B_E237G, and NS5B_S473T.

METHODS

HCV Viral Load Assay

In Phase 2 Study P7977-0523, the Roche COBAS® AmpliPrep/COBAS® HCV TaqMan® assay (research use only version) was used to determine HCV RNA results. The established LOD of this assay was 15 HCV IU/mL (defined by a 95% hit rate with World Health Organization standards). In the Studies GS-US-337-0118, GS-US-337-0122, GS-US-337-0102, GS-US-337-0109, and GS-US-337-0108, the COBAS® TaqMan® HCV Test v2.0 for use with the High Pure System assay was used to quantify HCV RNA in this study. The LLOQ of the assay was 25 IU/mL.

RESISTANCE ANALYSIS

Population and/or deep sequencing of NS5A was performed at baseline for all subjects enrolled in the phase 2 and 3 studies as shown in Table 1. NS5B and NS3 deep sequencing was performed at baseline for a subset of subjects enrolled in Phase 2/3 studies. Resistance testing was performed for subjects who met the criteria of the resistance analysis population. The resistance analysis population includes any subject who received at least one dose of a LDV/SOF-containing regimen, but did not achieve SVR12 due to virologic failure or early discontinuation, had HCV RNA ≥1,000 IU/mL and had a plasma sample available for analysis. Resistance analyses included NS5A and NS5B deep sequencing that was performed at failure, and phenotypic analysis that was attempted for the majority of virologic failure subjects and was successful for a subset of subjects

Virologic failure is defined as follows:

- **On-treatment virologic failure**
  Breakthrough: HCV RNA ≥ LLOQ after having previously had HCV RNA <LLOQ, while on-treatment, confirmed with 2 consecutive values (note, second confirmation value could be post-treatment), or last available on-treatment measurement with no subsequent follow-up values
  Rebound: >1 $\log_{10}$ IU/mL increase in HCV RNA from nadir while on treatment, confirmed with 2 consecutive values (note, second confirmation value could be post-treatment), or last available on-treatment measurement with no subsequent follow-up values
  Non-responsive: HCV RNA persistently ≥LLOQ through 8 weeks of treatment
- **Relapse**: HCV RNA ≥LLOQ during the post-treatment period having achieved HCV RNA <LLOQ at end-of-treatment, confirmed with 2 consecutive values or last available post-treatment measurement

Population Nucleotide Sequencing

Baseline population sequencing of the full-length HCV NS5A or NS5B coding region was performed (b) (4) using RT-PCR and standard Sanger population sequencing. The obtained sequence was also used to confirm the results of genotyping/ subtyping by the INNO-LiPA assay performed at screening. For post-baseline resistance analyses of subjects with virologic failure, the HCV NS5A or NS5B gene was amplified from subject samples if the serum/plasma level of HCV RNA was ≥1,000 IU/mL. Population sequencing results were reported as change from reference in the data sets. In addition to the reference, the conservation and common variants for each genotype are also reported in alphabetical order for each amino acid position. For genotype 1, the conservation and the common variants were determined based on sequencing results from DAA-untreated subjects enrolled in Gilead's HCV studies. For genotype 4, conservation and common variants were determined based on Gilead's data as well as sequences obtained from the European Hepatitis C Virus Database.

Next Generation Sequence Analysis

For most baseline and all virologic failures, NS5A, NS5B, or NS3 PCR amplicons at baseline and post-baseline timepoints were subjected to deep sequencing using the Illumina MiSeq deep sequencing platform. For baseline samples where population sequencing only or both population and deep sequencing data were available, analyses using the population sequence were reported. For baseline samples that only had deep sequencing data, consensus sequences were generated and used for analysis. In cases where multiple deep sequencing runs were performed for a given sample, the data from multiple runs were combined to create the consensus sequence. Consensus sequences of post-baseline samples were generated with inclusion of mixed amino acid calls present between 15 and 85%. In cases where the baseline amino acid differed from reference and the post-baseline substitution was a change to the reference amino acid, this was still considered as a post-baseline substitution. Deep sequencing results were reported for potential LDV, SOF, and RBV resistance-associated variant positions from subjects in the resistance analysis population with an assay cut-off at 1% and for all changes observed in >1 subjects in the resistance analysis population. The development of predominant substitutions was analyzed by study and per treatment arm across multiple studies.

Phenotypic Analysis

Phenotypic analyses of samples from subjects with virologic failure using a chimeric replicon encoding the NS5A or NS5B region derived from subject plasma/serum were performed by Gilead Sciences, Inc. (b) (4)

Baseline Sequence Data Analysis

Baseline sequences were analyzed for the presence of previously identified NS5A (Table 25), NS5B (nucleotide inhibitor, non-nucleotide inhibitor or RBV) (Table 26) and NS3 resistance associated variants (Table 27).

Table 25. List of specific resistance-associated NS5A substitutions analyzed by the sponsor.

Genotypes 1a and 1b	L31 I/F/M/V, P32L, A92K, Y93 C/F/H/N/S
Genotype 1a only	K24G/N/R, M28A/G/T, Q30E/G/H/K/L/R/T, S38F, H58D, A92T
Genotype 1b only	P58D

Table 26. List of specific resistance-associated or treatment-emergent NS5B substitutions analyzed by the sponsor.

Nucleoside/Nucleotide Inhibitor	
Genotypes 1a and 1b	S96T, N142T, L159T, S282T, M289L, L320F, V321A
Nonnucleoside Inhibitor	
Genotypes 1a and 1b	C316N/Y, M414I/V/T, L419M/S, R422K, M423A/I/T/B, C445F, Y448H, Y452H, I482L, A486I/T, V494A, P495A/T, P496S, G554S, S556G, D559G
Genotype 1b only	V499A
Ribavirin-Associated	
Genotypes 1a and 1b	T390I
Genotype 1a only	F415Y

Table 27. List of specific resistance-associated or treatment-emergent NS3 substitutions analyzed by the sponsor.

Genotypes 1a and 1b	V36 A/G/M/L/M, F43S, T54 A/C/G/S, V55 A/I, Q80 K/R/L, S122R, R155 C/G/K/M/T/Q/S, A156 F/G/N/T/V/S, D168 A/E/F/G/H/I/N/K/L/P/V/T/Y
Genotype 1a only	I170A/L/T
Genotype 1b only	V170A/L/T, M175L

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 205834 **SDN** 002 **DATE REVIEWED:** 06/27/2014

CONCLUSIONS

DAVP performed an independent analysis of the NGS data submitted for three pivotal phase 3 and two phase 2 clinical trials and compared the results to those reported by the sponsor. In general, there was good agreement between these results. However, the different analysis pipelines used much different default filtering and mapping criteria and this was apparent when comparing frequency table values such as Total Coverage, Variant Coverage, and Amino Acid Frequency. However, despite these differences, the general trends observed were very similar.

Overall, the LDV/SOF fixed dose combination had high SVR rates greater than 90% and resulted in very few virologic failures. Most of the subjects who failed treatment relapsed after treatment had been completed. In those subjects, most had NS5A polymorphisms at baseline that likely inhibited the LDV component of the FDC, resulting in a functional monotherapy with SOF. In other cases, NS5A resistance-associated substitutions emerged over the course of treatment. Polymorphisms/substitutions at Y93 were the predominant polymorphisms/substitutions associated with failure in both HCV GT1 subtypes. Treatment-emergent substitutions associated with SOF failure were also detected, including substitutions at V321 (n=3), L159 (n=1), S282 (n=1), V320 (n=1), and V321 (n=1). In addition, NS5B_D61G (n=8) was associated with treatment failure, and this substitution was previously described in the liver pre-transplant study (P7977-2025). NS5B_A112T, NS5B_E237G, and NS5B_S473T were novel treatment-emergent substitutions that may play a role in SOF resistance. Taken together the results of this independent analysis confirm that LDV/SOF was highly effective against HCV GT1, and has a high resistance to barrier. However, additional substitutions should be evaluated to determine if these impact the efficacy of LDV/SOF FDC. These include: NS5B_A112T, NS5B_E237G, and NS5B_S473T.

For complete labeling details, please see the review of NDA 205834 SDN 000 by Senior Clinical Virology Reviewer Lisa Naeger, Ph.D.

POST MARKETING RECOMMENDATIONS

1. Phenotypic assessment of NS5B_A112T, NS5B_E237G, and NS5B_S473T in the HCV GT1a replicon

ADMINISTRATIVE

Reviewer's Signature(s)

Eric F. Donaldson
Eric F. Donaldson, Ph.D.
Clinical Virology Reviewer

Concurrence(s)

Date: _____
HFD-530/Clin Micro TL/J O'Rear

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

APPENDICES

APPENDIX 1: P7977-0523

USUBJID	GT/ST	TXCAT	REG	GENE	SUBS	STAT	VDAG
P7977-0523-1069-5504	1a	Prior Null	SOF/LDV 12	5A	Q30R	E	2
					Y93H/C	E	3
					N392D	E	2
				5B	S62N	E	1(Q)
					A112T	E	3
P7977-0523-1069-5506	1a	Prior Null	SOF/LDV 12	5A	F19C	E	2
					K24R	E	1(G)
					H58R	E	3
					S415A	E	2
				5B	G61D	B	1(Q)
					S62N	B	2
p7977-0523-1069-5513	1a	Prior Null	SOF/LDV 12	5A	V28M	B	3
					Q30H	B	3
				5B	D61G	E	2
					S62N	B and E	2
P7977-0523-1069-5534	1a	Tx Naïve	SOF/LDV 6	5B	E87D	E	2
					A334G	E	2
P7977-0523-1069-5541	1a	Tx Naïve	SOF/LDV 6	5A	M28T	E	1(G)
				5B	D61G	E	3
					S62N	E	2
					E202D	E	2
P7977-0523-1069-5545	1a	Tx Naïve	SOF/LDV 6	5A	H58P	B and E	3
					E62D	B and E	3
				5B	D61G	B	1(Q)
					S62D	B and E	1(G)
P7977-0523-1069-5547	1a	Tx Naïve	SOF/LDV 6	5A	M28V	B	3
					L31M	E	3
				5B	D61G	B	3
					S62N	B	2
P7977-0523-1069-5548	1a	Tx Naïve	SOF/LDV 6	5A	M202T	E	2
				5B	N35S	E	2
					G61D	B	2
					N62S	B	2
					L314P	E	2
					S473T	B	3
p7977-0523-1069-5551	1a	Tx Naïve	SOF/LDV 6	5B	D61G	B	2
					S62N	B and E	3
					L320F	E	1(Q)
					A540P	B	2
P7977-0523-1069-5555	1a	Tx Naïve	SOF/LDV 6	5A	G403S	E	3
				5B	D61G	E	1(Q)
					S62N	B	2
P7977-0523-5868-5537	1a	Tx Naïve	SOF/LDV 6	5A	M28V	B	2
					Q30R	E	3
					S349T	E	3
					C404R	E	2
				5B	D61G	B	2
					S62N	B	2

USUBJID, unique subject identification code; **GT/ST**, HCV genotype and subtype; **TXCAT**, prior treatment category; **REG**, regimen; **GENE**, viral gene sequenced; **SUBS**, substitutions detected at 0.10 or greater frequency at failure timepoint but not detected at baseline; **STAT**, status of substitution; **VDAG**, number of variant detection algorithms that detected the substitution out of a possible of three; **E**, emergent substitution; **B**, baseline polymorphism.

APPENDIX 2: GS-US-337-0109

USUBJID	GT/ST	TXCAT	REG	GENE	SUBS	STAT	VDAG
GS-US-337-0109-0334-79378	1a	TE	SOF/LDV 12	NS5A	Q30H	B	1
					Y93H	B	2
				NS5B	S62N	B	1
GS-US-337-0109-0519-79034	1a	TE	SOF/LDV 12 + RBV	NS5A	Q30H	E	1(G)
					M31L	B	2
				NS5B	D61G	E	2
					S62N	B and E	2
					P353S	E	3
GS-US-337-0109-1302-79062	1a	TE	SOF/LDV 12	NS5A	T21I	E	3
					Q30H	E	2
					Y93H	E	3
				NS5B	S473T	B	2
GS-US-337-0109-2191-79303	1a	TE	SOF/LDV 12	NS5A	Q30R	B	2
					L31M	B	1(P)
				NS5B	E335K	E	2
					G543S	E	3
GS-US-337-0109-2493-79041	1a	TE	SOF/LDV 12 + RBV	NS5A	M28T	E	3
					Q30R	E	3
					A213T	E	3
					T367I	E	3
				NS5B	N62S	B	2
GS-US-337-0109-3060-79063	1a	TE	SOF/LDV 12 + RBV	NS5A	Q30K	E	1(G)
GS-US-337-0109-4434-79070	1a	TE	SOF/LDV 12 + RBV	NS5A	Q30L/R	E	3
					Y93H	E	3
				NS5B	D61G	B	1(Q)
					S62N	B and E	2
GS-US-337-0109-7393-79214	1a	TE	SOF/LDV 12	NS5A	M28V	B	2
					Y93N	B	2
				NS5B	K151R	E	3
GS-US-337-0109-7864-79383	1a	TE	SOF/LDV 12 + RBV	NS5A	K24R	B and E	2
					M28V	B and E	3
					Q30R	B and E	3
					S384T	E	3
				NS5B	S62N	B	3
					K124Q	E	3
GS-US-337-0109-4238-79179	1b	TE	SOF/LDV 12	NS5A	L28M	B	2
					Y93H	B and E	1(G)
					T200A	E	2
				NS5B	D61G	E	1(Q)
					D62N	E	1(Q)
					E440G	B	2
GS-US-337-0109-5518-79003	1b	TE	SOF/LDV 12	NS5A	L31M	E	1(G)
					Y93H	E	3
					L31F	B	1(P)
				NS5B	H118R	E	3
					Q184R	E	3
					I321V	B	2
					E440G	B	2
					Y561C	E	2
GS-US-337-0109-5847-79051	1b	TE	SOF/LDV 12	NS5A	L31V	E	1(G)
					A92T	B	2
					V156M/ (G)	E	3
					P223L	E	3
					E293D	E	3
					V33NS5A	E	3
				NS5B	N142S	E	3
					E440G	B	2
					S556G	E	3

USUBJID, unique subject identification code; **GT/ST**, HCV genotype and subtype; **TXCAT**, prior treatment category; **REG**, regimen; **GENE**, viral gene sequenced; **SUBS**, substitutions detected at 0.10 or greater frequency at failure timepoint but not detected at baseline; **STAT**, status of substitution; **VDAG**, number of variant detection algorithms that detected the substitution out of a possible of three; **E**, emergent substitution; **B**, baseline polymorphism.

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/s/

ERIC F DONALDSON
07/10/2014

JULIAN J O REAR
07/10/2014

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA: 205834 **SDN**: 000 **DATE REVIEWED:** 07/10/14
Virology Reviewer: Lisa K. Naeger, Ph.D.

NDA#: 205,834 **Serial #:** 000
Reviewer's Name(s): Lisa K. Naeger, Ph.D.

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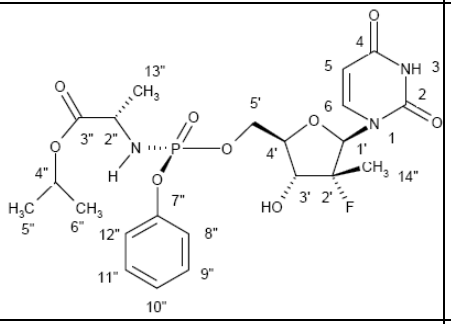
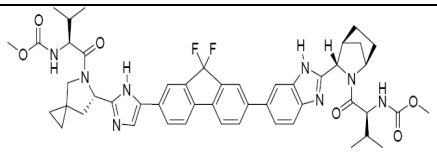
024	07/01/2014	07/01/2104	07/07/2104

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	529.46	889.00 Da

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA: 205834 **SDN**: 000 **DATE REVIEWED:** 07/10/14
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 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 α; LDV, ledipasvir; NGS, next generation sequencing; NRTIs, nucleoside reverse transcriptase inhibitors; PBL, peripheral blood lymphocytes; PDVF, protocol defined virologic failure; PEG, pegylated human interferon; PR, protease; P/R, pegylated interferon/ribavirin; RBV, ribavirin; SDM, site-directed mutants; SOF, sofosbuvir; SVR, sustained virologic response; SVR12, sustained virologic response at 12 week after end of treatment; WT, wild-type

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DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA: 205834 **SDN**: 000 **DATE REVIEWED:** 07/10/14
Virology Reviewer: Lisa K. Naeger, Ph.D.

EXECUTIVE SUMMARY

This NDA for fixed-dose combination (FDC) of ledipasvir (LDV) and sofosbuvir (SOF) is approvable with respect to virology for the treatment of HCV genotype 1. Sofosbuvir is a nucleotide prodrug of 2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate that is converted to the active uridine triphosphate form (GS-461203) within the hepatocyte. SOF had EC_{50} values ranging from 14-110 nM in stable full-length replicon cells of genotype 1a, 1b, 2a, 3a and 4a; and chimeric GT1b Con-1 replicons carrying NS5B coding sequences from genotypes 2b, 5a, or 6a. 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). In infectious virus assays, the EC_{50} values of sofosbuvir against genotype 1a and 2a were 30 and 20 nM, respectively.

Ledipasvir (LDV) inhibits HCV replication by interfering with the viral NS5A protein. The EC_{50} values of LDV against HCV genotypes 1a and 1b were 0.031 nM and 0.004 nM, respectively. In addition, LDV has EC_{50} values ranging from 0.15 to 530 nM against genotypes 2 to 6 replicons. LDV has an EC_{50} value of 21 nM against the GT2a JFH-1 replicon with L31 in NS5A, but has a reduced activity with an EC_{50} value of 249 nM against the GT2a J6 HCV strain with M31, a common resistance polymorphism. It has 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 has substantially lower activity against genotypes 3a and 6e with EC_{50} values of 168 nM and 264 nM, respectively.

Data from three phase 3 studies, Study 102 (ION-1; treatment-naïve subjects), Study 108 (ION-3; treatment-naïve non-cirrhotic subjects), and Study 109 (ION-2; treatment-experienced subjects), and two phase 2 studies Study 532 (ELECTRON) and Study 118 (LONESTAR) were submitted for virology resistance analyses. The FDA virology resistance analysis focused on NS5A amino acid positions K24, M28, Q30, L31, P32L, H58, A92 and Y93. Polymorphisms or mixtures at these NS5A positions were detected at baseline in the viruses from 23% (370/1615) of the subjects in the Phase 3 studies (ION-1, ION-2 and ION-3).

For the virology analyses, relapse rates were used as the measure of efficacy outcome for the three phase 3 studies and the two phase 2 studies. The overall relapse rate was 2.7% in all the studies submitted. In GT1a-infected subjects, the relapse rate was 3% (41/1378). In GT1b-infected subjects, the relapse rate was 1.7% (7/411). When the effect of individual baseline NS5A polymorphisms on relapse rates was examined, the highest relapse rates were seen in subjects whose viruses had baseline polymorphisms at positions Q30, L31, and Y93 where relapse rates were 6.6% (5/76), 10% (5/50), and 15% (8/54), respectively. Relapse rates for subjects whose viruses had one baseline NS5A resistance-associated polymorphism were 3.6%, but were higher for subjects whose viruses had 2 or 3 baseline NS5A resistance-associated polymorphisms with relapse rates of 9.5% and 9%, respectively.

The effect of baseline NS5A resistance-associated polymorphisms on relapse rates for each arm in each phase 3 study was analyzed. In treatment-naïve subjects (Study 108,

ION-3), overall relapse rates were 5.1% (11/215), 4.2% (9/216) and 1.4% (3/214) in the LDV/SOF 8-week arm, LDV/SOF+RBV 8-week arm, and LDV/SOF 12-week arm, respectively. Relapse rates for treatment-naïve subjects whose viruses lacked baseline NS5A resistance polymorphisms were similar to the overall response rates in each treatment arm. However, relapse rates for treatment-naïve subjects whose viruses had baseline NS5A resistance polymorphisms were 6.3% (3/48) for the LDV/SOF 8-week arm, 8% (4/49) for the LDV/SOF+RBV 8-week arm, and 0% (0/56) for the LDV/SOF 12-week arm. Therefore, for subjects whose viruses had NS5A polymorphisms at baseline, relapse rates were reduced in the longer 12-week treatment duration arm.

Overall, relapse rates were not higher for treatment-experienced subjects (2.5%; 11/440) compared to treatment-naïve subjects (3.6%; 23/645) with the caveat that different durations were studied in each of these subject groups. The number of baseline NS5A resistance polymorphisms was also similar in treatment-naïve and treatment-experienced subjects with 24% (153/645) of treatment-naïve subjects and 19% (83/440) of treatment-experienced subjects having viruses with baseline NS5A polymorphisms. However, relapse rates for treatment-experienced subjects whose viruses had baseline NS5A resistance polymorphisms were slightly higher at 7.2% (6/83) compared to 4.6% (7/153) for treatment-naïve subjects whose viruses had baseline NS5A resistance polymorphisms.

In treatment-experienced subjects (Study 109, ION 2), the effect of baseline NS5A resistance polymorphisms on relapse rates in the 12- and 24- week duration arms was examined. For subjects whose viruses lacked baseline NS5A polymorphisms, relapse rates were low; 2.3% (2/86) and 3.4% (3/89) for LDV/SOF 12-week arm and LDV/SOF +RBV 12-week arm and 0% (0/90) for the LDV/SOF 24-week arm. For treatment-experienced subjects whose viruses had baseline NS5A polymorphisms, relapse rates remained 0% (0/19) for the LDV/SOF 24-week arm, but were 22% (5/23) for the LDV/SOF 12-week arm and 4.5% (1/22) for the LDV/SOF +RBV 12-week arm. Adding RBV to a 12-week LDV/SOF regimen appeared to contribute to a decrease in relapse rates for this subset of subjects. Furthermore, the longer treatment duration of 24-weeks for subjects whose viruses had baseline NS5A polymorphisms reduced relapse rates to 0%.

Relapse rates were similar between treatment-experienced subjects overall in Study 109 and the NS3/4A inhibitor-experienced subjects in this study with a 2.5% (11/440) rate in Study 109 subjects compared to a 3% (6/203) rate in NS3/4A inhibitor-experienced subjects. Similarly, for subjects whose viruses had baseline NS5A polymorphisms, relapse rates were 7.2% (6/83) for Study 109 overall compared to 8% (3/37) for the subgroup of NS3/4A inhibitor-experienced subjects. Therefore, from the analysis of Study 109, relapse rates were not higher in NS3/4A inhibitor-experienced subjects.

Relapse rates were analyzed in treatment-experienced subjects with multiple baseline factors (i.e., baseline NS5A resistance polymorphisms, IL28B non-CC, high baseline viral load and cirrhosis). Although the subgroups were small, the trends show that in treatment-experienced subjects with multiple baseline factors such as NS5A resistance polymorphisms, IL28B non-CC genotype and/or cirrhosis, the longer 24-week duration of LDV/SOF has relapse rates of 0% compared to higher relapse rates (22-33%) for the 12-week duration arms.

There were 50 virologic failures (GT1a n=42; GT1b =8) in total from all the studies submitted. One GT1a-infected subject in Study 109 and one GT1b-infected subject in Study 102 experienced breakthrough due to documented non-compliance. All remaining virologic failures were relapsers: 41 GT1a and 7 GT1b-infected subjects. Most of the relapsers were non-CC and 24% of the GT1a relapsers and 29% of the GT1b relapsers were cirrhotic. In addition, 40% of the relapsers had viruses with baseline NS5A resistance polymorphisms. Many of these subjects whose viruses had baseline NS5A polymorphisms had additional NS5A substitutions emerge at failure (40%, 7/20) and 5 subjects (25%, 5/20) had substitutions emerge from mixtures at baseline. The percentage of subjects who had emergent NS5A substitutions without already existing baseline NS5A polymorphisms was 60% (18/30).

Overall, 55% (23/42) of the GT1a failures and 88% (7/8) of the GT1b failures had viruses with emergent NS5A resistance substitutions at amino acid positions K24, M28, Q30, L31, H58, A92 and Y93. In the phase 3 studies, 62% (23/37) of the failures had viruses with emergent NS5A resistance substitutions. A higher proportion of the failure subjects whose viruses had treatment-emergent NS5A substitutions were treatment-experienced subjects compared to treatment-naïve subjects (75% vs. 56%).

In GT1a failure subjects, the most common emergent NS5A substitutions were substitutions at Q30 and Y93. These substitutions each emerged in 43% (10/23) of the failures. L31M substitutions and substitutions at M28 emerged in 22% (5/23) and 17% (4/23) of failures, respectively. Other substitutions at K24 and H58 emerged in less than 10% of failures. In GT1b failure subjects, 43% (3/7) had viruses with emergent L31 substitutions and 86% (6/7) had emergent Y93H substitutions.

In total from the phase 3 studies, NS5A resistance-associated substitutions were observed in post-baseline isolates from 81% (30/37) of the virologic failure subjects. In phenotypic analyses, post-baseline isolates from subjects whose viruses harbored NS5A resistance-associated substitutions at failure showed 20- to >243-fold reduced susceptibility to LDV.

Retreatment of relapse subjects with NS5A substitutions looks promising based on very limited data reviewed to date. Twenty subjects who failed on SOF-containing regimens in Phase 2 studies, LONESTAR and ELECTRON, were retreated with LDV/SOF+RBV for either 12 (n=19) or 24 weeks (n=1). All 20 achieved SVR12 upon retreatment. However, only 4 of these subjects had viruses with NS5A resistance-associated substitutions before retreatment. Three subjects' viruses had only one NS5A resistance substitution each (Q30R, M28T or L31M) and received 12-week LDV/SOF + RBV. The fourth subject's virus had three NS5A substitutions (Q30L, L31M, and Y93H) in addition to NS5B substitutions, S282T, L320I and L320V and received 24-weeks LDV/SOF + RBV. While retreatment of relapse subjects who have NS5A substitutions looks possible, it is unclear based on the limited data available at this time what is the appropriate duration for retreatment, whether RBV is necessary, and whether the number or certain types of NS5A substitutions will affect successful retreatment.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA: 205834 **SDN**: 000 **DATE REVIEWED:** 07/10/14
Virology Reviewer: Lisa K. Naeger, Ph.D.

1. RECOMMENDATIONS

1.1. Recommendation and Conclusion on Approvability

This NDA for the fixed-dose combination of ledipasvir (LDV) and sofosbuvir (SOF) is approvable with respect to virology for the treatment of GT1 HCV infection. We recommend the 12-week duration of LDV/SOF for treatment-naives and treatment-experienced subjects without cirrhosis. For subjects with cirrhosis, we recommend the 24-week duration of LDV/SOF. In addition, we recommend 24-week duration of LDV/SOF for treatment-experienced subjects with multiple poor predictors of response at baseline including NS5A polymorphisms associated with resistance to NS5A inhibitors, IL28B non-CC genotype, high baseline viral load, and cirrhosis.

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

Please submit longitudinal data on persistence of NS5A resistance substitutions from subjects who did not achieve SVR12 in the Phase 2 and 3 LDV/SOF studies in Sequence Registry Study GS-US-248-0122 and from subjects who did not achieve SVR12 in Phase 2 studies of LDV with other DAAs.

2. SUMMARY OF OND VIROLOGY ASSESSMENTS

2.1 Non-Clinical Virology

Sofosbuvir is a nucleotide prodrug of 2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate that is converted to the active uridine triphosphate form (GS-461203) within the hepatocyte. SOF had EC_{50} values ranging from 14-110 nM in stable full-length replicon cells of genotype 1a, 1b, 2a, 3a and 4a; and chimeric GT1b Con-1 replicons carrying NS5B coding sequences from genotypes 2b, 5a, or 6a. 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). In infectious virus assays, the EC_{50} values of sofosbuvir against genotype 1a and 2a 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 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, but had a 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. 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 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 LDV, no antiviral antagonism was observed with the NS3 protease inhibitors, the NS5A inhibitor daclatasvir, IFN-α, RBV, nucleotide polymerase inhibitor R7128, non-nucleotide 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

The FDA virology resistance analysis focused on NS5A amino acid positions K24, M28, Q30, L31, P32L, H58, A92 and Y93. Polymorphic substitutions including mixtures at these NS5A positions were detected at baseline in 23% (370/1615) of the subjects in the Phase 3 studies (ION-1, ION-2 and ION-3). In the US GT1a population, it is estimated that variants at Y93 are present in 1 to 2% of subjects and the L31M, Q30H and Q30R variants (not mixtures) are present in 1.5 to 4% of subjects. Among 310 GT1b subjects in the US, the NS5A variants Y93H and L31M are estimated in 9% and 5% of the subjects, respectively.

Data from three phase 3 studies, Study 102 (ION-1; treatment-naïve subjects), Study 108 (ION-3; treatment-naïve non-cirrhotic subjects), and Study 109 (ION-2; treatment-experienced subjects), and two phase 2 studies Study 532 (ELECTRON) and Study 118

(LONESTAR) were submitted for virology resistance analyses. In these studies, 19% of the subject isolates had 1 baseline NS5A resistance-associated polymorphism, 3.5% had 2 baseline NS5A resistance-associated polymorphisms and 0.6% had 3 baseline NS5A resistance-associated polymorphisms. Relapse rates for subjects with one baseline NS5A resistance-associated polymorphisms were 3.6%, but were higher for subjects with 2 or 3 baseline NS5A resistance polymorphisms. Relapse rates were 9.5% and 9% for subjects with 2 and 3 baseline NS5A resistance polymorphisms, respectively. When the effect of individual baseline NS5A polymorphisms on relapse rates was examined, the highest relapse rates were seen in subjects with baseline polymorphisms at positions Q30, L31, and Y93 where relapse rates were 6.6% (5/76), 10% (5/50), and 15% (8/54), respectively.

Relapse rates were analyzed for the three phase 3 studies and the two phase 2 studies. The overall relapse rate was 2.7% in all the studies submitted. In GT1a subjects, the relapse rate was 3% (41/1378). In GT1b subjects, the relapse rate was 1.7% (7/411).

The effect of baseline NS5A resistance-associated polymorphisms on relapse rates for each arm in each phase 3 study was analyzed. In treatment-naïve subjects (Study 108, ION-3), overall relapse rates were 5.1% (11/215), 4.2% (9/216) and 1.4% (3/214) in LDV/SOF 8-week arm, LDV/SOF+RBV 8-week arm, and LDV/SOF 12-week arm, respectively. Relapse rates for treatment-naïve subjects without baseline NS5A resistance polymorphisms were similar to the overall response rates in each treatment arm (4.8%, 3%, and 1.9%). Relapse rates for treatment-naïve subjects with baseline NS5A resistance polymorphisms were 6.3% (3/48) for the LDV/SOF 8-week arm, 8% (4/49) for the LDV/SOF+RBV 8-week arm, and 0% (0/56) for the LDV/SOF 12-week arm. Therefore, for subjects with NS5A substitutions at baseline, relapse rates were reduced in the longer 12-week treatment duration arm.

Overall, relapse rates were not higher for treatment-experienced subjects (2.5%; 11/440) compared to treatment-naïve subjects (3.6%; 23/645) with the caveat that different durations were studied in each of these subject groups. The number of baseline NS5A resistance polymorphisms was also similar in treatment-naïve and treatment-experienced subjects with 24% (153/645) of treatment-naïve subjects and 19% (83/440) of treatment-experienced subjects having baseline NS5A polymorphisms. However, relapse rates for treatment-experienced subjects with baseline NS5A resistance polymorphisms were slightly higher at 7.2% (6/83) compared to 4.6% (7/153) for treatment-naïve subjects with baseline NS5A resistance polymorphisms.

In treatment-experienced subjects (Study 109, ION 2), the effect of baseline NS5A resistance polymorphisms on relapse rates in the 12- and 24- week duration arms was examined. For subjects without baseline NS5A polymorphisms, relapse rates were low; 2.3% (2/86) and 3.4% (3/89) for LDV/SOF 12-week arm and LDV/SOF+RBV 12-week arm and 0% (0/90) for the LDV/SOF 24-week arm. For treatment-experienced subjects with baseline NS5A polymorphisms, relapse rates remained 0% (0/19) for the LDV/SOF 24-week arm, but were 22% (5/23) for the LDV/SOF 12-week arm and 4.5% (1/22) for the LDV/SOF+RBV 12-week arm. Adding RBV to a 12-week LDV/SOF regimen appeared to contribute to a decrease in relapse rates for this subset of subjects. Furthermore, the longer treatment duration of 24-weeks for subjects with baseline NS5A polymorphisms reduced relapse rates to 0%.

Relapse rates were similar between treatment-experienced subjects overall in Study 109 and the NS3/4A inhibitor-experienced subjects in this study with a 2.5% (11/440) rate in Study 109 subjects compared to a 3% (6/203) rate in NS3/4A inhibitor-experienced subjects. Similarly, for subjects with baseline NS5A polymorphisms, relapse rates were 7.2% (6/83) for Study 109 overall compared to 8% (3/37) for the subgroup of NS3/4A inhibitor-experienced subjects. Therefore, from the analysis of Study 109, relapse rates were not higher in NS3/4A inhibitor-experienced subjects.

The effect of multiple baseline factors (i.e., baseline NS5A resistance-associated polymorphisms, IL28B non-CC and cirrhosis) on relapse rates in treatment-experienced subjects was examined. Although the subgroups were small, the trends show that in treatment-experienced subjects with multiple baseline factors such as NS5A resistance polymorphisms, IL28B non-CC genotype, high baseline viral load and/or cirrhosis, the longer 24-week duration of LDV/SOF has relapse rates of 0% compared to higher relapse rates (22-33%) for the 12-week duration arms.

In total from all the studies submitted, there were 50 virologic failures (GT1a n=42; GT1b =8). One GT1a-infected subject in Study 109 and one GT1b-infected subject in Study 102 experienced breakthrough due to documented non-compliance. All remaining virologic failures (n=48) were relapsers: 41 GT1a and 7 GT1b subjects. Most of the relapsers were non-CC (90% GT1a, 100% GT1b). Twenty-four percent of the GT1a-infected relapsers and 29% of the GT1b-infected relapsers were cirrhotic (cirrhotics were not studied in Study 108). In addition, 40% (19/48) of the relapsers (39% of the GT1a relapsers and 43% of the GT1b relapsers) had baseline NS5A resistance polymorphisms.

The emergent NS5A substitutions included substitutions at NS5A amino acid positions K24, M28, Q30, L31, H58, A92 and Y93. Overall, 55% (23/42) of the GT1a-infected failures and 88% (7/8) of the GT1b-infected failures had emergent NS5A resistance substitutions. In the phase 3 studies, 62% (23/37) of the failures had emergent NS5A resistance substitutions. A higher proportion of the failure subjects who had treatment-emergent NS5A substitutions were treatment-experienced subjects compared to treatment-naïve subjects (75% vs. 56%).

In GT1a-infected failure subjects, the most common emergent NS5A substitutions were substitutions at Q30 and Y93. These substitutions each emerged in 43% (10/23) of the failures. L31M substitutions and substitutions at M28 emerged in 22% (5/23) and 17% (4/23) of failures, respectively. Other substitutions at K24 and H58 emerged in less than 10% of failures. In GT1b-infected failure subjects, 43% (3/7) had emergent L31 substitutions and 86% (6/7) had emergent Y93H substitutions.

The proportion of failure subjects with baseline NS5A resistance-associated polymorphisms from all the studies was 40% (20/50) [13 subjects with 1 polymorphism, 6 subjects with 2 polymorphisms, and 1 subject with 3 polymorphisms]. Many of these subjects with baseline NS5A polymorphisms had additional NS5A substitutions emerge at failure (40%, 7/20) and 5 subjects (25%, 5/20) had substitutions emerge only from mixtures at baseline. The percentage of subjects who had emergent NS5A substitutions without already existing baseline NS5A polymorphisms was 60% (18/30).

In the phase 3 studies, the proportion of virologic failure subjects' isolates with baseline NS5A resistance polymorphisms was 41% (15/37). Twenty-seven percent (4/15) of these virologic failure subjects with baseline NS5A polymorphisms had additional NS5A resistance substitutions emerge at failure. The percentage of subjects without already existing baseline NS5A polymorphisms who had NS5A resistance-associated substitutions emerge at failure was 41% (15/37). In total, NS5A resistance-associated substitutions were observed in post-baseline isolates from 81% (30/37) of the virologic failure subjects; 38% (14/37) had 2 or more NS5A resistance-associated substitutions. In phenotypic analyses, post-baseline isolates from subjects who harbored NS5A resistance-associated substitutions at failure showed 20- to >243-fold reduced susceptibility to LDV.

Retreatment of relapse subjects with NS5A substitutions looks promising based on very limited data reviewed to date. Twenty GT1-infected subjects who failed on SOF-containing regimens in Phase 2 studies, LONESTAR and ELECTRON, were retreated with LDV/SOF LDV/SOF + RBV for either 12 (n=19) or 24 weeks (n=1). All 20 achieved SVR12 upon retreatment. However, only 4 of these subjects had NS5A resistance-associated substitutions before retreatment. Three subjects had only one NS5A resistance substitution each (Q30R, M28T or L31M) and received 12-week LDV/SOF LDV/SOF + RBV. The fourth subject had 3 NS5A substitutions (Q30L, L31M, and Y93H) in addition to NS5B substitutions, S282T, L320I and L320V and received 24-weeks LDV/SOF LDV/SOF+RBV. While retreatment of relapse subjects who have NS5A substitutions looks possible, it is unclear based on the data at this time what is the appropriate duration for retreatment, whether RBV is necessary, and whether the number or certain types of NS5A substitutions will affect successful retreatment.

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 **7/10/14**

4. VIROLOGY REVIEW

4.1 Important Milestones in Product Development

Sofosbuvir (SOF) was originally developed by Pharmasset as PSI-7977 until Pharmasset was bought by Gilead Sciences. SOF was approved by the FDA in December 2013 (NDA-204671).

We requested persistence data on NS5A substitutions that emerged on LDV treatment in Phase 2 studies. The sponsor responded that the majority of subjects who did not achieve SVR12 in the Phase 2 LDV/SOF studies (ELECTRON and LONESTAR) were successfully retreated with LDV/SOF-based therapy prior to any analysis of longitudinal NS5A sequence data. The subjects from the Phase 3 program who are not retreated in GS-US-337-1118 will be followed in the Sequence Registry (GS-US-248-0122). Longitudinal analyses of NS5A substitutions from these subjects will be provided when they are available. The analysis of samples from subjects in the Sequence Registry who did not achieve SVR12 in Phase 2 studies of LDV with DAAs other than SOF is ongoing. These data will also be provided to FDA when they are available.

4.2 Methodology

HCV Viral Load Assay
In Phase 2 Study P7977-0523, the Roche COBAS® AmpliPrep/COBAS® HCV TaqMan® assay (research use only version) was used to determine HCV RNA results. The established LOD of this assay was 15 HCV IU/mL (defined by a 95% hit rate with World Health Organization standards). In the Studies GS-US-337-0118, GS-US-337-0122, GS-US-337-0102, GS-US-337-0109, and GS-US-337-0108, the COBAS® TaqMan® HCV Test v2.0 for use with the High Pure System assay was used to quantify HCV RNA in this study. The LLOQ of the assay was 25 IU/mL.

RESISTANCE ANALYSIS
Population and/or deep sequencing of NS5A was performed at baseline for all subjects enrolled in the phase 2 and 3 studies as shown in Table 1. NS5B and NS3 deep sequencing was performed at baseline for a subset of subjects enrolled in Phase 2/3 studies. Resistance testing was performed for subjects who met the criteria of the resistance analysis population. The resistance analysis population includes any subject who received at least one dose of a LDV/SOF-containing regimen, but did not achieve SVR12 due to virologic failure or early discontinuation, had HCV RNA ≥1,000 IU/mL and had a plasma sample available for analysis. Resistance analyses included NS5A and NS5B deep sequencing that was performed at failure, and phenotypic analysis that was attempted for the majority of virologic failure subjects and was successful for a subset of subjects

Table 1. Summary of Genotypic Analyses for Baseline Samples from LDV/SOF Phase 2 and 3 Studies

Study	NS3/4A	NS5A	NS5B
GS-US-337-0118 LONESTAR PI-naïve or PI-experienced	Deep sequencing for all subjects[a]	Deep sequencing for all subjects[a]	Population sequencing for all subjects
P7977-0523 ELECTRON	N/A	Deep or population sequencing for all subjects who received LDV[b]	Deep or population sequencing for all subjects[b]
GS-US-337-0102 ION-1	N/A	Deep or population sequencing for all subjects[b]	Deep sequencing for a subset of subjects in Part B[b]
GS-US-337-0109 ION-2 PI-naïve or PI-experienced	Deep sequencing for all subjects	Deep sequencing for all subjects	Deep sequencing for all subjects
GS-US-337-0108 ION-3	N/A	Deep sequencing for all subjects	Deep sequencing for all subjects

a Population sequencing data also available for these subjects
b Deep sequencing data available for later enrolling subjects in these studies
PI: protease inhibitor, N/A: not applicable

Virologic failure is defined as follows:

- **On-treatment virologic failure**
  Breakthrough: HCV RNA ≥ LLOQ after having previously had HCV RNA <LLOQ, while on-treatment, confirmed with 2 consecutive values (note, second confirmation value could be post-treatment), or last available on-treatment measurement with no subsequent follow-up values
  Rebound: >1 $\log_{10}$ IU/mL increase in HCV RNA from nadir while on treatment, confirmed with 2 consecutive values (note, second confirmation value could be post-treatment), or last available on-treatment measurement with no subsequent follow-up values
  Non-responsive: HCV RNA persistently ≥LLOQ through 8 weeks of treatment
- **Relapse**: HCV RNA ≥LLOQ during the post-treatment period having achieved HCV RNA <LLOQ at end-of-treatment, confirmed with 2 consecutive values or last available post-treatment measurement

Population Nucleotide Sequencing

Baseline population sequencing of the full-length HCV NS5A or NS5B coding region was performed (b) (4) using RT-PCR and standard Sanger population sequencing. The obtained sequence was also used to confirm the results of genotyping/ subtyping by the INNO-LiPA assay performed at screening. For post-baseline resistance analyses of subjects with virologic failure, the HCV NS5A or NS5B gene was amplified from subject samples if the serum/plasma level of HCV RNA was ≥1,000 IU/mL. Population sequencing results were reported as change from reference in the data sets. In addition to the reference, the conservation and common variants for each genotype are also reported in alphabetical order for each amino acid position. For genotype 1, the conservation and the common variants were determined based on sequencing results from DAA-untreated subjects enrolled in Gilead's HCV studies. For genotype 4, conservation and common variants were determined based on Gilead's data as well as sequences obtained from the European Hepatitis C Virus Database.

Deep Nucleotide Sequence Analysis (NGS)

For most baseline and all virologic failures, NS5A, NS5B, or NS3 PCR amplicons at baseline and post-baseline timepoints were subjected to deep sequencing using the Illumina MiSeq deep sequencing platform. For baseline samples where population sequencing only or both population and deep sequencing data were available, analyses using the population sequence were reported. For baseline samples that only had deep sequencing data, consensus sequences were generated and used for analysis. In cases where multiple deep sequencing runs were performed for a given sample, the data from multiple runs were combined to create the consensus sequence. Consensus sequences of post-baseline samples were generated with inclusion of mixed amino acid calls present between 15 and 85%. In cases where the baseline amino acid differed from reference and the post-baseline substitution was a change to the reference amino acid, this was still considered as a post-baseline substitution. Deep sequencing results were reported for potential LDV, SOF, and RBV resistance-associated variant positions from subjects in the resistance analysis population with an assay cut-off at 1% and for all changes observed in >1 subjects in the resistance analysis population. The development of predominant substitutions was analyzed by study and per treatment arm across multiple studies.

Phenotypic Analysis

Phenotypic analyses of samples from subjects with virologic failure using a chimeric replicon encoding the NS5A or NS5B region derived from subject plasma/serum were performed by Gilead Sciences, Inc. (b) (4)

Baseline Sequence Data Analysis

Baseline sequences were analyzed for the presence of previously identified NS5A (Table 2; Study Report PC-337-2005, page 19), NS5B (nucleotide inhibitor, non-nucleotide inhibitor or RBV) (Table 3; Study Report PC-337-2005, page 20) and NS3 resistance-associated polymorphisms/substitutions (Table 4; Study Report PC-337-2005, page 20).

Table 2. List of Specific Resistance-Associated NS5A Polymorphisms/ Substitutions to Be Analyzed

Genotypes 1a and 1b	L31 I/F/M/V, P32L, A92K, Y93 C/F/H/N/S
Genotype 1a only	K24G/N/R, M28A/G/T, Q30E/G/H/K/L/R/T, S38F, H58D, A92T
Genotype 1b only	P58D

Table 3. List of Specific Resistance-Associated or Treatment-Emergent NS5B Substitutions to Be Analyzed

Nucleoside/Nucleotide Inhibitor	
Genotypes 1a and 1b	S96T, N142T, L159T, S282T, M289L, L320F, V321A
Nonnucleoside Inhibitor	
Genotypes 1a and 1b	C316N/Y, M414I/V/T, L419M/S, R422K, M423A/I/T/B, C445F, Y448H, Y452H, I482L, A486I/T, V494A, P495A/T, P496S, G554S, S556G, D559G
Genotype 1b only	V499A
Ribavirin-Associated	
Genotypes 1a and 1b	T390I
Genotype 1a only	F415Y

Table 4. List of Specific Resistance-Associated or Treatment-Emergent NS3 Substitutions to Be Analyzed

Genotypes 1a and 1b	V36 A/G/M/L/M, F43S, T54 A/C/G/S, V55 A/I, Q80 K/R/L, S122R, R155 C/G/K/M/T/Q/S, A156 F/G/N/T/V/S, D168 A/E/F/G/H/I/N/K/L/P/V/T/Y
Genotype 1a only	I170A/L/T
Genotype 1b only	V170A/L/T, M175L

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 IND115268 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 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, positive-sense RNA genome which encodes both structural proteins necessary for virus particle formation and nonstructural proteins

necessary for replication. Viral RNA encodes a single, long open reading frame producing a polyprotein. Structural proteins are cleaved by cellular proteases and the nonstructural proteins are cleaved by the viral encoded NS2 and NS3/4A proteases.

There are 6 major HCV genotypes with different geographic distributions. Genotypes 1a and 1b are most common in the 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). Therefore, DAAs have being studied in chronic HCV patients in combination with pegylated recombinant human IFN-α plus RBV (P/R), ribavirin (RBV) or other DAAs to reduce the selection of resistant variants, improve SVR rates and potentially shorten treatment duration. Boceprevir and simeprevir are currently marketed DAAs targeting the NS3/4A protease. Sofosbuvir (SOF) is a currently marketed uridine nucleoside polymerase inhibitor of NS5B, which was studied with RBV for 12-, 16- and 24-weeks for the treatment of GT2 and GT3 and with P/R for 12-weeks for the treatment of GT1. SOF was approved by the FDA in December 2013.

The current standard-of-care for adults with GT1 chronic hepatitis C (CHC) virus infection is treatment with SOF in combination with P/R for 12-weeks with overall SVR12 rates of 89%. The 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 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 study (COSMOS), overall SVR12 rates for this 12-week combination were 93% and 96% in patients with METAVIR scores F0-F2 and 93% in patients with GT1 HCV and advanced liver fibrosis (METAVIR scores F3 and F4).

Currently, the standard-of-care for treatment of GT4 CHC infection is SOF+P/R for 12-weeks. Because of the small number of subjects with these genotypes in the phase 3 studies, SOF is not indicated for subjects with GT5 or GT6 infection.

The current standard-of-care for adults with GT2 and GT3 CHC virus infection is 12 and 24 weeks of SOF+RBV, respectively. The SVR12 response rates for the 12-week SOF+RBV regimen in GT2-infected subjects are 93 - 95%. In GT3-infected subjects, response rates range from 60% in treatment-experienced cirrhotics to 93% in non-cirrhotic treatment-naïve subjects.

Thus, standard-of-care treatment for GT1-infected subjects, which is the predominant population of CHC infection in the US, still contains pegylated interferon-α. There are limitations in P/R treatment for some groups of patients. Patients with high viral loads, cirrhosis, homozygous or heterozygous "T" allele in the polymorphic IL28B gene, and African-Americans have lower rates of SVR with P/R treatment. In addition, P/R treatment is not tolerable in all chronic HCV infected patients, because of the significant adverse events associated with interferon and ribavirin. Furthermore, subjects who have previously failed to respond to P/R therapy have poorer treatment response to retreatment. Therefore, there is an unmet need for safe and effective treatment options without P/R in certain HCV patient populations, including GT1-infected subjects, and shorter easier treatment options without drug interactions for most subjects with chronic HCV infection.

4.5 NON-CLINICAL VIROLOGY

Sofosbuvir

Sofosbuvir is an HCV NS5B polymerase uridine nucleotide polymerase inhibitor that inhibits HCV RNA replication in cell culture. In human hepatocytes, SOF is converted to an active uridine triphosphate form (GS-461203) that has been shown to directly inhibit NS5B polymerase activity in a biochemical assay, at IC_{50} values ranging from 0.7 to 2.6 µM. Sofosbuvir demonstrates activity against stable genotypes 1a, 1b, 2a, 2b, 3a, 4a, 5a, and 6a HCV replicons at EC_{50} values of 0.014 to 0.11 µM. In addition, SOF is also active against genotype 1a and 2a HCV cell culture virus (EC_{50} = 0.03 and 0.02 µM in genotype 1a and 2a viral systems, respectively).

Please see the NDA-204671 virology review for complete review of SOF non-clinical virology.

Ledipasvir

MECHANISM OF ACTION

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. Resistance selection studies in cell culture, 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.

Table 5. Biochemical Activity of LDV against HCV Recombinant Enzymes and the HCV IRES

Compound (Target)	IC_{50} (nM)[a, b, c]			
	NS3/4A Protease	NS5B Polymerase	NS3 Helicase	HCV IRES
GS-5885 (NS5A)	>20,000	> 2700	> 11,000	> 100,000
BILN-2061 (NS3 protease)	1.2 ± 0.5	> 2700	> 100,000	81,380 ± 8739
GS-430319 (NS5B pol., site 2)	N.T.	62 ± 16	N.T.	N.T.
GS-441103 (NS3 helicase)	N.T.	N.T.	1430 ± 8	N.T.
IIId Oligo (HCV IRES)	N.T.	N.T.	N.T.	393 ± 37

a Values represent the arithmetic mean ± standard deviation of two or more independent experiments.
b All IC_{50} values preceded with ">" sign represent the highest soluble concentration of the compound observed in the assay.
c N.T. indicates "not tested".

LDV (GS-5885) was tested for its ability to inhibit recombinant HCV enzymes and the HCV IRES in biochemical assays (Table 5; Study Report PC-256-2019, page 11). No significant inhibition of the NS3/4A protease, NS3 helicase, NS5B polymerase or HCV IRES activities was observed with GS-5885. All control compounds inhibited these targets at concentrations consistent with historical results, including the NS3 protease

inhibitor BILN-2061, the (b) (4) thiophene NS5B polymerase inhibitor (b) (4) (GS-430319), the Gilead NS3 helicase inhibitor GS-441103, and the DNA oligonucleotide IRES inhibitor IIId oligo. These results showing that LDV does not target other HCV proteins are compatible with LDV inhibiting HCV through a mechanism involving NS5A.

ANTIVIRAL ACTIVITY IN CELL CULTURE

Replicons

The antiviral activity of LDV (GS-5885) was tested in multiple HCV replicon cell lines. Cytotoxicity was tested at the same time in these assays (see below). In standard HCV replicon antiviral assays in GT1a HRlucP (strain H-77) replicon cells and GT1b Rluc-2 (strain Con-1), which are both clones of Huh-7 replicons, LDV had an EC_{50} value of 0.053 nM against GT1a replicons and 0.004 nM against GT1b.

The antiviral activity of LDV was tested in additional HCV replicon cell lines including:

- HSG-51 and HSG-57 (independent Huh-7 clonal cell lines replicating GT1a (H-77 strain) replicon without a reporter gene)
- 1a-HGluc-1 and HGluc-2 (independent Huh-7 clonal cell lines replicating GT1a (H-77 strain) replicon encoding the Gaussia luciferase reporter gene)
- 1a-Sf9Rluc-1 and Sf9Rluc-2 (independent Huh-7 clonal cell lines replicating GT1a (SF-9 strain) replicon encoding the Renilla luciferase reporter gene)
- Huh-luc and GFP1b-7 (Huh-7 derived cell lines replicating a GT1b (Con-1 strain) replicon encoding firefly luciferase and GFP reporter genes, respectively)
- SL3 (HeLa (cervical carcinoma) cell line replicating a GT1b (Con-1 strain) replicon)
- 2aLucNeo-25 (Huh-7 derived cell line replicating a GT2a (JFH-1 strain) replicon

All these replicon antiviral assays were performed in 96-well format. Overall, in the seven GT1a replicon cells tested, EC_{50} values ranged from 0.010 to 0.020 nM with a median EC_{50} value of 0.012 nM. In the four individual GT1b cell lines tested, EC_{50} values ranged from 0.003 to 0.027 nM with median EC_{50} values of 0.003 nM. An increase in EC_{50} value of 11 nM was observed in the GT2a replicon cell line 2aLucNeo-25 (3600-fold increase in EC_{50} value compared to the median GT1b EC_{50} value).

In addition, the antiviral activity of LDV was determined using a panel of HCV replicons, representing the prevalent NS5A sequences for genotypes 2-6 in standard 3-day cell-based replicon assays. LDV had EC_{50} values ranging from 0.15 nM to 530 nM against genotype 2 to 6 (Table 6; Report PC-256-2037, page 12). 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, but had a reduced activity with an EC_{50} value of 249 nM against the GT2a J6 HCV strain, which has methionine at position 31 (M31), associated with resistance to LDV. Similarly, LDV had different antiviral activities against GT2b replicons depending on whether L31 or M31 was present in NS5A, with EC_{50} values of 16 nM for GT2b MD2b8-2 (L31 in NS5A) and 530 nM in GT2b MD2b-1 (M31 in NS5A). LDV has weaker 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 (Table 6; Study Report PC-256-2037, page

12). LDV has substantially lower activity against genotypes 3a and 6e with EC_{50} values of 168 nM and 264 nM, respectively.

Table 6. Antiviral Activity of LDV against Genotype 1-6 HCV

Genotype	HCV Replicon Strain	EC_{50} nM[a]
1a[b]	H77	0.031
1b[b]	Con1	0.004
2a[b,c]	JFH-1 (L31 in NS5A)	21
2a[d,f]	J6 (M31 in NS5A)	249
2b[d,f]	MD2b8-2 (L31 in NS5A)	16
2b[d,f]	MD2b-1 (M31 in NS5A)	530
3a[d,e]	S52	168
4a[b]	ED43	0.39
5a[d,f]	SA13	0.15
6a[b]	HK6a Consensus	1.1
6e[d,f]	D88	264

a Values represent the geometric mean from at least three independent experiments
b Stable subgenomic replicon cell lines assayed in HTS 384-well format
c Transiently transfected JFH-1 (L31 in NS5A) yielded an EC_{50} of 6.8 nM
d Transiently transfected replicons in 1C cells assayed in either 96-well (2a, 2b and 3a) or 384-well (5a and 6e) format
e Subgenomic Rluc-encoding replicon
f NS5A chimeric replicons either encoding full-length NS5A (2a and 2b) or NS5A amino acids 9-184 (5a and 6e)

The antiviral activity of LDV was also tested against an infectious GT2a HCV strain (J6/JFH-1) in Lunet-CD81 cells. In this system, LDV had an EC_{50} value of 3.2 nM, which is relatively similar to the EC_{50} value of 11 and 21 nM previously observed in GT2a subgenomic replicon cells. LDV has decreased antiviral activity against GT2a in cell culture compared to GT1a and GT1b.

The antiviral activity of LDV was tested in GT3a and GT4a NS5A chimeric replicons. In the transient replicon replication assay, the EC_{50} value of LDV against the GT3a NS5A chimeric replicon was 10.1 nM compared to 0.002 nM for GT1b (Table 7; Report PC-256-2021, page 9). In contrast, the EC_{50} value of the GT4a NS5A chimeric replicon was 0.045 nM (23-fold vs. GT1b).

Table 7. Antiviral Activity of LDV against Genotype 3a and 4a NS5A Chimeric Replicons in Transient Replicon Replication Assays

Compound	EC_{50} (nM)[a]		
	Genotype 1b	1b/ Genotype 3a NS5A	1b/ Genotype 4a NS5A
GS-5885	0.0020 ± 0.0002	10.1 ± 0.6	0.045 ± 0.008

Antiviral Activity against a Panel of Treatment-Naïve GT1 Clinical Isolates

Thirty-three baseline isolates from clinical studies were tested for activity to LDV. The entire population of the NS5A gene was amplified from individual subject plasma samples and cloned into the replicon-based shuttle vector. This pooled population of

molecules was utilized for transient replication assays. In total, NS5A from 33 subject serum samples that did not have a resistance-associated variant at baseline were tested for their sensitivity to LDV. Of the 33 samples tested, 30 were from GT1a-infected subjects and 3 were from GT1b-infected subjects. Mean EC_{50} values for these subjects were 0.022 nM and 0.006 nM against GT1a and GT1b replicons, respectively. Among these samples, the EC_{50} values ranged from 0.008 to 0.085 nM against GT1a isolates, and 0.004 nM to 0.007 nM against GT1b isolates.

Antiviral Activity against Other Viruses

The antiviral activity of LDV was tested against HCV, BVDV, RSV, HBV and HIV-1 in their respective cell-based assay systems. 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 (Table 8; Report PC-256-2018, page 10).

Table 8. Antiviral Activity and Cytotoxicity of LDV against HCV, BVDV, RSV, HBV and HIV-1

Virus	Description	Cell line	EC_{50} nM[a]	CC_{50} nM[a]
HCV Replicon (genotype 1b)	Hepacivirus (positive ssRNA)	Huh-7	0.0040 ± 0.0008	> 44,400
BVDV Replicon	Pestivirus (positive ssRNA)	Huh-7	19,268 ± 7343	> 50,000
RSV	Paramyovirus (negative ssRNA)	Hep-2	> 10,000	> 10,000
HBV	Hepadnavirus (DNA reverse-transcribing)	AD-38	> 10,000	> 10,000
HIV	Lentivirus (RNA reverse transcribing)	MT-4	> 2791	2791 ± 985

a Values represent the arithmetic mean ± standard deviation of two or more independent experiments.

Table 9. Antiviral Activity of Ledipasvir, Sofosbuvir, and GS-5816 against Various Viruses

	LDV	SOF	GS-5816
Virus	EC_{50}[a] (μM)	EC_{50}[a] (μM)	EC_{50}[a] (μM)
Human Rhinovirus[b,c]	>50	>50	>50
Influenza A PC/1/73	>100	>100	>100
Influenza B LEE/40	>100	88	>100
West Nile virus Kern 2002	>100	>100	>100
Yellow Fever virus 17D	>100	73	>100
Dengue type 2	>41	>82	>48
Banzi virus	>100	>100	>100

a All values based on two or more independent experiments.
b Infectious mixture of HRV1A, HRV16 and HRV14
c Top concentration tested 50 μM for HRV, 100 μM for all other viruses tested

The ability of ledipasvir to inhibit viruses other than Hepatitis C Virus (HCV) was investigated using cell-based assays. Ledipasvir along with sofosbuvir and Gilead compound GS-5816 were tested for antiviral activity 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. No antiviral activity was detected for LDV, SOF or GS-5816 against any of these viruses at the highest concentration tested or the highest concentration without cytotoxicity, except SOF had EC_{50} values of 88 μM and 73 μM for influenza B virus and Yellow Fever virus, respectively (Table 9; Report PC-256-2036, page 9).

CYTOTOXICITY

In the replicon assays, no cytotoxicity was observed in the GT1a HRlucP and GT1b Rluc-2 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.

The cytotoxicity of LDV was measured in multiple cell lines including 1b-Rluc-2, Huh-luc, 1a-HRlucP, HepG2 and SL3, after three and seven days of drug exposure. Among the different cell lines, the CC_{50} values ranged from 5910 to >50,000 nM at Day 3 and from 4029 to 27,959 nM at Day 7 (Table 10). The lowest CC_{50} value in the tested cell lines was 4029 nM observed in cell line HepG2, but had a selectivity index of 1,343,000 using the median GT1b EC_{50} value (0.003 nM). Cytotoxicity in MT-4 cells was also tested in a five-day assay and yielded a CC_{50} value of 2791 nM for GS-5885 (Study report PC-256-2018).

Table 10. Cytotoxicity of LDV after Three- and Seven-Day Exposure in Multiple Cell Lines (arithmetic mean CC_{50} value (nM))

	1b-Rluc-2	Huh-luc	1a-HRlucP	HepG2	SL-3
Day 3 Exposure	36,647	>50,000	16,168	5910	>50,000
Day 7 Exposure	19,754	27,959	6314	4029	nd

ANTIVIRAL ACTIVITY IN COMBINATION WITH OTHER ANTIVIRAL AGENTS

To examine the effects on antiviral activity of LDV and SOF in combination with the HCV NS3/4A protease inhibitors boceprevir, simeprevir, or telaprevir, or the NS5A inhibitor daclatasvir, GT1a replicon cells were incubated with serially-diluted LDV and SOF in combination with each of the four additional anti-HCV agents. The results were analyzed using MacSynergy II, which provides surface plots displaying significant deviations from additivity. Synergy and antagonism volumes ($\mu M^2\%$) are calculated from deviations from the additive surface. At the 95% confidence interval, the mean synergy and antagonism volumes were between 25 and – 25 μM^2 % for all combinations tested, indicative of additive interactions. In cell culture, the combination of SOF and LDV exhibited additive antiviral activity (Table 11 and Table 12; Report PC-256-2035, page 9). No antiviral antagonism was observed, and no significant change in cell viability was observed in combination studies of SOF and LDV.

Table 11. Antiviral Combination Assessments for Drug Combinations with LDV

Compound Used in Combination with LDV	Class	Synergy Volume (nM^2)	Antagonism Volume (nM^2)	Interaction
Boceprevir	NS3 Protease Inhibitor	2.3 ± 2.3	-19.6 ± 5.4	Additive
Simeprevir	NS3 Protease Inhibitor	3.7 ± 3.8	-11.5 ± 10.2	Additive
Telaprevir	NS3 Protease Inhibitor	0.7 ± 1.2	-7.9 ± 12.9	Additive
Daclatasvir	NS5A Inhibitor	4.3 ± 6.7	-11.5 ± 5.1	Additive

a No significant cytotoxicity observed for any of the concentrations tested

Table 12. Antiviral Combination Assessments for Drug Combinations with SOF

Compound Used in Combination with SOF	Class	Synergy Volume (nM^2)	Antagonism Volume (nM^2)	Interaction
Boceprevir	NS3 Protease Inhibitor	0.7 ± 0.6	-16.0 ± 9.4	Additive
Simeprevir	NS3 Protease Inhibitor	0.3 ± 0.6	-11.9 ± 8.2	Additive
Telaprevir	NS3 Protease Inhibitor	2.7 ± 2.5	-12.7 ± 6.7	Additive
Daclatasvir	NS5A Inhibitor	0.7 ± 1.2	-16.0 ± 9.4	Additive

a No significant cytotoxicity observed for any of the concentrations tested

Co-infection of persons with both HCV and HIV-1 is highly prevalent, with estimates as high as 30% of HIV-infected individuals also infected with HCV. Therefore, co-infected individuals may be treated with ledipasvir-containing regimens for the treatment of HCV infection. The effects of various classes of HIV inhibitors on ledipasvir antiviral activity, as well as ledipasvir's effect on the antiviral activity of those HIV-1 inhibitors was assessed in both GT1a HCV replicon systems, as well as HIV_{IIIB} cell culture infection systems.

Table 13. EC_{50} Values for LDV in Combination with HIV-1 Inhibitors in GT1a Replicon Cells

HIV Inhibitor Concentration	LDV EC_{50} (nM)[a]							
	EFV	EVG	TFV	DRV	FTC	ATV	RPV	RAL
15 µM	0.055 ± 0.029	0.046 ± 0.007	0.035 ± 0.002	0.085 ± 0.029	0.019 ± 0.002	0.007 ± 0.0004	0.009 ± 0.003	0.014 ± 0.005
1.5 µM	0.030 ± 0.008	0.04 ± 0.007	0.03 ± 0.007	0.046 ± 0.022	0.024 ± 0.007	0.015 ± 0.003	0.01 ± 0.001	0.019 ± 0.002
0.15 µM	0.032 ± 0.011	0.046 ± 0.018	0.029 ± 0.005	0.04 ± 0.013	0.02 ± 0.005	0.017 ± 0.001	0.015 ± 0.001	0.017 ± 0.003
0 µM	0.038 ± 0.013	0.09 ± 0.014	0.031 ± 0.007	0.04 ± 0.011	0.015 ± 0.002	0.017 ± 0.003	0.014 ± 0.004	0.018 ± 0.002

a No cytotoxicity observed for any of the concentrations tested

GT1a replicon cells were incubated with serially-diluted LDV in the presence of multiple concentrations of EFV (NNRTI), EVG (INSTI), TFV (NRTI), DRV (PI), FTC (NRTI), ATV (PI), RPV (NNRTI), and RAL (INSTI). Concentrations of 0.15 to 15 µM were utilized for all HIV-1 inhibitors tested to match or exceed the physiological levels observed in patients. Additionally, the anti-HCV activity of SOF was similarly tested. LDV and SOF EC_{50} values were similar regardless of the presence of any of the HIV-1 inhibitors at the concentrations tested. No cell toxicity was observed in these experiments. These data indicate that there is no antagonistic effect of these HIV-1 inhibitors on LDV (Table 13; Report PC-256-2034, page 9) or SOF (Table 14 Report PC-256-2034, page 10) activity in cell culture.

Table 14. EC_{50} Values for SOF in Combination with HIV-1 Inhibitors in GT1a Replicon Cells

HIV Inhibitor Concentration	SOF EC_{50} (nM)[a]							
	EFV	EVG	TFV	DRV	FTC	ATV	RPV	RAL
15 µM	26.862 ± 11.564	23.221 ± 2.835	23.766 ± 3.846	20.962 ± 0.949	18.799 ± 3.353	26.533 ± 4.122	23.674 ± 9.893	15.766 ± 5.921
1.5 µM	21.587 ± 3.484	22.886 ± 5.977	25.291 ± 3.647	18.955 ± 1.925	21.321 ± 2.639	19.863 ± 3.853	19.343 ± 2.731	14.253 ± 3.884
0.15 µM	19.983 ± 1.216	18.443 ± 5.201	19.542 ± 2.34	18.823 ± 4.659	18.566 ± 1.056	19.274 ± 1.838	18.912 ± 4.329	19.916 ± 2.274
0 µM	19.271 ± 1.717	30.05 ± 2.171	16.688 ± 5.878	26.248 ± 5.381	18.056 ± 2.425	18.034 ± 1.163	17.632 ± 3.654	15.725 ± 1.432

a No cytotoxicity observed for any of the concentrations tested

To examine the effects of LDV on the anti-HIV activity of the HIV-1 inhibitors, HIV-infected MT-4 cells were incubated with serially-diluted EFV, EVG, TFV, DRV, FTC, ATV, RPV, and RAL in the presence of multiple concentrations of LDV or SOF. Values corresponding to 1X, 5X and 20X EC_{50} value in GT1a replicon cells were utilized for each of the anti-HCV compounds tested. The EC_{50} values for all of the HIV-1 inhibitors tested were similar regardless of the presence of LDV (Table 15; Report PC-256-2034, page 10) or SOF (Table 16; Report PC-256-2034, page 11) at the concentrations tested. These data indicate that there is no antagonistic effect of LDV or SOF on the anti-HIV activity of any of the HIV inhibitors tested. No cell toxicity was observed in these MT-4 experiments.

Table 15. EC_{50} Values for HIV-1 Inhibitors in Combination with LDV

LDV (EC_{50})	EC_{50} (nM)[a]							
	EFV	EVG	TFV	DRV	FTC	ATV	RPV	RAL
20X	1.97 ± 0.05	3.27 ± 0.37	8137 ± 1142	7.35 ± 0.12	1262.9 ± 200.6	9.90 ± 0.82	0.13 ± 0.003	10.79 ± 0.69
5X	2.02 ± 0.16	3.42 ± 0.13	9819 ± 1490	6.79 ± 0.56	1173.9 ± 107.0	9.57 ± 0.47	0.14 ± 0.02	8.88 ± 1.38
1X	1.92 ± 0.12	3.11 ± 0.07	10220 ± 636	7.32 ± 0.28	1186.6 ± 169.2	10.30 ± 0.40	0.12 ± 0.007	9.45 ± 1.30
None	1.21 ± 0.15	1.70 ± 0.15	7246 ± 292	6.31 ± 0.23	614.7 ± 189.6	8.18 ± 0.16	0.07 ± 0.012	7.26 ± 1.88

a No cytotoxicity observed for any of the concentrations tested

Table 16. EC_{50} Values for HIV-1 Inhibitors in Combination with SOF

SOF (EC_{50})	EC_{50} (nM)[a]							
	EFV	EVG	TFV	DRV	FTC	ATV	RPV	RAL
20X	1.78 ± 0.23	2.54 ± 0.10	8026 ± 436	6.24 ± 0.47	785.6 ± 39.0	9.54 ± 1.75	0.11 ± 0.018	8.30 ± 0.53
5X	1.93 ± 0.10	2.85 ± 0.54	9428 ± 444	6.18 ± 0.53	1075.9 ± 98.8	8.77 ± 0.41	0.12 ± 0.02	8.52 ± 0.70
1X	1.96 ± 0.22	3.10 ± 0.37	9701 ± 1102	7.08 ± 0.49	1104.6 ± 57.9	9.48 ± 0.84	0.15 ± 0.015	9.39 ± 1.05
None	1.21 ± 0.15	1.70 ± 0.15	7246 ± 292	6.31 ± 0.23	614.7 ± 189.6	8.18 ± 0.16	0.07 ± 0.012	7.26 ± 1.88

a No cytotoxicity observed for any of the concentrations tested

In addition, new anti-HCV agents may be used clinically in combination with pegylated interferon-α and/or ribavirin or with other specific HCV inhibitors. The antiviral activity of LDV was investigated in the HCV GT1b replicon system when combined with IFN-α, RBV and other HCV inhibitors (NS3/4A protease inhibitors GS-9256 and GS-9451, nucleoside (R7128) and non-nucleoside (GS-9190) NS5B polymerase inhibitors). The resulting data from two independent experiments were analyzed using MacSynergy II, which provides surface plots displaying significant deviations from additivity. Combinations of LDV with IFN-α or GS-9190 resulted in synergy volumes of 32 and 34 nM^2, respectively, indicating minor synergy (Table 17; Report PC-256-2015, page 9). Ribavirin, GS-9256 and R7128 yielded synergy volumes of 61, 52 and 51 nM^2% when combined with LDV, respectively, indicating a moderate synergistic interaction between LDV and these three HCV inhibitors. The combination of LDV with GS-9451 resulted in a synergy volume of 132 nM^2% signifying a strongly synergistic antiviral interaction. None of the compounds yielded antiviral antagonism volumes outside of the additive range (> -25 nM) when combined with LDV. No cellular toxicity was observed at the highest drug concentrations tested for all combinations. Therefore, LDV did not show antagonism with multiple classes of HCV inhibitors in cell culture.

Table 17. Antiviral Combination Activity for Drug Combinations with LDV

Drug(s) Used in Combination with GS-5885	Synergy Volume (nM^2)[a]	Antagonism Volume (nM^2)[a]	Interaction
IFN-α	32 ± 1.4	0.0 ± 0.0	Minor Synergy
RBV	61 ± 0.5	–0.5 ± 0.1	Moderate Synergy
GS-9190	34 ± 9.9	–17 ± 0.7	Minor Synergy
GS-9256	52 ± 5.1	–0.7 ± 0.7	Moderate Synergy
GS-9451	132 ± 44	–0.1 ± 0.2	Strong Synergy
R7128	51 ± 7.8	–0.2 ± 0.1	Moderate Synergy

a Values represent the arithmetic mean ± standard deviation of two independent experiments performed in triplicate.

CELL CULTURE RESISTANCE

GT1a

Drug-resistant colonies were selected by a 3-week treatment of GT1a replicon cells with LDV (GS-5885). GT1a replicon cells were treated with 10, 20, or 40 nM of LDV for three weeks. Ten clones from the 40 nM selection and 6 clones from the 20 nM selection were analyzed for genotypic changes in the NS5A gene (Table 18; Report PC-256-2031, page 9). The colonies in the 10 nM selection were analyzed as a pool. A single substitution of glutamine to glutamic acid at position 30 (Q30E) was identified in 4/10 and 5/6 colonies of the 40 and 20 nM selections, respectively. A single substitution of tyrosine to histidine at amino acid 93 (Y93H) was identified in 6/10 and 1/6 clones isolated from 40 and 20 nM selections, respectively. Both Q30E and Y93H were identified in the pooled cells from the 10 nM selection. The sponsor states that substitutions at other residues were also observed, but that none of these were identified in more than one clone. These substitutions were not identified in the report.

Table 18. Amino Acid Substitutions Identified in the NS5A Genes of GT1a Replicons after Selection with LDV

GS-5885 Concentration	40 nM	20 nM	10 nM
# Clones Analyzed	10	6	pool
NS5A Mutations[a]	Q30E (n = 4)	Q30E (n = 5)	Q30E or Y93H
	Y93H (n = 6)	Y93H (n = 1)	

a Mutations detected only in a single colony are not listed

To examine the phenotypic susceptibility of colonies selected by LDV, colonies were expanded and tested in an EC_{50} assay using LDV (GS-5885) as well as the investigational NS3/4A inhibitor BILN-2061 as a control. The pooled selected cells from the 10 nM LDV selection were also tested. All expanded clones as well as the pool demonstrated significantly reduced susceptibilities to LDV ranging from 2,730-fold to 8,430-fold increases in EC_{50} values compared to a GT1a wild-type control. By comparison, no significant changes in EC_{50} values were observed using BILN-2061. All tested colonies as well as the pooled replicon cells demonstrated significantly reduced susceptibility to the investigational NS5A inhibitor, BMS-790052 (daclatasvir), indicating cross-resistance of these substitutions.

Lunet cells were transiently transfected with RNA from GT1a replicons encoding the Q30E or Y93H NS5A substitutions to ascertain the effect of these individual mutants on LDV susceptibility and explore potential cross-resistance to GS-7977 (SOF) (a nucleoside inhibitor), or ribavirin (RBV) (Table 19; Report PC-256-2031, page 11). The fold-change in EC_{50} values of LDV (GS-5885) for the Q30E and Y93H GT1a mutants were 997-fold and 3,029-fold, respectively. In contrast, no changes in EC_{50} values were observed for GS-7977 and RBV against the Q30E or Y93H mutant replicons.

Table 19. Fold-change in EC_{50} Values for GS-5885 (LDV), GS-7977 (SOF), and RBV against Q30E and Y93H Mutant GT1a Replicons

Compound	Fold Shift in EC_{50}[a,b]	
	Q30E	Y93H
GS-5885	997	3029
GS-7977	1.0	0.7
RBV	0.8	1.0

a Values represent the arithmetic mean of two or more independent experiments, and were generated in 96-well assay

b Fold resistance is calculated as the ratio of mutant EC_{50} to wild-type EC_{50}

In summary, genotypic analyses of resistant replicon RNA substitutions emerging in the NS5A gene identified Q30E and Y93H as the primary resistance substitutions selected. Engineering NS5A Q30E or Y93H substitutions individually into a GT1a replicon resulted in approximately 1,000- and 3,000-fold increases, respectively, in the EC_{50} values of LDV relative to wild-type 1a replicon, indicating reduced susceptibility to the drug. The mutant NS5A replicons exhibited cross-resistance to another investigational NS5A inhibitor (BMS-790052), but remained sensitive to the uridine nucleotide inhibitor GS-7977 (SOF) and ribavirin.

GT1b

Drug-resistant replicon colonies were selected by a 3-week treatment of GT1b replicon cells with LDV (GS-5885). Resistant colonies were selected following a 3 week treatment of the GT1b HCV replicon cell line 1b-Rluc-2 with 0.3125, 0.625, and 1.25 nM of LDV (75, 150 and 300 × EC_{50} value, respectively). Fifteen individual colonies were isolated, expanded, and confirmed to have reduced LDV susceptibility. The remaining colonies were pooled. All individual colonies and the pool colonies were analyzed for genotypic changes in the NS5A gene (Table 20; PC-256-2016, page 10).

Table 20. Amino Acid Substitutions Identified in the NS5A Gene after Selection with LDV

NA5A Mutations Identified	Note
Y93H	Observed in all resistant replicon clones (n= 15/15) and in the pool of resistant cells
Q54H, P299T/Q	Observed in two resistant replicon clones (n=2/15); each mutation was found in combination with Y93H
C446R, M2I[a]	Observed in a single resistant clone (n=1/15) but both located in the NS5A-NS5B junction; each mutation was found in combination with Y93H
F127L, T135N, R262Q, N276S, S297P, A300E, A393T, S401Y, D430N, S437R	Observed in a single resistant replicon clone only (n=1/15); each mutation was found in combination with Y93H

a Substitution M2I was a mutation identified in the very N-terminus of NS5B.

A single substitution of tyrosine to histidine at amino acid 93 (Y93H) was identified in all LDV-resistant clones and in the pool of resistant replicon cells. Substitutions at other

residues in combination with Y93H were also observed, including Q54H, F127L, T135N, R262Q, N276S, S297P, P299T/Q, A300E, A393T, S401Y, D430N, S437R, C446R in NS5A, or M2I in NS5B. However, all substitutions appeared only once, except changes at residues Q54 and P299, which were observed twice. The selection of LDV resistance within the NS5A gene provides evidence that NS5A is the target for this compound.

Individual substitutions were introduced into a wild-type replicon (PI-hRluc) by site-directed mutagenesis. Y93H, Q54H, P299T and P299Q were selected for these analyses, since substitutions at these residues were observed more than once. Although C446R and M2I appeared once only, they were phenotyped because both substitutions are located in the NS5A-5B junction and could potentially be related to resistance.

The Y93H substitution conferred 3,310-fold resistance to LDV with an EC_{50} value of 4.3 nM in transient replicon assays (Table Report PC-256-2016, page 11). The single substitutions, P299T, P299Q, or M2I in NS5B had a <4-fold change in LDV susceptibility, while replicons carrying either Q54H or C446R failed to replicate. Since these substitutions appeared only in combination with Y93H and at low frequency, the sponsor argues that the Q54H, P299T, P299Q, C446R and M2I substitutions are either random substitutions or potential compensatory substitutions for Y93H.

In summary, 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 (Table 21; Report PC-256-2016, page 11). Y93H was cross-resistant to the NS5A inhibitor GS-432567, but was fully susceptible to the adenosine nucleoside NS5B inhibitor 2'-C-Me-A.

Table 21. Phenotypic Analyses of Individual Substitutions with LDV in Transient Replicon Replication Assay

Compound	EC_{50}(nM)[a]	Fold Resistance[a,b]			
	WT	Y93H	P299Q	P299T	M2I[c]
GS-5885	0.001	3310	3.6	2.2	2.0
GS-432567	0.003	44	2.4	1.3	1.6
2'-C-Me-A	120	1.5	1.5	1.6	1.3

a Values represent the arithmetic mean of two or more independent experiments, and were generated in 96-well assays.
b Fold resistance is calculated as the ratio of mutant EC_{50} to wild-type EC_{50}.
c M2I was a mutation identified in the very N-terminus of NS5B.

CROSS-RESISTANCE

Transient replicon replication assays were performed to evaluate the antiviral activity of LDV against a panel of resistance substitutions. A panel of GT1b replicons encoding resistance substitutions to NS3/4A protease inhibitors (V36M, T54A, R155K, A156T, or D168V) was transiently transfected into Huh-Lunet cells and tested for susceptibility to LDV (GS-5885). Results indicated that LDV retained full activity against all of the protease inhibitor-resistant mutants tested (EC_{50} values elevated <2-fold compared to wild-type) (Table 22; Report PC-256-2017, page 9). Wild-type EC_{50} values and mutant

EC_{50} shifts for the control NS3/4A protease inhibitors (BILN-2061 and VX-950) and adenosine nucleoside NS5B polymerase inhibitor (2'-C-Me-A) agreed with historical results.

Table 22. Cross-resistance of Known NS3/4A Protease Inhibitor Resistance Substitutions to LDV

	EC_{50} (nM)[a]	Fold Resistance[a,b,c]				
Compound	WT	V36M	T54A	R155K	A156T	D168V
GS-5885	0.0047	0.9	0.9	0.7	0.5	0.8
BILN-2061	3.3	N.T	N.T.	> 2000	1302	1705
VX-950	236	10	7.9	N.T.	N.T.	N.T.
2'-C-Me-A	133	2.3	1.3	1.1	0.6	0.9

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

LDV was also tested against several replicons encoding nucleoside and nonnucleoside NS5B polymerase inhibitor resistance substitutions, including sofosbuvir-resistant (S282T), GS-9190-resistant (Y448, Y448H/Y452H, C316Y/C445F/Y452H), thiadiazine-resistant (M414T), or thiophene-resistant (M423T) in transient transfection replicon assays. LDV showed <1-fold decreases in susceptibility compared to wild-type against all of the NS5B polymerase inhibitor resistance substitutions tested indicating full susceptibility (Table 23; Report PC-256-2017, page 10). Wild-type and mutant EC_{50} values for 2'-C-Me-A, (b) (4) benzothiadiazine inhibitor (b) (4) (GS-331523), (b) (4) thiophene inhibitor (b) (4) (GS-331537), and GS-9190 agreed with historical results, and confirmed that each inhibitor was resistant to signature resistance substitution(s).

Table 23. Cross-resistance of Known NS5B Polymerase Inhibitor Resistance Substitutions to LDV

	EC_{50} (nM)[a]	Fold Resistance[a,b,c]					
Compound	WT	S282T	M414T	M423T	Y448H	Y448H/ Y452H	C316Y/C445F/Y452H
GS-5885	0.0047	0.7	0.6	0.6	0.6	0.7	0.6
2'-C-Me-A	133	36	1.7	0.6	1.2	0.8	0.9
GS-331523 (Benzothiadiazine)	225	N.T.	> 8.8	N.T.	N.T.	N.T.	N.T.
GS-331537 (Thiophene)	7.0	N.T.	N.T.	286	N.T.	N.T.	N.T.
GS-9190	2.6	N.T.	N.T.	N.T.	8.9	44	182

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

LDV was further tested against replicons encoding resistance subsitutions to NS5A inhibitors (L31V, L31F, L31M, or Y93H). Results of transient replicon replication assays indicated that LDV has reduced antiviral activity against all of the NS5A inhibitor-resistant mutants tested (EC_{50} values >10-fold compared to wildtype) (Table 24; Report PC-256-2017, page 10). The L31V substitution conferred 133-fold reduced susceptibility to LDV and the Y93H substitution conferred 3,310-fold reduced susceptibility to LDV. The (b) (4) NS5A inhibitor (b) (4) (GS-432567) was tested as a comparator and also showed decrease susceptibility against the NS5A mutants. There was no EC_{50} value shift between wild-type and NS5A mutants for the negative control compound 2'-C-Me-A.

Table 24. Cross-resistance of Known NS5A Inhibitor Resistance Substitutions to LDV

Compound	EC_{50}(nM)[a]	Fold Resistance[a,b]			
	WT	L31V	L31F	L31M	Y93H
GS-5885	0.001	133	10	12	3310
GS-432567	0.003	17	13	11	44
2'-C-Me-A	120	1.3	1.6	1.5	1.5

a Values represent the arithmetic mean of two or more independent experiments using 96-well assays.

b Fold resistance is calculated as the ratio of mutant EC_{50} to wild-type EC_{50}.

In addition, a panel of HCV mutant replicons bearing signature NS5A inhibitor and/or NS3/4A inhibitor and/or NS5B non-nucleotide inhibitor and/or NS5B nucleotide resistance-associated variants was investigated via transient replicon assays for their cross-resistance between SOF, LDV and other classes of direct-acting antivirals.

SOF and LDV were tested against a panel of single GT1a and GT1b NS3 mutants that confer resistance to NS3/4A inhibitors. The fold-change in EC_{50} values of SOF and LDV were all less than 2-fold for the NS3/4A inhibitor resistance substitutions tested (V36A/M, Q41R, F43S, T54S/A, Q80K, R155K/T/C/I/M/S/G/L/Q/W, A156T/S/V/D/G, D168A/N/T/V/E/G/H/Y, or I170T) indicating they remained fully active to SOF and LDV and are not cross-resistant with NS3/4A inhibitors.

SOF and LDV were tested against GT1a and 1b replicons encoding single non-nucleotide NS5B inhibitor site II resistance substitutions or the SOF-associated S282T mutant. All single non-nucleotide NS5B inhibitor resistance substitutions tested (L419S, L419M, R422K, M423V/T/I, I482L, A486T/V/I, and V494A) did not confer any resistance to SOF, LDV, GS-5816 or RBV. SOF had 4-fold and 7-fold reduced susceptibility to the S282T substitution in GT1a and GT1b replicons, respectively, but had <1-fold decreased susceptibility to the non-nucleotide NS5B inhibitor substitutions. LDV had < 1-fold change in susceptibility to all the mutants studied and was fully active against the S282T nucleotide inhibitor mutant.

SOF, LDV and GS-5816 (NS5A inhibitors), GS-9669 (non-nucleotide NS5B inhibitor site II), GS-9451 (NS3/4A inhibitor) and RBV were tested against a panel of double and triple class GT1a and GT1b mutants that confer resistance to NS3/4A inhibitors, NS5A

inhibitors, nucleotide inhibitors and/or non-nucleotide NS5B inhibitor site II inhibitors. Reductions in susceptibility to SOF were observed for all double and triple class mutants containing S282T in combination with NS5A and/or non-nucleotide NS5B inhibitor resistance substitutions with an EC_{50} fold-changes ranging between 3.5 and 19. LDV and GS-5816 were fully active against all double class mutants of S282T and NS5B site II resistance substitutions. However, 2-fold to >5,000-fold decreases in susceptibility to LDV were detected for double and triple class mutants that contained NS5A resistance substitutions. As expected, RBV was fully active against all NS5A and NS5B mutants. All double and triple resistant variants with S282T had significantly lower replication capacity than double and triple variants that did not have the S282T.

In summary, GT1 replicons encoding substitutions conferring resistance to NS5A inhibitors, NS3/4A inhibitors, or non-nucleotide NS5B inhibitors remained fully susceptible to SOF in cell culture indicating that there is no cross-resistance between SOF and HCV NS3/4A inhibitors, non-nucleotide NS5B inhibitors, or NS5A inhibitors. 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.

4.5 Clinical Studies

PHASE II STUDIES

P7977-0523 (ELECTRON)

This multi-center, open-labeled exploratory study investigated the safety, tolerability, pharmacokinetics and pharmacodynamics following oral administration of SOF 400 mg and ribavirin for 12 weeks with and without pegylated interferon in treatment-naive subjects with chronic HCV infection genotype 2 or genotype 3. Multiple new arms were added to this study including arms of subjects with genotype 1 HCV infection.

A total of 25 treatment-naive subjects with genotype 1 HCV infection were enrolled into the following treatment group:

- LDV/SOF LDV/SOF+RBV 6 Weeks: LDV/SOF (400 mg/90 mg) once daily + RBV 1000 to 1200 mg/day divided BID for 6 weeks in noncirrhotic treatment-naive subjects with genotype 1 HCV infection (Group 21)

The results from Group 21 (6 weeks of treatment) contrast with the SVR12 rates observed in Group 13, in which treatment-naive subjects were treated with SOF+LDV+RBV for 12 weeks. A reduction in response rates from 100% (Group 13) to 68% (Group 21) was observed when the treatment duration was decreased from 12 to 6 weeks (Table 25; Summary of Clinical Efficacy report, page 34).

Table 25. P7977-0523: Proportion of Subjects with SVR4 and/or SVR12 Following Treatment with LDV/SOF Administered with and without RBV for 6 or 12 Weeks in Null-Responder, Treatment-Naive, and Hemophiliac Subjects with Genotype 1, 2, or 3 HCV Infection (Part 6, Groups 16 to 18, 20, and 21)

	Null Responder Genotype 1 with Cirrhosis		Treatment Naive Genotype 2/3	Hemophilia Genotype 1	Treatment Naive Genotype 1
	Group 16	Group 17	Group 18	Group 20	Group 21
	SOF/LDV 12 Weeks (N = 10)	SOF/LDV+RBV 12 Weeks (N = 9)	SOF/LDV 12 Weeks (N = 10)	SOF/LDV+RBV 12 Weeks (N = 14)	SOF/LDV+RBV 6 Weeks (N = 25)
SVR4, n/N (%)	8/10 (80.0)	9/9 (100.0)	8/10 (80.0)	14/14 (100.0)	22/25 (88.0)
95% CI	44.4–97.5	66.4–100.0	44.4–97.5	76.8–100.0	68.8–97.5
SVR12, n/N (%)	7/10 (70.0)	9/9 (100.0)	8/10 (80.0)	—	17/25 (68.0)
95% CI	34.8–93.3	66.4–100.0	44.4–97.5	—	46.5–85.1

A total of 3 subjects in Group 16 (cirrhotic nulls LDV/SOF 12 WK) experienced viral relapse. One of these subjects had baseline NS5A resistance polymorphisms (M28V, Q30H). Deep sequencing analysis of NS5A showed that NS5A resistance substitutions were detected post-treatment in the virus from all 3 subjects who relapsed in Group 16. No S282T or other NS5B nucleoside inhibitor or RBV resistance polymorphisms/substitutions were detected at baseline or relapse for these subjects.

In Group 21, NS5B was analyzed at baseline for all 25 subjects by population sequencing. No S282T or other NS5B nucleotide inhibitor resistance polymorphisms were detected. Among the 8 subjects who experienced viral relapse after completion of 6 weeks of treatment with LDV/SOF+RBV, 2 subjects' virus had NS5A resistance polymorphisms at baseline. At the time of relapse no S282T was detected by deep sequencing, but emergent NS5A LDV resistance substitutions were detected in the viruses from 3 of the 8 subjects who relapsed.

GS-US-337-0118 (LONESTAR)

This Phase 2, randomized, open-label study assessed the safety, tolerability, and antiviral efficacy of LDV/SOF administered with and without RBV for 8 or 12 weeks. The study had 2 parallel cohorts. Cohort 1 consisted of noncirrhotic treatment-naive subjects (3 treatment groups: LDV/SOF 8 WK, LDV/SOF+RBV 8 WK, LDV/SOF 12 WK) and Cohort 2 consisted of cirrhotic and noncirrhotic treatment-experienced subjects (2 treatment groups: LDV/SOF 12 WK, LDV/SOF+RBV 12 WK).

A total of 97% subjects achieved SVR12 across all groups. No subjects in any group had on-treatment virologic failure (i.e., breakthrough, rebound, or nonresponse). In treatment-naive subjects, the highest proportion of subjects who achieved SVR12 was in the LDV/SOF+RBV 8 Week group (100%, 21/21), followed by the LDV/SOF 8 Week group (95%, 19/20; 1 subject relapsed), and the LDV/SOF 12 Week group (95%, 18/19; 1 subject withdrew consent before the post-treatment Week 12 visit) (Table 26; Summary Clin Eff, page 51). In treatment-experienced subjects, SVR12 was achieved by 100% (21/21) of subjects in the LDV/SOF+RBV 12 Week group and in 95% (18/19) of subjects in the LDV/SOF 12 Week group (1 subject relapsed). Of note, 21 of the 22 cirrhotic subjects who had previously failed treatment with a NS3/4A inhibitor-based regimen achieved SVR12.

Table 26. GS-US-337-0118: Sustained Virologic Response Rate at Posttreatment Follow-Up Week 12 (Full Analysis Set)

	Treatment-Naive			Treatment-Experienced	
	Group 1	Group 2	Group 3	Group 4	Group 5
	SOF/LDV 8 Weeks (N = 20)	SOF/LDV+RBV 8 Weeks (N = 21)	SOF/LDV 12 Weeks (N = 19)	SOF/LDV 12 Weeks (N = 19)	SOF/LDV+RBV 12 Weeks (N = 21)
SVR12	19/20 (95.0%)	21/21 (100.0%)	18/19 (94.7%)	18/19 (94.7%)	21/21 (100.0%)
95% CI	75.1% to 99.9%	83.9% to 100.0%	74.0% to 99.9%	74.0% to 99.9%	83.9% to 100.0%

Nine subjects were identified as having virus with baseline NS5A resistance polymorphisms and 7 of them achieved SVR12. The presence of NS3/4A inhibitor resistance-associated substitutions was detected in the virus from 33 subjects by deep sequencing with a 1% assay cut off. All subjects whose virus had baseline NS3/4A inhibitor substitutions achieved SVR12. The NS5B nucleotide inhibitor resistance-associated substitution S282T was not detected in any subject's virus at baseline.

Two subjects relapsed: 1 in the LDV/SOF 8 Week treatment-naive group and 1 in the LDV/SOF 12 Week treatment-experienced group. Both subjects who relapsed had virus with NS5A resistance polymorphisms at baseline. One subject who relapsed had virus with L31M at baseline; following relapse, Y93H and Q30L NS5A substitutions and the NS5B substitution S282T were detected in addition to L31M. Subsequently, the levels of S282T decreased 11-fold in 5 days. Despite the presence of resistance substitutions to both LDV and SOF, the subject experienced a rapid reduction of HCV viral load following rescue treatment with LDV/SOF+RBV for 24 weeks and subsequently achieved SVR12. The other subject who relapsed had virus with Q30H+Y93H resistance polymorphisms at baseline and relapsed without the emergence of additional NS5A resistance substitutions. No NS5B S282T was detected by deep sequencing in this subject's virus. The other 7 subjects whose virus had the NS5A resistance polymorphisms L31M, Q30H, or Y93H, or other variants at these positions at baseline, achieved SVR12.

PHASE III STUDIES

STUDY 102 (ION-1)

This ongoing Phase 3, randomized, open-label, multicenter study assessed the antiviral efficacy, safety, and tolerability of 12 or 24 weeks of LDV/SOF administered with and without RBV treatment in treatment-naive subjects with genotype 1 HCV infection.

Approximately 800 subjects were randomized in a 1:1:1:1 ratio to 1 of the following 4 treatment groups: LDV/SOF 24 Week group (Group 1): (n = 217): LDV/SOF tablet (SOF 400 mg/LDV 90 mg) once daily for 24 weeks; LDV/SOF+RBV 24 Week group (Group 2): (n = 218): LDV/SOF tablet (SOF 400 mg/LDV 90 mg) once daily + RBV (1000 or 1200 mg/day divided BID) for 24 weeks; LDV/SOF 12 Week group (Group 3): (n = 217): LDV/SOF tablet (SOF 400 mg/LDV 90 mg) once daily for 12 weeks; LDV/SOF+RBV 12 Week group (Group 4): (n = 218): LDV/SOF tablet (SOF 400 mg/LDV 90 mg) once daily + RBV (1000 or 1200 mg/day divided BID) for 12 weeks

The majority of subjects had GT1a HCV infection (67%), non-CC (CT or TT) IL28B alleles (70%), HCV RNA ≥ 800,000 IU/mL (79%), with a mean (SD) baseline HCV RNA value of 6.4 (0.66) $\log_{10}$ IU/mL. A total of 16% of subjects had cirrhosis at screening. The majority of subjects were IFN eligible (92%).

Table 27. GS-US-337-0102: Virologic Outcomes for Subjects in the 12-Week Treatment Groups

	SOF/LDV 12 Weeks (N = 214)	SOF/LDV+RBV 12 Weeks (N = 217)
SVR12	209/214 (97.7%)	211/217 (97.2%)
Overall Virologic Failure	1/214 (0.5%)	0/217
Relapse	1/213 (0.5%)	0/217
On-Treatment Virologic Failure	0/214	0/217
Other	4/214 (1.9%)	6/217 (2.8%)

In the LDV/SOF 12 Week group: 98% (95% CI: 94.6% to 99.2%) of subjects (209/ 214) achieved SVR12; 1 subject (0.5%) relapsed and of the 4 subjects (1.9%) who could not be assessed for SVR12, 2 subjects were lost to follow-up and 2 subjects completed treatment and achieved SVR4, but did not have a post-treatment Week 12 visit at the time of data finalization (Table 27; Summary of Clincial Efficacy, page 69). In the LDV/SOF+RBV 12 Week group: 97% (95% CI: 94.1% to 99.0%) of subjects (211/217) achieved SVR12; no subjects relapsed and of the 6 subjects (2.8%) who could not be assessed for SVR12, 5 subjects were lost to follow-up or withdrew consent and 1 subject completed treatment and achieved SVR4, but did not have a post-treatment Week 12 visit at the time of data finalization.

The one subject who relapsed in the LDV/SOF 12-Week arm had cirrhosis. The addition of RBV to a LDV/SOF regimen did not appear to have an impact on the SVR rate in treatment-naïve subjects. Another subject, Subject 5663-71589, a noncirrhotic subject with GT1b chronic HCV infection and IL28 CT allele was randomized to the LDV/SOF 24-week treatment arm and completed 12 weeks treatment with 88% adherence. The subject was suppressed at Week 6, but at Week 8 had breakthrough with detected HCV RNA and emergence of the Y93H substitution. At Week 12, the subject discontinued treatment due to documented noncompliance. Y93H was also detected 4 weeks post-treatment (>99%). No NS5B nucleotide inhibitor resistance associated substitutions were detected at any of these visits. Phenotypic analysis of NS5A and NS5B genes showed >310-fold resistance to LDV, but no resistance to SOF.

STUDY 109 (ION-2)

This Phase 3, randomized, open-label, multicenter study evaluated the antiviral efficacy, safety, and tolerability of 12 or 24 weeks of LDV/SOF±RBV treatment in treatment-experienced subjects with genotype 1 HCV infection. Approximately 50% of subjects had received a prior NS3/4A inhibitor+Peg-IFN+RBV regimen.

Following screening, 441 subjects were randomized in a 1:1:1:1 ratio to 1 of the following 4 treatment groups: LDV/SOF (400 mg/90 mg) for 24 weeks (Group 1), LDV/SOF (400 mg/90 mg) + RBV 1000 or 1200 mg/day divided BID for 24 weeks (Group 2), LDV/SOF (400 mg/90 mg) for 12 weeks (Group 3), or LDV/SOF (400 mg/90 mg) + RBV 1000 or 1200 mg/day divided BID for 12 weeks (Group 4). The randomization

schedule was stratified by genotype (1a or 1b; subjects with mixed genotype 1a/1b were stratified as 1a), the presence or absence of cirrhosis at screening, and response to prior HCV therapy (relapse/breakthrough or nonresponse at screening). The majority of subjects in the safety analysis set had genotype 1a HCV infection (79%), non-CC (CT or TT) IL28B alleles (88%), and HCV RNA ≥ 800,000 IU/mL (89%), with a mean (SD) baseline HCV RNA value of 6.5 (0.54) $\log_{10}$ IU/mL. A total of 88 subjects (20%) had cirrhosis at screening.

Table 28. GS-US-337-0109: Virologic Outcomes

	SOF/LDV 12 Weeks (N = 109)	SOF/LDV+RBV 12 Weeks (N = 111)	SOF/LDV 24 Weeks (N = 109)	SOF/LDV+RBV 24 Weeks (N = 111)
SVR12	102/109 (93.6%)	107/111 (96.4%)	108/109 (99.1%)	110/111 (99.1%)
Overall Virologic Failure	7/109 (6.4%)	4/111 (3.6%)	0/109	1/111 (0.9%)
Relapse	7/108 (6.5%)	4/111 (3.6%)	0/109	0/110
On-Treatment Virologic Failure	0/109	0/111	0/109	1/111 (0.9%)
Other	0/109	0/111	1/109 (0.9%)	0/111

In the LDV/SOF 12 Week group: 94% (102/109) (95% CI: 87.2% to 97%) of subjects achieved SVR12; 7 subjects (6.5%) relapsed. In the LDV/SOF+RBV 12 Week group: 96% (107/111) (95% CI: 91.0% to 99.0%) of subjects achieved SVR12, 4 subjects (3.6%) relapsed (Table 28; Summary of Clinical Efficacy, page 85). In the LDV/SOF 24 Week group: 99% (108/109) (95% CI: 95.0% to 100.0%) of subjects achieved SVR12; no subjects relapsed and 1 subject (0.9%) did not return for the post-treatment Week 12 visit (due to withdrawal of consent) and was, therefore, classified as a failure. In the LDV/SOF+RBV 24 Week group: 99% (110/111) (95% CI: 95.1% to 100.0%) of subjects achieved SVR12, and 1 subject (0.9%) experienced on-treatment virologic failure (rebound), which was associated with documented study drug noncompliance.

STUDY 108 (ION-3)

This phase 3 randomized, open-label, multicenter study evaluated the antiviral efficacy, safety, and tolerability of LDV/SOF+RBV for 8 weeks and LDV/SOF (without RBV) for 8 and 12 weeks in treatment-naive subjects with genotype 1 HCV infection. Following screening, 647 subjects were randomized in a 1:1:1 ratio to 1 of the following 3 treatment groups: LDV/SOF (400 mg/90 mg) for 12 weeks (Group 1), LDV/SOF (400 mg/90 mg) + RBV 1000 or 1200 mg/day divided BID for 8 weeks (Group 2), and LDV/SOF (400 mg/90 mg) for 8 weeks (Group 3) and stratified by genotype (1a or 1b; subjects with mixed genotype 1a/1b were stratified as 1a). Most subjects in this study had genotype 1a (80%) HCV infection; 1 subject in the LDV/SOF 8 Week group did not have a confirmed subtype for their genotype 1 HCV infection. No subjects had cirrhosis. Most subjects had non-CC (CT or TT) IL28B alleles (73%) and HCV RNA ≥ 800,000 IU/mL (81%), with a mean baseline HCV RNA value of 6.5 $\log_{10}$ IU/mL.

In the LDV/SOF 8 Week group: 94% (202/215) (95% CI: 89.9% to 96.7%) of subjects achieved SVR12; 11 subjects (5.1%) relapsed and 2 subjects (0.9%) were lost to follow-up or withdrew consent (Table 29; Summary of Clinical Efficacy, page 102). In the LDV/SOF+RBV 8 Week group: 93% (201/216) (95% CI: 88.8% to 96.1%) of subjects achieved SVR12; 9 subjects (4.2%) relapsed and of the 6 subjects (2.8%) who could not be assessed for SVR12, 5 subjects were lost to follow up and 1 subject discontinued

after Day 2 due to an AE of road traffic accident. In the LDV/SOF 12 Week group: 95% (206/216)(95% CI: 91.7% to 97.8%) of subjects achieved SVR12; 3 subjects (1.4%) relapsed and of the 7 subjects (3.2%) who could not be assessed for SVR12, 5 subjects were lost to follow up and 2 subjects had HCV RNA <LLOQ at the posttreatment Week 4 visit and the posttreatment Week 12 visit pending. The 8-week, RBV-free LDV/SOF regimen was noninferior to the 8-week RBV-containing regimen and the 12-week LDV/SOF regimen, as demonstrated by lower-bound 95% CI of −3.9% and lower bound 97.5% CI of −6.4%, respectively.

Table 29. GS-US-337-0108: Virologic Outcomes (Full Analysis Set)

	SOF/LDV 8 Weeks (N = 215)	SOF/LDV+RBV 8 Weeks (N = 216)	SOF/LDV 12 Weeks (N = 216)
SVR12	202/215 (94.0%)	201/216 (93.1%)	206/216 (95.4%)
Overall Virologic Failure	11/215 (5.1%)	9/216 (4.2%)	3/216 1.4%)
Relapse	11/215 (5.1%)	9/214 (4.2%)	3/216 (1.4%)
On-Treatment Virologic Failure	0/215	0/216	0/216
Other	2/215 (0.9%)	6/216 (2.8%)	7/216 (3.2%)

4.6 Clinical Virology

PHASE III STUDIES

SCREENING GENOTYPE/SUBTYPE VERSUS ANALYSIS GENOTYPE/SUBTYPE

Screening of genotype/subtype was performed by Siemens VERSANT HCV genotype assay Version 2.0 INNO-LiPA 2.0. Analysis screening of genotype/subtype was done by NS5A direct sequencing and phylogenetic analysis. The concordance between the screening and analysis assays for GT1a was 99.6% (1376/1381) (Table 30). The concordance between the screening and analysis assays for GT1b was 96% (411/426). Three subjects were screened as GT1 and by analysis were GT1a. These subjects are included in the statistical analysis. There was one subject who screened as GT4 by the Siemens assay and by NS5A sequencing analysis and this subject achieved SVR12. This GT4 subject is excluded from the statistical analysis.

Table 30. Screening and Analysis GT Subtype Results

Screening	# Subjects	Analysis (NS5A)	SVR
1a	1376	1a	
1a	1	1b	SVR12
1a	1	1a/1b	SVR12
1b	411	1b	
1b	14	1a	
1	3	1a	SVR12
GT1a	1	NS5A GT1h	SVR12
GT1a	2	NS5A GT1c	SVR12
GT1b	1	NS5A GT1l	SVR12
GT4	1	NS5A GT4d	SVR12

Overall, in all the studies submitted, the relapse rate was 2.7%. In GT1a subjects, the relapse rate was 3% (41/1378) (Table 31). In GT1b subjects, the relapse rate was 1.7% (7/411).

Table 31. Relapse Rate by Screening Genotype/Subtype

	Relapse
Overall	2.7% (48/1795*)
GT1a	3% (41/1378)
GT1b	1.7% (7/411)

*6 subjects were screened as GT1 no subtype (n=3) or had no screening GT (n=3), but by analysis, 4 subjects were GT1a and 2 subjects were GT1b

BASELINE NS5A RESISTANCE ANALYSIS

Sequencing for the HCV NS5A gene was performed for all enrolled subjects from ION-1 by next generation population or population sequencing, and all subjects in ION-2 and ION-3 by deep sequencing. Next generation sequencing of the NS5B gene was performed on samples from all subjects in ION-2 and ION-3 and a subset of subjects from ION-1. In addition, the ION-2 study contains NS3/4A-experienced subjects, and deep sequencing of NS3 protease coding region was performed for all enrolled subjects from this study. HCV consensus sequences were generated for all successfully sequenced samples from next generation sequencing.

Proportion of Baseline NS5A Polymorphisms in Phase 3 Studies

NS5A resistance-associated polymorphic variants that were analyzed in the FDA virology analysis included any substitution at positions K24, M28, Q30, L31, P32, H58, A92 and Y93. These NS5A resistance-associated polymorphisms or mixtures at these amino acid positions were detected at baseline in the virus from 23% (410/1795) of the subjects from all the submitted studies (ION-1, ION-2, ION-3, 523 and 118) and 23% (370/1615) of the subjects in the Phase 3 studies (ION-1, ION-2, and ION-3) (Table 32). The proportion of NS5A resistance-associated polymorphisms at baseline in each of the phase 3 studies ranged from 19-25%.

Table 32. Subjects with Baseline NS5A Resistance-Associated Polymorphisms

Study	Proportion of Subjects with Baseline NS5A Substitutions
102 (ION-1)	25% (134/530)
108 (ION-3)	24% (153/645)
109 (ION-2)	19% (83/440)
Phase 3 Studies	23% (370/1615)
All submitted studies + 523 +118	23% (410/1795)

The sponsor provided the prevalence of NS5A resistance-associated variants in the United States (US) and European Union (EU). Approximately 15% of GT1a HCV-infected subjects in the US carried NS5A resistance-associated variants compared to approximately 21% in the EU. For GT1b, approximately 16% of US subjects and 17% of EU subjects had baseline NS5A resistance-associated variants. The prevalence of specific NS5A resistance-associated variants varies and is dependent on subtype.

Among 1,118 GT1a subjects in the US, five NS5A resistance-associated variants that confer >1,000-fold resistance to LDV in cell culture were observed in 0.3% to 2.3% of subjects: Y93H, 2.3%; Y93N, 1.2%; Y93C, 1%; M28A, 0.4%; and H58D, 0.3% (Table 33 and Figure 1; Integrated Phase 2 and 3 Virology Report, page 96).

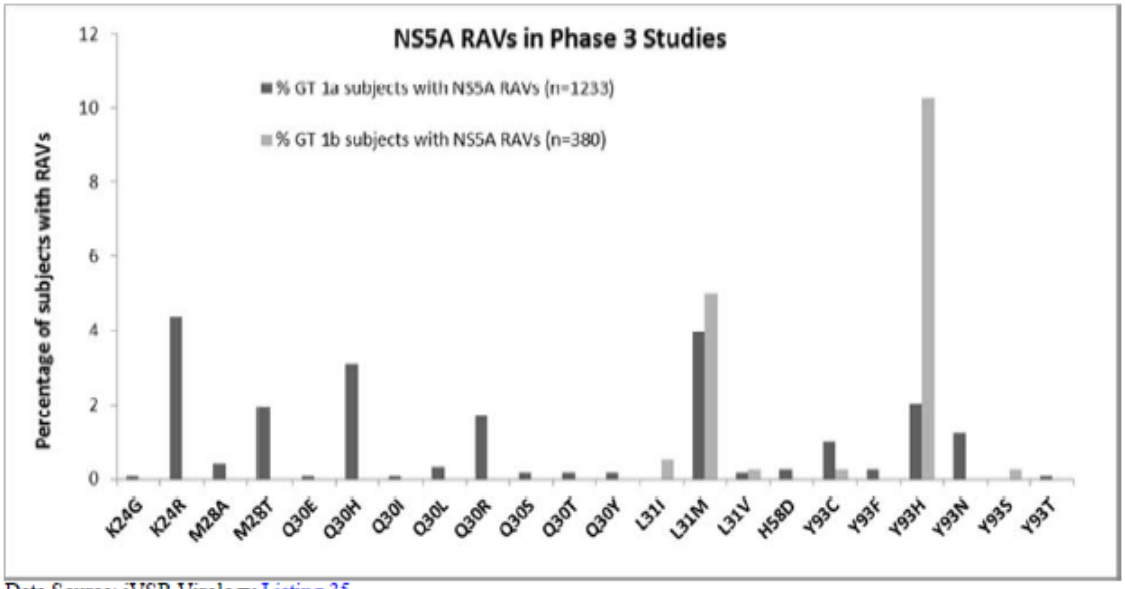
Table 33. Specific NS5A Resistance-Associated Variants Categorized by Geographic Distribution in Phase 3 Studies

	Fold change from Wild Type		USA				EU			
NS5A RAV	GT 1a	GT 1b	Total # of GT 1a subjects with NS5A RAVs (n=1118)	Total # of GT 1b subjects with NS5A RAVs (n=310)	% of GT 1a subjects with NS5A RAVs (n=1118)	% of GT 1b subjects with NS5A RAVs (n=310)	Total # of GT 1a subjects with NS5A RAVs (n=115)	Total # of GT 1b subjects with NS5A RAVs (n=70)	% GT 1a subjects with NS5A RAVs	% GT 1b subjects with NS5A RAVs
Y93H	>1000	>1000	26	27	2.3	8.7	3	8	2.6	11.4
M28A	>1000	NA	5	0	0.4	0	0	0	0	0
Q30E	>1000	NA	0	0	0	0	1	0	0.9	0
H58D	>1000	NA	3	0	0.3	0	0	0	0	0
Y93C	>1000	NA	11	1	1	0.3	1	0	0.9	0
Y93N	>1000	NA	13	1	1.2	0.3	1	0	0.9	0
Y93S	>1000	100-1000	0	1	0	0.3	0	0	0	0
L31M	100-1000	2.5-10	42	14	3.8	4.5	7	5	6.1	7.1
Q30H	100-1000	NA	33	1	3	0.3	4	0	3.5	0
Q30R	100-1000	NA	17	0	1.5	0	4	0	3.5	0
Y93T	100-1000	NA	1	0	0.1	0	0	0	0	0
L31I	100-1000	10-50	1	1	0.1	0.3	0	0	0	0
L31V	100-1000	10-50	1	1	0.1	0.3	1	0	0.9	0
M28T	50-100	NA	20	1	1.8	0.3	3	0	2.6	0
K24G	50-100	NA	1	0	0.1	0	0	0	0	0
Y93F	10-50	NA	3	0	0.3	0	0	0	0	0
Q30S	10-50	NA	2	0	0.2	0	0	0	0	0
K24R	2.5-10	NA	41	3	3.7	1	10	0	8.7	0
Q30I	2.5-10	NA	0	0	0	0	1	0	0.9	0
Q30L	2.5-10	NA	2	0	0.2	0	2	0	1.7	0
Q30T	2.5-10	NA	2	0	0.2	0	0	0	0	0
Q30Y	NA	NA	2	0	0.2	0	0	0	0	0

Some subjects had more than one baseline NS5A RAV

The L31M, Q30H and Q30R variants, which confer 100- to 1,000-fold resistance in cell culture, were detected in 1.5 to 3.8% of GT1a subjects in the US. The M28T or K24R substitutions, which confer <100-fold resistance in cell culture, were observed in 1.8% or 3.7% of US subjects, respectively. Among 310 GT1b subjects in the US, the NS5A variants Y93H and L31M were observed in 8.7% and 4.5% of the subjects, respectively. Similar or slightly higher prevalence of specific resistance-associated variants was observed among the EU subjects.

Figure 1. Prevalence of Specific NS5A Baseline Polymorphisms in Phase 3 Studies



Data Source: iVSR Virology Listing 35

Effect of Baseline NS5A Resistance-Associated Polymorphisms on Relapse

The effect of the presence of baseline NS5A polymorphisms on relapse rates for the different durations (8, 12 and 24 weeks) with and without RBV was assessed. Overall, relapse rates for the 8-week duration arms with the fixed dose combination (LDV/SOF) of LDV/SOF were 8% for subjects whose virus had any baseline NS5A resistance-associated polymorphisms, similar to the relapse rate of 7% for subjects in the LDV/SOF + RBV arms (Table 34). Treatment with longer durations of LDV/SOF decreased relapse rates. Relapse rates for subjects receiving 12-weeks LDV/SOF were 5.6% without RBV and 1.2% with RBV. No subjects with baseline NS5A resistance polymorphisms relapsed in 24-week duration LDV/SOF arms with or without RBV.

As shown earlier in this review, 23% of the subjects studied in phase 3 studies had virus with baseline NS5A polymorphisms. Overall of all subjects in the phase 3 studies, 19% had virus with 1 NS5A resistance polymorphism, 3.5% had virus with 2 NS5A polymorphisms and 0.6% had virus with 3 NS5A polymorphisms (Table 34). Most of the subjects whose virus had NS5A resistance polymorphisms (82%) had one NS5A polymorphism with 15% having 2 NS5A polymorphisms and only 3% having 3 NS5A polymorphisms. Overall, relapse rates for subjects whose virus had one NS5A

polymorphisms were 3.6%. Relapse rates were higher for subjects whose virus had 2 or more NS5A polymorphisms. Relapse rates were 9.5% and 9% for subjects whose virus had 2 and 3 NS5A resistance polymorphisms, respectively.

Table 34. Relapse Rates by Number of Baseline NS5A Polymorphisms*

Baseline NS5A Polymorphisms	All	1	2	3
Overall	4.6% (19/410)	3.6% (12/336)	9.5% (6/63)	9% (1/11)
LDV/SOF 8 WK	8% (4/50)	9.1% (4/44)	0/4	0/2
LDV/SOF 12WK	5.6% (8/144)	1.7% (2/115)	22% (6/27) 4 PI exp	0/2
LDV/SOF 24WK	0% (0/39)	0% (0/34)	0/5	0
LDV/SOF +RBV 8WK	7% (4/55)	6.5% (3/46)	0/6	33% (1/3)
LDV/SOF +RBV 12WK	1.2% (1/85)	1.6% (1/64)	0/18	0/3
LDV/SOF +RBV 24WK	0% (0/31)	3.7% (1/27)	0/3	0/1

*Any change at positions K24, M28, Q30, L31, P32, H58, A92 and Y93

The effect of individual baseline NS5A polymorphisms on relapse rates was examined. The highest relapse rates were seen in subjects whose viruses had polymorphisms at Q30, L31, and Y93 at baseline. Relapse rates were 6.6%, 10%, 15%, 4.8%, 4.3% and 1.4% for subjects with viruses having baseline NS5A substitutions at Q30, L31, Y93 M28, A92 or H58, respectively (Table 35). No subjects with substitutions at K24 relapsed. There were no subjects in the phase 3 studies who had the P32L substitution at baseline. In general, relapse rates were 0% for each individual baseline NS5A polymorphisms when subjects received durations of 24-weeks LDV/SOF or 12 weeks LDV/SOF with RBV.

Table 35. Relapse Rates by Baseline NS5A Polymorphisms

Baseline NS5A Substitution	GT1
K24R/Q/G/E	0% (0/25)
M28V/T/L/I/A	4.8% (6/125)
LDV/SOF 8 WK	5.2% (1/19)
LDV/SOF 12WK	5.9% (3/51)
LDV/SOF 24WK	0% (0/10)
LDV/SOF+RBV 8WK	6.3% (1/16)
LDV/SOF+RBV 12WK	0% (0/18)
LDV/SOF+RBV 24WK	0% (0/10)
Q30H/R/S/T/L	6.6% (5/76)
LDV/SOF 8 WK	0% (0/4)
LDV/SOF 12WK	18% (4/22)
LDV/SOF 24WK	0% (0/9)

LDV/SOF+RBV 8WK	7.7% (1/13)
LDV/SOF+RBV 12WK	0% (0/22)
LDV/SOF+RBV 24WK	0% (0/5)
L31M/V	10% (5/50)
LDV/SOF 8 WK	25% (2/8)
LDV/SOF 12WK	11% (2/18)
LDV/SOF 24WK	0% (0/4)
LDV/SOF+RBV 8WK	0% (0/3)
LDV/SOF+RBV 12WK	7.7% (1/13)
LDV/SOF+RBV 24WK	0% (0/4)
P32L	0
H58P/D/Q/R/S/T/Y	1.4% (2/142)
A92P/V	4.3% (1/23)
Y93H/N/C	15% (8/54)
LDV/SOF 8 WK	17% (1/6)
LDV/SOF 12WK	19% (4/21)
LDV/SOF 24WK	0% (0/3)
LDV/SOF+RBV 8WK	38% (3/8)
LDV/SOF+RBV 12WK	0% (0/9)
LDV/SOF+RBV 24WK	0% (0/7)
Q30 + Y93	17% (3/18)

NS5A substitutions: at positions 24, 28, 30, 31, 32, 58, 92, 93

Response by Baseline NS5A Polymorphisms and Other Baseline Factors

Relapse rates were analyzed for subjects in the phase 3 studies with the baseline factors IL28B non-CC and cirrhosis. For subjects with IL28B non-CC genotypes, relapse rates in the phase 3 studies was 2.7%. Relapse rates were higher overall (5.7-5.8%) for the 8-week duration arms with and without RBV compared to the 12-week duration arms where relapse rates were 1.7% with RBV and 2.6% without RBV and the 24-week duration arms where relapse rates were 0% (Table 36). Subjects with cirrhosis had overall relapse rates of 4.6% in the phase 3 studies. Relapse rates were 7% in the 12-week arms compared to 0% for the 24-week arms. The combination of non-CC genotype and cirrhosis resulted in overall relapse rates of 5.6% in the phase 3 studies. In the treatment-experienced subjects in Study 109, relapse rates were 14-21% for the 12-week arms compared to 0% for the 24-week arm.

Table 36. Relapse Rates by Baseline IL28B non-CC and Cirrhosis

	non-CC	**cirrhotic**	**non-CC, cirrhotic**
GT1 (NS5A)			
All Studies	3.2% (44/1373)	4.9% (12/246)	5.4% (11/201)
Phase 3 Studies	2.7% (33/1229)	4.6% (8/173)	5.6% (8/143)
LDV/SOF 8 WK	5.7% (9/159)	na	na
LDV/SOF 12WK	2.6% (11/415)	7.1% (4/56)	8.7% (4/46)
LDV/SOF 24 WK	0% (0/131)	0% (0/30)	0% (0/27)
LDV/SOF+RBV 8WK	5.8% (9/156)	na	na
LDV/SOF+RBV 12WK	1.7% (4/238)	7.3% (4/55)	9.8% (4/41)
LDV/SOF+RBV 24WK	0% (0/130)	0% (0/32)	0% (0/29)

Study 109	2.9% (11/385)	8% (7/88)	8.8% (7/80)
LDV/SOF 12WK	7% (7/99)	14% (3/22)	14% (3/21)
LDV/SOF 24 WK	0% (0/93)	0% (0/22)	0% (0/20)
LDV/SOF+RBV 12WK	4% (4/100)	18% (4/22)	21% (4/19)

Effect of Baseline NS5A Polymorphisms on Relapse Rates in Treatment-Naïve Subjects

The effect of baseline NS5A resistance-associated polymorphisms on relapse rates for each arm in each study was analyzed. In treatment-naïve subjects (Study 108, ION-3), overall relapse rates were 5.1%, 4.2% and 1.4% in LDV/SOF 8-week arm, LDV/SOF+RBV 8-week arm, and LDV/SOF 12-week arm, respectively (Table 37). Relapse rates were similar for treatment-naïve subjects whose viruses lacked baseline NS5A resistance polymorphisms. Relapse rates for treatment-naïve subjects whose viruses had baseline NS5A resistance polymorphisms were 6.3% for the LDV/SOF 8-week arm, 8% for the LDV/SOF+RBV 8-week arm, and 0% for the LDV/SOF 12-week arm. Relapse rates for the subgroup of subjects whose viruses had baseline NS5A polymorphisms and who had IL28B non-CC genotype were a little higher for the 8-week arms; 9% without RBV and 12.5% with RBV. However, relapse rates were 0% for this subgroup with the 12-week duration LDV/SOF regimen.

Overall, relapse rates were not higher for treatment-experienced subjects (2.7%) compared to treatment-naïve subjects (3.6%) with the caveat that different durations were studied in each of these subject groups. The number of baseline NS5A resistance polymorphisms was similar in treatment-naïve and treatment-experienced subjects with 24% (153/645) of treatment-naïve subjects and 19% (83/440) of treatment-experienced subjects having baseline NS5A polymorphisms. However, relapse rates for treatment-experienced subjects with baseline NS5A resistance polymorphisms was slightly higher at 7.2% compared to 4.6% for treatment-naïve subjects with baseline NS5A resistance polymorphisms (Table 37).

Effect of Baseline NS5A Polymorphisms on Relapse Rates in Treatment-Experienced Subjects

The effect of baseline NS5A resistance polymorphisms on relapse rates in the 12- and 24- week duration arms for treatment-experienced subjects was examined. For subjects whose viruses lacked baseline NS5A polymorphisms, relapse rates were low; 2.3% and 3.4% for LDV/SOF 12-week arm and LDV/SOF+RBV 12-week arm and 0% for the LDV/SOF 24-week arm (Table 37). For treatment-experienced subjects whose viruses had baseline NS5A polymorphisms, relapse rates remained 0% for the LDV/SOF 24-week arm, but were 22% for the LDV/SOF 12-week arm and 4.5% for the LDV/SOF+RBV 12-week arm. Adding RBV to a 12-week LDV/SOF regimen appeared to contribute to a decrease in relapse rates for this subset of subjects.

Relapse Rates in NS3/4A Inhibitor-Experienced Subjects

In Study 109 (ION-2), relapse rates were compared between treatment-experienced subjects overall and the NS3/4A-experienced subjects to determine if relapse rates are higher in NS3/4A-experienced subjects. Overall, relapse rates were similar between these two groups with a 2.5% rate in Study 109 subjects compared to a 3% rate in NS3/4A-experienced subjects (Table 37). Similarly, for subjects whose virus had

baseline NS5A polymorphisms, relapse rates were 7.2% for Study 109 overall compared to 8% for just the NS3/4A-experienced subjects. Therefore, from the analysis of Study 109, relapse rates were not higher in NS3/4A-experienced subjects.

Table 37. Relapse Rates by Baseline NS5A Polymorphisms

GT1 (NS5A)	ALL	No BL NS5A	BL NS5A
Study 108 naïve	3.6% (23/645)	3.3% (16/492)	4.6% (7/153)
LDV/SOF 8 WK	5.1% (11/215)	4.8% (8/167)	6.3% (3/48)
LDV/SOF 12WK	1.4% (3/214)	1.9% (3/158)	0% (0/56)
LDV/SOF+RBV 8WK	4.2% (9/216)	3% (5/167)	8.2% (4/49)
Study 109	2.5% (11/440)	1.4% (5/356)	7.2% (6/83)
LDV/SOF 12 WK	6.4% (7/109)	2.3% (2/86)	22% (5/23)
LDV/SOF 24 WK	0% (0/109)	0% (0/90)	0% (0/19)
LDV/SOF+RBV 12WK	3.6% (4/111)	3.4% (3/89)	4.5% (1/22)
LDV/SOF+RBV 24WK	0% (0/111) Note: 1 BT	0% (0/91)	0% (0/19)
NS3/4A-experienced	3% (6/203)	1.8% (3/166)	8% (3/37)
LDV/SOF 12 WK			20% (2/10)
LDV/SOF 24 WK			0% (0/9)
LDV/SOF+RBV 12WK			9% (1/11)
LDV/SOF+RBV 24WK			0% (0/7)
Study 102	1.9% (1/530)		1
LDV/SOF 12 WK	1		

In addition, the failure rate was examined by the presence at baseline of each individual NS3 substitution (V36, T54, R155, A156 and D168) as well as prior boceprevir or telaprevir use. Failure rates were highest (12.5%) for NS3 substitutions at position D168 followed by 7% for substitutions at T54 (Table 38). The number of subjects in each arm was too small to make any determinations about duration. Failure rates were 1.5% for subjects who failed a prior boceprevir regimen compared to 3.7% for subjects who failed on a prior telaprevir regimen.

Table 38. Response by Baseline NS3/4A Substitutions in Study 109 (n=439)

NS3 Substitution	SVR12 Rate	Relapse Rate
Any NS3 Resistance Substitution (36, 54, 155, 156, 168)		3/88 (3.4%)
V36L/M	95% (38/40)	0% (0/40)
LDV/SOF+RBV 12WK	13/13	0
LDV/SOF+RBV 24WK	5/5	0
LDV/SOF 12 WK	92% (11/12)	0
LDV/SOF 24 WK	90% (9/10)	0
T54A/S	93% (13/14)	7% (1/14)
LDV/SOF+RBV 12WK	75% (3/4)	1/4
LDV/SOF+RBV 24WK	4/4	0
LDV/SOF 12 WK	4/4	0
LDV/SOF 24 WK	2/2	0

155	94% (55/58)	3% (2/58)
LDV/SOF+RBV 12WK	13/13	0
LDV/SOF+RBV 24WK	14/14	0
LDV/SOF 12 WK	88% (15/17)	12% (2/17)
LDV/SOF 24 WK	93% (13/14)	0
156	1/1	0/1
LDV/SOF+RBV 24WK	1/1	0
168	88% (7/8)	12.5% (1/8)
LDV/SOF+RBV 12WK	2/2	0
LDV/SOF+RBV 24WK	3/3	0
LDV/SOF 12 WK	2/3	1/3
LDV/SOF 24 WK	-	
170	96% (43/45)	2.4% (1/41)
LDV/SOF+RBV 12WK	86% (6/7)	0
LDV/SOF+RBV 24WK	8/8	0
LDV/SOF 12 WK	93% (13/14)	7% (1/14)
LDV/SOF 24 WK	13/13	0
R80	98% (171/175)	2.3% (4/174)
LDV/SOF+RBV 12WK	96% (43/45)	4% (2/45)
LDV/SOF+RBV 24WK	44/44	0
LDV/SOF 12 WK	95% (38/40)	5% (2/40)
LDV/SOF 24 WK	45/45	0
Prior boceprevir use	97% (69/71)	1.5% (1/68)
LDV/SOF+RBV 12WK	95% (20/21)	
LDV/SOF+RBV 24WK	18/18	
LDV/SOF 12 WK	17/17	
LDV/SOF 24 WK	93% (14/15)	
Prior telaprevir use	96% (132/137)	3.7% (5/134)
LDV/SOF+RBV 12WK	97% (37/38)	
LDV/SOF+RBV 24WK	24/24	
LDV/SOF 12 WK	91% (42/46)	
LDV/SOF 24 WK	29/29	

RELAPSE RATES IN SUBJECTS WITH MULTIPLE BASELINE FACTORS

The effect of multiple baseline factors (i.e., baseline NS5A resistance polymorphisms, IL28B non-CC and cirrhosis) on relapse rates was analyzed. Although these subgroups are small, the trends show that in subjects with multiple baseline factors such as NS5A resistance subsitutions, IL28B non-CC genotype and/or cirrhosis, the longer 24-week duration of LDV/SOF has relapse rates of 0% compared to higher relapse rates (22-33%) for the 12-week duration arms (Table 39).

Table 39. Relapse Rates by Multiple Baseline Factors: Baseline NS5A Polymorphisms, IL28B non-CC and Cirrhosis

GT1 (NS5A)	ALL	BL NS5A CC	BL NS5A non-CC	BL NS5A Not cirrhotic	BL NS5A cirrhotic	BL NS5A, non-CC, cirrhotic
Study 108 naïve	3.6% (23/645)	0%	7% (7/98)	na	na	na
LDV/SOF 8 WK	5.1% (11/215)		9% (3/32)	na	na	na
LDV/SOF 12WK	1.4% (3/214)		0% (0/34)	na	na	na
LDV/SOF+RBV 8WK	4.2% (9/216)		12.5% (4/32)	na	na	na
Study 109	2.5% (11/440)	0% (0/17)	9.1% (6/66)	5.8% (4/69)	14% (2/14)	15.4% (2/13)
LDV/SOF 12 WK	6.4% (7/109)		28% (5/18)	21% (4/19)	25% (1/4)	33% (1/3)
LDV/SOF 24 WK	0% (0/109)		0% (0/14)	0% (0/16)	0% (0/3)	0% (0/3)
LDV/SOF+RBV 12WK	3.6% (4/111)		5% (1/20)	0% (0/18)	25% (1/4)	25% (1/4)
LDV/SOF+RBV 24WK	0% (0/111) Note: 1 BT		0% (0/14)	0% (0/16)	0% (0/3)	0% (0/3)
PI-experienced	3% (6/203)	0% (0/4)	9% (3/33)	6.7% (2/30)	14% (1/7)	14% (1/7)
LDV/SOF 12 WK			22% (2/9)	2		
LDV/SOF 24 WK			0% (0/8)			
LDV/SOF+RBV 12WK			10% (1/10)		1	1
LDV/SOF+RBV 24WK			0% (0/6)			
Study 102	1.9% (1/530)		1	1		
LDV/SOF 12 WK	1		1	1		

Study 108 Relapse Rates by Baseline Viral Load and NS5A Polymorphisms

In Study 108, examination of relapse rates by baseline viral load (< and ≥ median baseline viral load of 4 million IU/mL) showed that relapse rates were higher for treatment-naïve subjects with high baseline viral loads (≥4M) who had the shorter 8-week duration of treatment compared to the 12-week duration (8% vs. 1.9% relapse rates) (Table 40). Of the 7 relapser treatment-naïve subjects whose viruses had Baseline NS5A polymorphisms, 4 had baseline viral loads >4 million IU/mL and 3 had baseline viral loads <4 million IU/mL (3.8M, 2.6M and 1.7M). In the treatment-experienced study, relapse rates were also higher in subjects with high baseline viral loads receiving the 12-week duration of treatment compared to the 24-week duration.

However, most of the subjects with high baseline viral loads also had cirrhosis (6 of the 9 relapsers in this group were also cirrhotic).

Table 40. Relapse Rates by Baseline Viral Load and Baseline NS5A Polymorphisms

	<4 IU/mL Baseline Viral Load	≥4M IU/mL Baseline Viral Load	≥4M IU/mL and Baseline NS5A Polymorphisms
All Studies	1.1% (11/983)	4.6% (37/812)	8% (15/187)
Study 108	1.5% (5/320)	5.5% (18/325)	6.4% (5/78)
LDV/SOF 8 WK	1.9% (2/103)	8% (9/112)	7.7% (2/26)
LDV/SOF 12WK	0.9% (1/106)	1.9% (2/108)	0% (0/28)
LDV/SOF+RBV 8WK	1.8% (2/111)	6.7% (7/105)	12.5% (3/24)
Study 109	0.8% (2/247)	4.7% (9/193)*	13.5% (5/37)
LDV/SOF 12WK	3.3% (2/59)	10% (5/50)	28.6% (4/14)
LDV/SOF 24 WK	0% (0/62)	0% (0/47)	0% (0/7)
LDV/SOF+RBV 12WK	0% (0/71)	10% (4/40)	16.7% (1/6)

4M is median baseline viral load; *6 of the 9 relapsers in this group were also cirrhotic. The other 3 had BL VL of 5M, 15M and 16M and all 3 had 2 BL NS5A substitutions.

EFFECT OF BASELINE LEDIPASVIR PHENOTYPE ON SVR12

SVR12 Outcome by Phenotypic Levels of Resistance

The sponsor examined the baseline NS5A resistance polymorphisms by phenotypic levels of resistance (<100-fold, 100- to 1,000-fold, >1,000-fold) and outcome in both treatment-naïve and treatment-experienced subjects (See Table 41 and 42; Integrated Phase 2 and 3 Virology Report, page 102 and 103).

According to their analyses, NS5A resistance-associated substitutions Y93H, Y93N, Y93C, M28A or H58D in GT1a and A92K or Y93H in GT1b confer >1,000-fold resistance to LDV in cell culture. The L31M, L31I, L31V, Q30H, Q30R, Q30G, or P32L substitutions in GT1a and the P58D substitution in GT1b are in the 100 to 1,000-fold resistance category. The K24R, K24G, K24N, M28T, Q30L, Q30T, S38F, A92T, or Y93F substitutions in GT1a and L31M, L31V, L31I, or P32L substitutions in GT1b are in the <100-fold resistance category. They concluded that for treatment-naïve subjects, there were no significant reductions in SVR12 rates for subjects in each of these NS5A resistance polymorphisms groups, including those with >1,000-fold resistance to LDV, treated with LDV/SOF for 8, 12 or 24 weeks.

For treatment-experienced subjects, they concluded there was no significant effect on SVR12 observed for treatment-experienced subjects with NS5A resistance polymorphisms corresponding to <1,000-fold resistance to LDV. However, they concluded for a small group of treatment-experienced subjects, a statistically significant reduction in SVR12 was observed among subjects with LDV resistance polymorphisms corresponding to >1,000-fold resistance treated for 12 weeks with LDV/SOF.

Table 41. Summary of NS5A Resistance Associated Variants Detected in Treatment-Naïve Subjects from ION-1 and ION-3 Studies with a 1% Cutoff by Treatment Regimen

	SOF/LDV		SOF/LDV + RBV		Total for all Treatment Arms Phase 3 Studies	
	# of subjects with RAVs n/N	SVR12 for subjects with RAVs n/N (%)	# of subjects with RAVs n/N	SVR12 for subjects with RAVs n/N (%)	# of subjects with RAVs n/N (%)	SVR12 for subjects with RAVs n/N (%)
Total Subjects with RAVs >1% cutoff	116	-	78	-	194	-
Presence of Any Baseline NS5A RAV 2.5-10.0 fold	28/116	28/28 (100)	15/78	14[e]/15 (93.3)	43/194(22.2)	42[e]/43 (97.7)
Presence of Any Baseline NS5A RAV 10.0-50.0 fold	1/116	1/1 (100)	1/78	1/1 (100)	2/194(1.0)	2/2 (100)
Presence of Any Baseline NS5A RAV 50.0-100 fold	10/116	10/10 (100)	5/78	5/5 (100)	15/194(7.7)	15/15 (100)
Presence of Any Baseline NS5A RAV with 100-1000 fold	32/116	29[a,c]/32 (90.6)	30/78	29[a]/30 (96.7)	62/194(32)	58[i]/62 (93.5)
Presence of Any Baseline NS5A RAV >50 fold	87/116	80/87 (92)	62/78	56/62 (90.3)	149/194(76.8)	136/149 (90.3)
Presence of Any Baseline NS5A RAV >100 fold	77/116	70/77 (90.9)	57/78	51/57 (89.5)	134/194(69.1)	121/134 (90.3)
Presence of Any Baseline NS5A RAV with >1000-fold	45/116	41[a,b]/45 (91.1)	27/78	22[h]/27 (81.5)	72/194(37.1)	63[a,h,h]/72 (87.5)
SVR12 for Subjects with RAVs (%)	-	109[d]/116 (94.0)	-	71[j]/78 (91.0)	-	180[d,j]/194 (92.8)

a One subject relapsed
b Three subjects relapsed
c One subject relapsed, and one subject had HCV RNA < LLOQ at the posttreatment Week 4 visit and have the posttreatment Week 12 visit pending
d Six subjects relapsed and one had HCV RNA < LLOQ at the posttreatment Week 4 visit and have the posttreatment Week 12 visit pending
e One Subject had Early discontinuation
f One Subject GT 1l
g One subject GT 4d and one subject GT 1h
h Four subjects relapsed and one discontinued study treatment
i Five subjects relapsed and two discontinued study treatment
j Two subjects relapsed and one had HCV RNA < LLOQ at the posttreatment Week 4 visit and have the posttreatment Week 12 visit pending

n/N = Number of subjects with RAVs / Number of subjects successfully sequenced
Data Source: iVSR Virology Listing 35

Table 42. Summary of NS5A Resistance-Associated Variants Detected in Treatment-Experienced Subjects from ION-2 Study with a 1% Cutoff by Treatment Regimen

	SOF/LDV		SOF/LDV + RBV		Total for all Treatment Arms Phase 3 Studies	
	# of subjects with RAVs 12 and 24 Weeks n/N	SVR12 for subjects with RAVs n/N (%)	# of subjects with RAVs 12 and 24 Weeks n/N	SVR12 for subjects with RAVs n/N (%)	# of subjects with RAVs 12 and 24 Weeks n/N (%)	SVR12 for subjects with RAVs n/N (%)
Total Subjects with RAVs >1% cutoff	30	-	32	-	62	-
Presence of Any Baseline NS5A RAV 2.5-10.0 fold	7/30	7/7 (100)	10/32	10/10(100)	17/62 (27.4)	17/17 (100)
Presence of Any Baseline NS5A RAV 10.0-50.0 fold	2/30	2/2 (100)	2/32	2/2(100)	4/62 (6.5)	4/4 (100)
Presence of Any Baseline NS5A RAV 50.0-100 fold	2/30	2/2 (100)	1/32	1/1(100)	3/62 (4.8)	3/3 (100)
Presence of Any Baseline NS5A RAV with 100-1000 fold	9/30	8[a]/9 (88.9)	6/32	4[c,e]/6 (66.7)	15/62 (24.2)	12[a,c,e]/15 (80)
Presence of Any Baseline NS5A RAV >50 fold	21/30	17/21 (81)	20/32	18/20 (90)	41/62(66.1)	34/41 (82.9)
Presence of Any Baseline NS5A RAV >100 fold	19/30	15/19 (78.9)	19/32	17/19 (89.5)	38/62(61.3)	31/38 (81.6)
Presence of Any Baseline NS5A RAV with >1000-fold	10/30	7[b]/10 (70.0)	13/32	12[d]/13(92.3)	23/62 (37.1)	19[b,d]/23 (82.6)
SVR12 for Subjects with RAVs (%)	-	26[a,b]/30 (86.7)	-	29[c,d,e]/32 (90.6)	-	55/62 (88.7)

a One GT1a subject with M28T(1.03%); Q30R(>99%); L31M(>99%) at BL relapsed
b Three subjects relapsed: One GT 1a subject with Q30H(98.76%); Y93H(98.07%); One GT 1a with Q30R(1.43%) and Y93N(97.60%); and one GT 1b subject with Y93H(59.82%).
c One GT 1a subject with L31M(>99%) relapsed
d One GT 1a subject with Y93H(1.20%) relapsed
e Subject discontinued study treatment due to lack of efficacy associated with documented noncompliance

n/N = Number of subjects with RAVs / Number of subjects successfully sequenced
Data Source: iVSR Virology Listing 35

In comparison, FDA virology performed an analysis examining relapse rates by similar groups with any change at K24 or M28 included in the <100-fold resistance group, any change at Q30 or L31 included in the 100 to 1,000-fold resistance group, and Y93H/N/C and any change at H58 included in the >1,000-fold resistance group (Table 43). From this analysis and review of the sponsor's submitted data above, FDA virology concludes that relapse still occurs in the 8-week arms when NS5A resistance-associated polymorphisms in the <1,000-fold resistance groups are present at baseline. The data are not supportive of using the NS5A resistance polymorphism groups of <100-fold, 100 to 1,000-fold or >1,000-fold for determining appropriate regimen duration. Furthermore, longer durations of 12-weeks for treatment-naives and 24-weeks for treatment-experienced reduce relapse rates to 0% for subjects with any baseline NS5A resistance polymorphisms.

Table 43. Relapse Rates by Phenotypic Resistance Groups

GT1 (NS5A)	Any BL NS5A	<100-fold NS5A RAVs	100-1000-fold NS5A RAVs	>1000-fold NS5A RAVs
Study 108 naïve	4.6% (7/153)	3.3% (2/60)	5.1% (2/39)	7.1% (5/70)
LDV/SOF 8 WK	6.3% (3/48)	5.3% (1/19)	10% (1/10)	4.5% (1/22)
LDV/SOF 12WK	0% (0/56)	0% (0/25)	0% (0/14)	0% (0/22)
LDV/SOF+RBV 8WK	8% (4/49)	6.7% (1/15)	6.7% (1/15)	15% (4/26)
Study 109	7.2% (6/83)	6.9% (2/29)	30% (3/10)	9.7% (3/31)
LDV/SOF 12 WK	22% (5/23)	18% (2/11)	25% (2/8)	43% (3/7)
LDV/SOF 24 WK	0% (0/19)	0% (0/5)	0% (0/9)	0% (0/9)
LDV/SOF+RBV 12WK	4.5% (1/22)	0% (0/6)	14% (1/7)	0% (0/7)
LDV/SOF+RBV 24WK	0% (0/19)	0% (0/7)	0% (0/6)	0% (0/8)

<100-fold RAVs: M28L/V/T/A/I or K24R/Q/G
100-1,000-fold RAVs: L31M/I/V, Q30H/R/L/H/T/S/Y
>1,000-fold RAVs: Y93H, Y93N, Y93C, H58P/C/R/S/T/Q/N/Y

VIROLOGIC FAILURES

In total from all the studies submitted, there were 50 virologic failures (GT1a n=42; GT1b =8). See Appendix A for details of virologic failures. One GT1b-infected subject in Study 102 and one GT1a-infected subject in Study 109 experienced breakthrough. All remaining failures (n=48) were relapsers: 41 GT1a and 7 GT1b.

In Study 102, Subject 5663-71589, a non-cirrhotic subject with GT1b chronic HCV infection and IL28 CT allele was randomized to the LDV/SOF 24-week treatment arm and completed 12 weeks treatment with 88% adherence. The subject was suppressed at Week 6, but at Week 8 had breakthrough with detected HCV RNA and emergence of the Y93H substitution. At Week 12, the subject discontinued treatment due to documented noncompliance. Y93H was also detected 4 weeks post-treatment (>99%). No NS5B nucleotide inhibitor resistance-associated substitutions were detected at any of these visits. Phenotypic analysis of NS5A and NS5B genes showed >310-fold resistance to LDV, but no resistance to SOF.

The subject in Study 109 who had breakthrough (Subject 79383) never achieved undetectable levels of HCV RNA and rebounded at Week 2. This subject had IL28B genotype CC without cirrhosis. At baseline this subject had the NS5A polymorphism M28M/V. At the breakthrough Week 2 timepoint, this subject had emergent NS5A substitutions K24K/R, M28V, and Q30R. The sponsor states the breakthrough in this subject was due to documented noncompliance.

There was only 1 relapser in Study 102 and this subject was GT1a, IL28B non-CC and cirrhotic with a baseline viral load of 19,600,000 IU/mL. In the other phase 3 studies, Studies 108 and 109, there were 23 (19 GT1a and 4 GT1b) and 11 (8 GT1a and 3 GT1b) relapsers, respectively (Table 44). In the phase 2 studies, Study 118 (LONESTAR) and Study 0523 (ELECTRON), there were 2 and 11 relapsers, respectively, (all GT1a).

Table 44. Subjects with No SVR from All Submitted Studies

	Study 102	**Study 108**	**Study 109**	**Study 118**	**Study 523**	**Total**
NS5A GT1a						
No SVR	11	34	10	3	11	69
DAA Breakthrough			**1**			**1**
Relapse	1	**19**	**8**	2	11	**41**
NS5A GT1b						
No SVR	1	4	3			8
DAA Breakthrough	1					1
Relapse		**4**	**3**			**7**
TOTAL FAILURES	2	23	12	2	11	50

Most of the relapsers were non-CC (90% GT1a, 100% GT1b) (Table 45). Twenty-four percent of the GT1a relapsers and 29% of the GT1b relapsers were cirrhotic, with the caveat that cirrhotics were not studied in Study 108. In addition, 40% (19/48) of the relapsers (39% of the GT1a relapsers and 43% of the GT1b relapsers) had baseline NS5A resistance polymorphisms.

Table 45. Number of Relapsers and Baseline Characteristics in Each Study/Arm

GT	**STUDY**	**ARM**	**RELAPSES (N)**	**NON-CC**	**CIRR**	**BASELINE NS5A**
1A	102	LDV/SOF 12WK	1	1	1	1
	108	LDV/SOF+ RBV 8 WK	7	7	0	3
	108	LDV/SOF 12 WK	2	2	0	0
	108	LDV/SOF	10	8	0	3

		8WK				
	109	LDV/SOF+ RBV 12 WK	4	4	4	1
	109	LDV/SOF 12 WK	4	4	1	3
	118	LDV/SOF 12 WK	1	1	1	1
	118	LDV/SOF 8 WK	1	1	0	1
	0523	LDV/SOF 12 WK	3	2	3	1
	0523	LDV/SOF+ RBV 6 WK	8	7	0	2
	TOTAL		41	37 (90%)	10 (24%)	16 (39%)
1B	108	LDV/SOF+ RBV 8 WK	2	2	0	1
	108	LDV/SOF 12 WK	1	1	0	0
	108	LDV/SOF 8WK	1	1	0	0
	109	LDV/SOF+ RBV 12 WK				
	109	LDV/SOF 12 WK	3	3	2	2
	TOTAL		7	7 (100%)	2 (29%)	3 (43%)

Relapse rates in treatment-naïve subjects and treatment-experienced subjects were similar: 3.6% in treatment-naïve and 2.5% in treatment-experienced (Table 46). As shown above, the relapse rates for treatment-experienced subjects with baseline NS5A resistance polymorphisms was slightly higher at 7.2% compared to 4.6% for treatment-naïve subjects with baseline NS5A resistance polymorphisms. The proportion of treatment-experienced failure subjects that had baseline NS5A resistance polymorphisms was 55%, higher than the 30% of treatment-naïve subjects with baseline NS5A resistance polymorphisms.

Table 46. Relapse Rates in Treatment-Naïve vs. Treatment-Experienced Subjects

	Relapse Rates	**#Subjects with Baseline NS5A Substitutions**	**Relapse Rates for Subjects with Baseline NS5A Substitutions**	**Proportion of Relapsers with Baseline NS5A Subsitutions**
Study 108 Naive	3.6% (23/645)	24% (153/645)	4.6% (7/153)	30% (7/23)
Study 109 Treatment-exp	2.5% (11/440)	19% (83/440)	7.2% (6/83)	55% (6/11)

EMERGENT NS5A SUBSTITUTIONS

Treatment-emergent NS5A resistance substitutions at NS5A amino acid positions K24, M28, Q30, L31, P32, H58, A92 and Y93 were analyzed in the 50 LDV/SOF treatment failures. Overall, 55% (23/42) of the GT1a failures and 88% (7/8) of the GT1b failures had emergent NS5A resistance substitutions at these amino acid posititons. In the phase 3 studies, 62% (23/37) of the failures had emergent NS5A resistance substitutions. A higher proportion of the failure subjects who had treatment-emergent NS5A substitutions were treatment-experienced subjects compared to treatment-naïve subjects (75% vs. 56%) (Table 47).

Table 47. Proportion of Failures* with NS5A Substitutions

	Proportion of Failures* with Emergent NS5A Substitutions	Proportion of Failures* with Baseline NS5A Polymorphisms
GT1a	55% (23/42)	40% (17/42)
GT1b	88% (7/8)	38% (3/8)
GT1 Phase 3 Studies	62% (23/37)	41% (15/37)
Naive	56% (14/25)	32% (8/25)
Treatment-exp	75% (9/12)	58% (7/12)

NS5A substitutions: at positions K24, M28, Q30, L31, P32, H58, A92, Y93
***Includes relapsers and breakthrough subjects (71589) from Study 102 and (79383) from Study 109**

In GT1a failure subjects, the most common emergent NS5A substitutions were substitutions at Q30 and Y93. Of the failures with emergent NS5A substitutions, Q30 and Y93 substitutions each emerged in 43% (10/23) of the failures (Table 48). L31M substitutions and substitutions at M28 emerged in 22% (5/23) and 17% (4/23) of failures, respectively. Other substitutions at K24 and H58 emerged in less than 10% of failures. In GT1b failure subjects, 43% (3/7) had emergent L31 substitutions and 86% (6/7) had emergent Y93 substitutions (Table 48).

The proportion of failure subjects whose viruses had baseline NS5A polymorphisms was 40% (20/50) [13 subjects with 1 polymorphism, 6 subjects with 2 polymorphisms, and 1 subject with 3 polymorphisms]. Many of these subjects whose viruses had baseline NS5A polymorphisms had additional emergent NS5A substitutions at failure (40%, 7/20) and 5 subjects (25%, 5/20) had emergent substitutions from mixtures at baseline. Additionally, 18 subjects had virus with emergent NS5A substitutions when there were no baseline NS5A polymorphisms present. The percentage of subjects whose virus had emergent NS5A substitutions without already existing baseline NS5A polymorphisms was 60% (18/30).

In the phase 3 studies, 37 subjects (29 with genotype 1a and 8 with genotype 1b) qualified for resistance analysis due to virologic failure (35 relapsers, 2 breakthroughs on-treatment because of documented non-compliance). Post-baseline NS5A and NS5B deep sequencing data (assay cutoff of 1%) were available for 37/37 and 36/37 subjects, respectively.

Of the 29 genotype 1a virologic failure subjects, 55% (16/29) of subjects' viruses had emergent NS5A resistance-associated substitutions at positions K24, M28, Q30, L31, or Y93 at failure. The most common variants were Q30R, Y93H or N, and L31M. Of the 8 genotype 1b virologic failure subjects, 88% (7/8) had emergent NS5A resistance-associated substitutions at positions L31 and Y93 at failure. The most common variant was Y93H. In phenotypic analyses, post-baseline isolates from subjects whose viruses harbored NS5A resistance-associated substitutions at failure showed 20- to >243-fold reduced susceptibility to ledipasvir.

In the phase 3 studies, the proportion of virologic failure subjects whose viruses had baseline NS5A resistance-associated polymorphisms was 41% (15/37). Twenty-seven percent (4/15) of these failure subjects whose viruses had baseline NS5A polymorphisms had additional NS5A resistance substitutions emerge at failure. The percentage of subjects without already existing baseline NS5A polymorphisms whose viruses had NS5A resistance substitutions emerge at failure was 41% (15/37). In total, NS5A resistance-associated substitutions were observed in post-baseline isolates from 81% (30/37) of the virologic failure subjects; 38% (14/37) had 2 or more NS5A resistance-associated substitutions.

Table 48. Emergent NS5A Substitutions in SVR12 Failures

	Proportion of Failures with Emergent NS5A Substitutions	**Proportion of Failures with Baseline NS5A Polymorphisms**
GT1a	55% (23/42)	40% (17/42)
K24	4.3% (1/23)	0
M28	17% (4/23)	29% (5/17)
Q30	43% (10/23)	29% (5/17)
L31M	22% (5/23)	29% (5/17)
H58	8.7% (2/23)	12% (2/17)
Y93	43% (10/23)	35% (6/17)
GT1b	88% (7/8)	38% (3/8)
K24	0	
L28M	0	33% (1/3)
Q30	0	
L31M/V/I	43% (3/7)	
H58	0	
A92		33% (1/3)
Y93	86% (6/7)	67% (2/3)

The proportion of failures with no NS5A substitutions at failure was 24% (n=12) (Table 49). At failure, 76% of the subjects had 1 or more NS5A substitutions at failure. At failure, 40% (n=20) of subjects had 1 NS5A substitutions, 34% (n=17) had 2 NS5A substitutions and 2% (n=1) had 3 NS5A substitutions at failure.

Table 49. Number of NS5A Substitutions at Failure (n=50)

# NS5A Substitutions at Failure	Proportion of Failures (n)
0	24% (12)
1	40% (20)
2	34% (17)
3	2% (1)

NS5B RESISTANCE ANALYSIS

Emergent NS5B Substitutions

There was one subject (Subject GS-US-337-0118-2760-2504), in the LONESTAR Study 118 whose virus had emergent S282T at relapse (after Follow-up Week 4 and before Follow-up Week 8). This treatment-naïve GT1a non-CC subject received 8-week LDV/SOF and relapsed at follow-up Week 8 after EOT. The phenotypic fold-change in SOF resistance at failure was 11-fold and 25-fold change from baseline. No subject's virus in the phase 3 subjects had emergent S282T substitutions. In addition, no subject's virus had emergent L159F, C316N, L320F or V321A substitutions. These substitutions were previously identified as related to treatment-failure in the NDA review of the SOF phase 3 studies.

Baseline NS5B Substitutions

In all the submitted studies, there were no subjects whose virus had the S282T polymorphism at baseline. Twenty-two subjects' viruses had the L159F polymorphism in NS5B at baseline (all were GT1b; F is a common polymorphism at this site in GT1b). All 22 subjects achieved SVR12.

There were no subjects whose virus had L320F at baseline. There were 6 subjects whose virus had the V321I polymorphism at baseline. All these subjects except Subject GS-US-337-0109-5518-79003 (non-CC, cirrhotic, 12-weeks LDV/SOF) achieved SVR12. Subject GS-US-337-0109-5518-79003 who received 12-weeks LDV/SOF was IL28B non-CC, cirrhotic and also had the C316H as well as the V321I substitution at baseline and failure. This subject's virus had emergent L31M and Y93H substitutions in NS5A at relapse.

There were two GT1a subjects whose viruses had C316Y at baseline and both achieved SVR12. There were 38 GT1b subjects whose viruses had C316N at baseline and 3 GT1b subjects whose viruses had C316H at baseline. All these subjects except GS-US-337-0109-5518-79003 (described above) achieved SVR12.

There were two other subjects besides Subject GS-US-337-0109-5518-79003 whose viruses had both C316H and V321I polymorphisms at baseline, one treatment-naïve and one treatment-experienced non-cirrhotic, and they both achieved SVR12.

RE-TREATMENT WITH LDV/SOF

Twenty subjects who failed an initial sofosbuvir-containing regimen in the LONESTAR or ELECTRON studies were retreated with LDV/SOF+RBV for 12 weeks (n=19) or 24 weeks (n=1). All twenty subjects achieved SVR upon retreatment.

One subject from the LONESTAR Study GS-US-337-0118 (Subject 2504), was initially treated with LDV/SOF for 8 weeks, and then was retreated with LDV/SOF+RBV for 24 weeks. After the original treatment with SOF and LDV, this subject's virus was found to have multiple resistance substitutions in both NS5A (Q30L, L31M and Y93H) and NS5B, including the S282T SOF resistance substitution. Despite the presence of multiple NS5A resistance substitutions and 8% of S282T at retreatment baseline, this subject achieved SVR12 following retreatment with LDV/SOF+RBV for 24 weeks. This subject received the LDV/SOF + RBV and extended treatment duration of 24 weeks in the retreatment regimen.

The remaining 19 subjects were originally treated in three different arms of the ELECTRON study (P7977-0523) with SOF+RBV (n=10) or SOF+GS-9669+RBV (n=1) for 12 weeks, or with LDV/SOF+RBV for 6 weeks (n=8). Eight subjects in the ELECTRON study who did not achieve SVR12 after 6 weeks of treatment with LDV/SOF+RBV were retreated with the same regimen for 12 weeks. Among these 8 subjects, 3/8 had virus with NS5A resistance substitutions at relapse while 5/8 had no NS5A resistance substitutions. All 8 subjects achieved SVR after retreatment with LDV/SOF+RBV for 12 weeks.

In total, 4 subjects had NS5A substitutions before retreatment (Table 50). The three subjects who received LDV/SOF+RBV for 12 weeks as the retreatment regimen each had only one detectable NS5A resistance substitution before retreatment. The one subject with multiple NS5A substitutions received a 24-week regimen of LDV/SOF+RBV.

Table 50. 4 Subjects with NS5A Substitutions before Retreatment with LDV/SOF+RBV

PID	Studies	First Trt	NS5A Substitutions before Retreatment	Retreatment Regimen	Outcome
2504	118	LDV/SOF 8 WK	**Q30L** (4.5%), **L31M** (>99%), **Y93H** (97%) NS5B: [S282T (91%), L320I (2.7%) L320V (6%)]	LDV/SOF+RBV 24 WK	SVR12
5537/ 77242	523/ 122	LDV/SOF 6 WK	**Q30R** (31%)	LDV/SOF+RBV 12 WK	SVR12
5541/ 77244	523/ 122	LDV/SOF 6 WK	**M28T** (8%)	LDV/SOF+RBV 12 WK	SVR12
5547/ 77241	523/ 122	LDV/SOF 6 WK	**L31M** (>99%)	LDV/SOF+RBV 12 WK	SVR12

NS5A resistance-associated substitutions are bolded

The other 11 subjects who failed initial treatment in the ELECTRON study received study drugs in the retreatment study for the same length of time as in the original trial (12 weeks), but with a modified drug combination. Ten of these subjects initially received

SOF+RBV; addition of LDV to the retreatment regimen led to SVR in 10/10 cases. The other subject had originally been treated with a regimen that included GS-9669, a nonnucleoside NS5B polymerase inhibitor, along with SOF plus RBV. Replacing GS-9669 with LDV and retreating for 12-weeks led to SRV12 in this subject. Because none of these 11 subjects had initially been treated with LDV, the NS5A viral region was not sequenced. Two of the 11 subjects' viruses had the F415Y RBV-associated substitution, and another subject's virus had the L159F nucleotide resistance associated substitution at the time of retreatment. All 11 subjects achieved SVR upon retreatment with LDV/SOF+RBV for 12 weeks.

Interestingly, in their report, the sponsor specifically singles out the 13 treatment-experienced subjects who had viruses containing baseline NS5A resistance polymorphisms and were treated with 24 weeks of LDV/SOF in Study 109 (ION-2) (Table 51; Report PC-337-2005, page 151).

Table 51. Subjects with Baseline NS5A Resistance Substitutions Treated with 24 Weeks of LDV/SOF

Treatment-experienced subjects (Baseline NS5A RAVs detected by deep sequencing)					
Study	Subject ID	Subtype	Baseline NS5A RAVs	Resistance Mutation Category Fold Change	Treatment Outcome
GS-US-337-0109	0057-79200	1a	K24G (>99%)	10-50	SVR12
GS-US-337-0109	1640-79046	1a	K24R (1.04%)	2.5-10	SVR12
GS-US-337-0109	7864-79411	1a	K24R (1.86%)	2.5-10	SVR12
GS-US-337-0109	0529-79217	1a	K24R (10.19%)	2.5-10	SVR12
GS-US-337-0109	4007-79103	1a	K24R (5.04%)	2.5-10	SVR12
GS-US-337-0109	0407-79253	1a	L31M (>99%)	100-1000	SVR12
GS-US-337-0109	5501-79273	1a	L31M (92.24%)	100-1000	SVR12
GS-US-337-0109	2760-79309	1a	L31M (98.60%)	100-1000	SVR12
GS-US-337-0109	5518-79236	1a	Q30H (4.79%); Y93H (4.74%)	>1000	SVR12
GS-US-337-0109	2186-79239	1a	Q30R (2.67%)	100-1000	SVR12
GS-US-337-0109	6833-79354	1a	Q30T (28.98%)	2.5-100	SVR12
GS-US-337-0109	5847-79108	1a	Y93H (1.13%)	>1000	SVR12
GS-US-337-0109	2487-79434	1b	L31I (1.19%)	10-50	SVR12
Treatment-naïve subjects (Baseline NS5A RAVs detected by population sequencing)					
Study	Subject ID	Subtype	Baseline NS5A RAVs	Resistance Mutation Category Fold Change	Treatment Outcome
GS-US-337-0102	4488-71174	1a	L31L/M	100-1000	SVR12
GS-US-337-0102	5847-71106	1a	M28T	50-100	SVR12
GS-US-337-0102	4421-71057	1a	Q30H	100-1000	SVR12
GS-US-337-0102	3060-71132	1a	Y93Y/C	>1000	SVR12
GS-US-337-0102	2111-71032	1b	Y93Y/H	>1000	SVR12
GS-US-337-0102	1516-71199	1b	Y93Y/H	>1000	SVR12

Twelve of these subjects were infected with GT1a HCV, and one had GT1b virus. In addition, the sponsor reports 6 treatment-naïve subjects in ION-1 study whose virus had baseline NS5A resistance associated polymorphisms (4 GT1a and 2 GT1b) and who received 24 weeks of LDV/SOF treatment. All 19 of these subjects achieved SVR12. Again, each of these subjects' viruses had one baseline NS5A resistance polymorphism, with the exception of one subject (79236) who had 2 NS5A polymorphisms.

The sponsor states, "LDV/SOF 24-week treatment resulted in SVR12 in all 6 treatment-experienced subjects having NS5A resistance substitutions corresponding to >100-fold resistance to LDV. These subjects can be considered surrogates for those who have failed a prior NS5A-inhibitor containing regimen, and suggest that re-treatment with 24 weeks of LDV/SOF may be successful in this group."

5. CONCLUSION

This NDA for the fixed-dose combination of LDV and SOF is approvable with respect to virology. LDV/SOF is highly effective in chronic HCV subjects with GT1 HCV and demonstrated overall relapse rates of 2.7% in 3 Phase 3 studies. Based on the virology review of this NDA and the lower relapse rates for subjects with baseline NS5A resistance polymorphisms, we recommend the 12-week duration for treatment-naïve HCV subjects. We recommend the 12-week duration of LDV/SOF for treatment-experienced subjects without cirrhosis or other poor baseline predictors such as baseline NS5A resistance polymorphisms, IL28B non-CC, high baseline viral load, and cirrhosis. For treatment-experienced subjects with cirrhosis and/or poor baseline predictors, we recommend the 24-week duration of LDV/SOF. Given the promising but still uncertain retreatment options for LDV/SOF virologic failures, we recommend minimizing the risk of relapse and giving patients the optimal chance of achieving SVR with their first LDV/SOF regimen, especially patients with cirrhosis.

6. PACKAGE INSERT

APPLICANT PROPOSED PACKAGE INSERT

12.4 Microbiology

Mechanism of Action

(b) (4)

Sofosbuvir is an inhibitor of the HCV NS5B RNA-dependent RNA polymerase, which is essential for viral replication. Sofosbuvir is a nucleotide prodrug that undergoes intracellular metabolism to form the pharmacologically active uridine analog triphosphate (GS-461203), which can be incorporated into HCV RNA by the NS5B polymerase and acts as a chain terminator. In a biochemical assay, GS-461203 inhibited the polymerase activity of the recombinant NS5B from HCV genotype 1b, 2a, 3a and 4a with IC_{50} values ranging from 0.7 to 2.6 μM. GS-461203 is (b) (4) 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 genotype 1a and 1b were 0.031 nM and 0.004 nM, respectively. The median EC_{50} of ledipasvir against chimeric replicons encoding NS5A sequences from clinical isolates (b) (4) 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 μM; N=3). (b) (4)

In HCV replicon assays, the EC_{50} values of sofosbuvir against full-length replicons from genotype 1a, 1b, 2a, 3a and 4a, and chimeric 1b replicons encoding NS5B from genotype 2b, 5a or 6a ranged from (b) (4) The median EC_{50} value of sofosbuvir against chimeric replicons encoding NS5B sequences from clinical isolates was (b) (4) for genotype 1a (range (b) (4); N=67), (b) (4) for genotype 1b (range (b) (4); N=29) (b) (4) for genotype 2 (range (b) (4); N=15) and (b) (4) for genotype 3a (range (b) (4); N=106). In (b) (4) virus assays, the EC_{50} values of sofosbuvir against genotype 1a and 2a were (b) (4) respectively. (b) (4)

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 genotype 1a and 1b. Reduced susceptibility to ledipasvir was associated with the primary NS5A substitution Y93H in both genotype 1a and 1b. Additionally a Q30E substitution emerged in genotype 1a replicons. Site-directed mutagenesis of the Y93H in both genotype 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, 2a, 2b, 3a, 4a, 5a and 6a. Reduced susceptibility to sofosbuvir was associated with the primary NS5B substitution S282T in all replicon genotypes examined. Site-directed mutagenesis of the S282T substitution in replicons of 8 genotypes conferred 2- to 18-fold reduced susceptibility to sofosbuvir (b) (4)

In Clinical Trials

In a pooled analysis of subjects who received [TRADENAME] in Phase 3 trials, 37 subjects (29 with genotype 1a and 8 with genotype 1b) qualified for resistance analysis due to virologic failure (b) (4) Post-baseline NS5A and NS5B deep sequencing data (assay cutoff of 1%) were available for 37/37 and 36/37 subjects, respectively.

(b) (4)

Cross Resistance

Ledipasvir was fully active against the sofosbuvir resistance-associated substitution S282T in NS5B while all ledipasvir resistance-associated substitutions in NS5A were fully susceptible to sofosbuvir. Both sofosbuvir and ledipasvir were fully active against substitutions associated with resistance to other classes of direct acting antivirals with different mechanisms of actions, such as NS5B non-nucleoside inhibitors and NS3 protease inhibitors.

Effect of Baseline HCV (b) (4) on Treatment Outcome

Analyses were conducted to explore the association between pre-existing baseline NS5A (b) (4) In the pooled analysis of the Phase 3 trials, 256/ (b) (4) (16%) subjects had baseline NS5A (b) (4) identified by population or deep sequencing irrespective of subtype.

In treatment-naïve subjects with NS5A RAVs, SVR12 rates of 89% (34/38) after 8 weeks and 96% (69/72) after 12 weeks of therapy were observed with [TRADENAME]. No association between any individual NS5A RAV or group of RAVs and treatment outcome was observed.

In treatment-experienced subjects who had baseline NS5A (b) (4)

(b) (4)

FDA PROPOSED PACKAGE INSERT

12.4 Microbiology

Mechanism of Action

(b) (4)

Sofosbuvir is an inhibitor of the HCV NS5B RNA-dependent RNA polymerase, which is (b) (4) for viral replication. Sofosbuvir is a nucleotide prodrug that undergoes intracellular metabolism to form the pharmacologically active uridine analog triphosphate (GS-461203), which can be incorporated into HCV RNA by the NS5B polymerase and acts as a chain terminator. In a biochemical assay, GS-461203 inhibited the polymerase activity of the recombinant NS5B from HCV genotype 1b, 2a, 3a and 4a with IC_{50} values ranging from 0.7 to 2.6 μM. GS-461203 is (b) (4) 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 genotype 1a and 1b were 0.031 nM and 0.004 nM, respectively. The median EC_{50} of ledipasvir against chimeric replicons encoding NS5A sequences from clinical isolates (b) (4) 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 μM; N=3). Ledipasvir has 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. (b) (4) has substantially lower activity against genotypes 2a, 2b, 3a and 6e with EC_{50} values of 21 - 249 nM, 16 - (b) (4) nM, 168 nM and 264 nM, respectively. In HCV replicon assays, the EC_{50} values of sofosbuvir against full-length replicons from genotype 1a, 1b, 2a, 3a and 4a, and chimeric 1b replicons encoding NS5B from genotype 2b, 5a or 6a ranged from 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), 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). In (b) (4) virus assays, the EC_{50} values of sofosbuvir against genotype 1a and 2a were 30 and 20 nM, respectively.

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 genotype 1a and 1b. Reduced susceptibility to ledipasvir was associated with the primary NS5A substitution Y93H in both genotype 1a and 1b. Additionally a Q30E substitution emerged in genotype 1a replicons. Site-directed mutagenesis of the Y93H in both genotype 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 1,000-fold).

HCV replicons with reduced susceptibility to sofosbuvir have been selected in cell culture for multiple genotypes including 1b, 2a, 2b, 3a, 4a, 5a and 6a. Reduced susceptibility to sofosbuvir was associated with the primary NS5B substitution S282T in all replicon genotypes examined. Site-directed mutagenesis of the S282T substitution in replicons of 8 genotypes conferred 2- to 18-fold reduced susceptibility to sofosbuvir.

In Clinical Trials

In a pooled analysis of subjects who received [TRADENAME] in Phase 3 trials, 37 subjects (29 with genotype 1a and 8 with genotype 1b) qualified for resistance analysis due to virologic failure (35 (b) (4), 2 breakthroughs on-treatment (b) (4) documented non- (b) (4)). Post-baseline NS5A and NS5B deep sequencing 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 emergent NS5A resistance-associated substitutions at positions (b) (4) at failure. The most common emergent NS5A substitutions were Q30R, Y93H or N, and L31M.

Of the 8 genotype 1b virologic failure subjects, 88% (7/8) had emergent NS5A resistance-associated substitutions at positions (b) (4) at failure. The most common (b) (4) was Y93H.

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.

(b) (4)

The sofosbuvir-associated resistance substitution S282T in NS5B was not detected in any failure isolate from the Phase 3 trials. (b) (4) in combination with NS5A substitutions L31M, Y93H and Q30L were detected in one subject at failure following 8 week treatment with [TRADENAME] from a Phase 2 trial [LONESTAR].

Cross Resistance

Ledipasvir was fully active against the sofosbuvir resistance-associated substitution S282T in NS5B while all ledipasvir resistance-associated substitutions in NS5A were fully susceptible to sofosbuvir. Both sofosbuvir and ledipasvir were fully active against substitutions associated with resistance to other classes of direct-acting antivirals with different mechanisms of actions, such as NS5B non-nucleoside inhibitors and NS3/4A protease inhibitors. NS5A substitutions conferring resistance to ledipasvir may reduce the antiviral activity of other NS5A inhibitors. The efficacy of (b) (4) has not been

studied in patients who have previously failed treatment with other regimens that include an NS5A inhibitor.

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.

Effect of Baseline HCV (b) (4) /Polymorphisms on Treatment (b) (4)

Analyses were conducted to explore the association between pre-existing baseline NS5A (b) (4) and relapse rates. In the pooled analysis of the Phase 3 trials, 23% (370/ (b) (4)) subjects had baseline NS5A (b) (4) polymorphisms(any change at NS5A amino acid positions (b) (4) identified by population or deep sequencing. (b) (4)

In treatment-naïve subjects (b) (4)

In treatment-experienced subjects whose virus had baseline NS5A (b) (4) , relapse rates were 22% (5/23) after 12 weeks and 0% (0/19) after 24 weeks of treatment with [TRADENAME].

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 NS5B nucleoside inhibitors. The sofosbuvir resistance-associated substitution S282T was not detected in the baseline NS5B sequence of any subject in Phase 3 trials by population or deep sequencing.

7. APPENDIX A: Listing of Virologic Failures

GT1A VIROLOGIC FAILURES (N = 42)

PID	Study	Arm	HCVHIS	NON RECAT	GT	IL28	Cirr	BL Viral Load	Baseline NS5A	Emergent NS5A Substitutions	Failure Pheno	NS5B and NS3/4A
71276	102	FDC 12WK	NAÏVE	Relapse	1a	TT	Y	19600000	**L31M** R44R/K S85N R331K/E V352V/I	R331E V352I	BL: LDV>42	
79383	109	FDC+RBV 24 WK	P/R EXP BT OR REL	DAA BT WK2	1a	CC	N	9860000	**M28M/V** M53M/V I63I/V T64A R73R/K I74I/V V75V/I R78K K107T S131T T135A I144V E171D R176R/K E181D P194L V196A/T A197V T200A G215G/R M226E/L/V A245T R293D S310A/V R311P V315I V326L T328T/A R348Q K357R V361V/M T367N/S L368V G390G/S T394T/P G403A V410A G439E D441G	**K24K/R M28V Q30R** V37M R73K R176K V196A G215R M226E T367S S384T T394S C404C/S P405P/S	LDV >42	
73185	108	FDC+RBV 8WK	NAIVE	Relapse	1a	TT	N	6540000	**M28V Q30H/R** T64A R78K M83V **Y93Y/H** C98S	**Q30R** P223P/A	BL: LDV>42	
73277	108	FDC+RBV 8WK	NAIVE	Relapse	1A	CT	N	7940000		**L31M**	LDV>58	
73408	108	FDC 8WK	NAIVE	Relapse	1A	TT	N	19900000	V37L/W R44K T64T/S S85N	V37L	NO POST BL	
73514	108	FDC 8WK	NAIVE	Relapse	1A	CT	N	13600000			LDV=2	SOF=1.7 NS5B: E237G

												G549S W573L A580T
73445	108	FDC+RBV 8WK	NAIVE	Relapse	1A	CT	N	1670000	F36L R44K **H58P**		LDV=2	
73416	108	FDC+RBV 8WK	NAIVE	Relapse	1A	CT	N	7930000	R44K V75A/V S85S/N	V75A S85N	LDV<1	
73564	108	FDC+RBV 8WK	NAIVE	Relapse	1A	TT	N	2470000	R44K S85N	S38F **Y93H**	LDV>42	
73033	108	FDC 8WK	NAIVE	Relapse	1A	CT	N	3850000	**L31L/M** R44R/K	**Q30R L31M** R44K	LDV>45 BL=7.4	
73313	108	FDC 8WK	NAIVE	Relapse	1A	CC	N	10700000	R44R/K R78R/K	**Q30Y** R78K **Y93H** L158I	LDV>52	SOF=2.3
73385	108	FDC+RBV 8WK	NAIVE	Relapse	1A	CT	N	4000000	V75T **Y93N**		BL LDV>58 NO POST BL	
73538	108	FDC 8WK	NAIVE	Relapse	1A	CT	N	7420000	R78K	**Q30R**	LDV>58	
73453	108	FDC 8WK	NAIVE	Relapse	1A	CC	N	6300000	K20R V37L V75A	K20G **Y93H**	LDV>42	
73490	108	FDC 8WK	NAIVE	Relapse	1A	CT	N	23800000	R44K/R V46V/A	**Q30R** R44K V46A	LDV>52	
73078	108	FDC 12WK	NAIVE	Relapse	1A	CT	N	15000000		I74I/V **Y93N**	LDV>42	
73227	108	FDC 8WK	NAIVE	Relapse	1A	CT	N	8520000	**Y93Y/N**	**Y93N**	LDV>42	
73610	108	FDC+RBV 8WK	NAIVE	Relapse	1A	CT	N	17700000	R78K S85S/N I121I/V	S85N I121V	LDV<1	
73274	108	FDC 8WK	NAIVE	Relapse	1A	CT	N	26400000	**M28T** R78K		LDV=20 BL LDV=18	
73114	108	FDC 8WK	NAIVE	Relapse	1A	CT	N	1520000	R78K		LDV= 1.6	
73230	108	FDC 12WK	NAIVE	Relapse	1A	CT	N	5620000	T64S V75A		LDV<1	
79378	109	FDC 12WK	PR EXP PARTIAL	Relapse	1A	CT	N	15000000	**Q30H** R44K S85N **Y93H**		BL LDV >42	NS3: R80K
79034	109	FDC+RBV 12WK	PR DAA (BOC) EXP NR	Relapse	1A	TT	Y	5170000	**L31M** R44K R78K	**Q30H**	LDV= 143	NS3: S181A
79062	109	FDC 12WK	PR DAA (TLV)EXP BT OR RELAPSE	Relapse	1A	CT	Y	844000		T21T/I **Y93H**	LDV=20	NS3: V36M Q80K R155K
79303	109	FDC 12WK	PR DAA (TLV)EXP BT OR RELAPSE	Relapse	1A	CT	N	734000	**Q30R L31M** R44K R73K V75A R78K R81W T99N		BL LDV>42	NS3: R155T D168N
79041	109	FDC+RBV 12WK	PR EXP BT OR RELAPSE	Relapse	1A	CT	Y	13700000	R44K R78K	**M28T Q30R**	LDV=30	NS3: R80K
79063	109	FDC+RBV 12WK	PR DAA (TLV)EXP NR	Relapse	1A	CT	Y	17500000	T64T/A S85D/N	**Q30K** T64A D85N	LDV=24	NS3: I170V
79070	109	FDC+RBV 12WK	PR EXP BT OR RELAPSE	Relapse	1A	CT	Y	13000000	G2E R6K T21S I37V/I V46T I79T/I S85N	**Q30L/R Y93H**	LDV>42	NS3: T54S R80K
79214	109	FDC 12WK	P/R DAA(TLV) EXP NR	Relapse	1A	CT	N	16400000	**M28V** R44K R78K **Y93N** T99S		LDV>42	
2504	118	FDC 8WK	NAIVE	Relapse	1A	CT	N	2940500	**L31L/M** R44K	**L31M Y93H**	LDV>79 1 BL LDV=	S282T (11X)

											2.4	
2635	118	FDC 12WK	PR DAA (BOC) EXP NR	Relapse	1A	CT	Y	8387599	**Q30H** R44K R78L R81K **Y93H**	I34I/V	BL LDV= 126	
5504	0523	FDC 12WK	PR EXP NULL	Relapse	1A	TT	Y	408000	F36L	**Y93C/H**	LDV>42	
5506	0523	FDC 12WK	PR EXP NULL	Relapse	1A	TT	Y	2520000	F36L I37L R48Q R78K	**H58R Y93H**	LDV>42	
5513	0523	FDC 12WK	PR EXP NULL	Relapse	1A	CC	Y	6950000	**M28V Q30H** P77/S R78K	**M28A** P77S	LDV>42 BL LDV=17	
5534	0523	FDC+RBV 6WK	NAIVE	Relapse	1A	CC	N	4370000	R48Q R78K		LDV= 1.2	
5541	0523	FDC+RBV 6WK	NAIVE	Relapse	1A	CT	N	6060000	R78K		LDV<1	
5545	0523	FDC+RBV 6WK	NAIVE	Relapse	1A	CT	N	4100000	F36L **H58H/P** E62E/D	**H58P** E62D	LDV= 2.8	
5547	0523	FDC+RBV 6WK	NAIVE	Relapse	1A	CT	N	8870000	**M28M/V** R44R/K R78K	**M28V L31M** R44K	LDV>42	
5548	0523	FDC+RBV 6WK	NAIVE	Relapse	1A	TT	N	9580000	F36L R78K/R	I37V/M	LDV<1	
5551	0523	FDC+RBV 6WK	NAIVE	Relapse	1A	CT	N	10200000	R78K	I37M	LDV<1	
5555	0523	FDC+RBV 6WK	NAIVE	Relapse	1A	CT	N	700000	I37M R78K		LDV= 1.4	
5537	0523	FDC+RBV 6WK	NAIVE	Relapse	1A	CT	N	10200000	R44R/K R78K S85N	**Q30Q/R**	LDV<1	

INSTI Resistance Polymorphisms/Substitutions Bolded

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA: 205834 **SDN**: 000 **DATE REVIEWED:** 07/10/14
Virology Reviewer: Lisa K. Naeger, Ph.D.

GT1B VIROLOGIC FAILURES (N = 8)

PID	Study	Arm	HCVHIS	NON RECAT	GT	IL28	Cirr	BL Viral Load	Baseline NS5A	Emergent NS5A Substitutions	Failure Pheno	NS5B and NS3/4 A
71589	102	FDC 24 WK	NAIVE	BT non-compliant	1B	CT	N	2440000		**Y93H**	>208	
73335	108	FDC+RBV 8 WK	NAIVE	Relapse	1B	TT	N	6070000	F37L Q54H R78K **Y93Y/H**	**Y93H**	LDV>192 BL LDV=2	
73124	108	FDC 12 WK	NAIVE	Relapse	1B	TT	N	1610000	T17T/S F37I T64A	T17S **L31I** **Y93H**	LDV>212	
73049	108	FDC+RBV 8 WK	NAIVE	Relapse	1B	TT	N	31700000	K44R T64S		LDV=1.5	
73300	108	FDC 8WK	NAIVE	Relapse	1B	TT	N	14200000	T17S F37L K44K/R Q54H Q62E T79T/A	K44R T79A **Y93H**	LDV >243	
79179	109	FDC 12WK	PR NULL	Relapse	1B	CT	N	5330000	T17S T21S **L28M** V34V/L F37V T64A R78K **Y93Y/H**	V34L **Y93H**	LDV>243 BL LDV =21	NS3: V170I
79003	109	FDC 12WK	PR +DAA (TLV) BT OR RELAPSE	Relapse	1B	CT	Y	4370000	T17S F37F/L T64T/A R78K	**L31M** F37L T64A **Y93H**	LDV=143	NS5B: C316H V321I
79051	109	FDC 12WK	PR NULL	Relapse	1B	CT	Y	5120000	F37L K44K/R Q54H R78K **A92T**	**L31V** K44R	LDV=109	

INSTI Resistance Polymorphisms/Substitutions Bolded

APPENDIX B:

Response by Baseline Factors

SVR12 Response Rate by Baseline IL28b Genotype and Cirrhosis

	CC	CT	TT	Non-CC	Cirrhosis	No Cirrhosis	Non-CC and Cirrhotic
GT1a (NS5A)							
Naive							
LDV/SOF+RBV 12WK (N=175)	97% (65/67)	96% (78/81)	100% (26/26)	**97% (104/107)**	**100% (42/42)**	96% (127/132)	100% (27/27)
LDV/SOF+RBV 24WK (n= 38)	92% (11/12)	100% (21/21)	100% (5/5)	**100% (26/26)**	**100% (8/8)**	97% (29/30)	100% (7/7)
LDV/SOF+RBV 8WK (n=189)	95% (52/55)	93% (105/113)	90% (19/21)	**93% (124/134)**		93% (176/189)	
LDV/SOF 12WK (n=329)	98% (86/88)	94% (168/179)	97% (60/62)	**95% (228/241)**	**90% (19/21)**	96% (293/306)	87% (13/15)
LDV/SOF 24WK (n=39)	(12/12)	(18/18)	(9/9)	**(27/27)**	**(7/7)**	(32/32)	(6/6)
LDV/SOF 8WK (n=186)	96% (45/47)	92% (97/106)	94% (31/33)	**92% (128/139)**		93% (173/186)	
Treatment-Experienced							
LDV/SOF+RBV 12WK (n=120)	100% (12/12)	96% (73/76)	97% (31/32)	**96% (104/108)**	**90% (38/42)**	100% (77/77)	90% (35/39)
LDV/SOF+RBV 24WK (n=87)	94% (15/16)	100% (54/54)	100% (17/17)	**100% (71/71)**	**100% (18/18)**	99% (68/69)	100% (16/16)
LDV/SOF 12WK (n=108)	92% (11/12)	93% (66/71)	92% (23/25)	**93% (89/96)**	**86% (30/35)**	96% (70/73)	87% (27/31)
LDV/SOF 24WK (n=85)	100% (12/12)	100% (53/53)	95% (19/20)	**99% (72/73)**	**100% (18/18)**	98% (65/66)	100% (16/16)
GT1b (NS5A)							
Naive							
LDV/SOF+RBV 12WK (N=72)	100% (20/20)	98% (40/41)	100% (11/11)	**98% (51/52)**	**100% (16/16)**	98% (54/55)	100% (11/11)
LDV/SOF+RBV 24WK (n=13)	2/2	7/7	4/4		**2/2**	11/11	
LDV/SOF+RBV 8WK (n=48)	12/12	26/26	80% (8/10)			96% (46/48)	
LDV/SOF 12WK (n=114)	20/20	70/70	96% (23/24)		**12/12**	99% (101/102)	10/10
LDV/SOF 24WK (n=12)	3/3	5/5	4/4		**1/1**	11/11	1/1
LDV/SOF 8WK (n=47)	12/12	25/25	90% (9/10)			98% (46/47)	
Treatment-Experienced							

LDV/SOF+RBV 12WK (n=33)	3/3	25/25	5/5		**10/10**	23/23	8/8
LDV/SOF+RBV 24WK (n=23)	2/2	13/13	8/8		**4/4**	19/19	4/4
LDV/SOF 12WK (n=30)	4/4	81% (13/16)	10/10		**75% (6/8)**	95% (21/22)	71% (5/7)
LDV/SOF 24WK (n=23)	4/4	14/14	5/5	**19/19**	**4/4**	19/19	4/4

Naïve studies = 523, 118, 102 and 108
Treatment-experienced studies = 523, 118 and 109

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/s/

LISA K NAEGER
07/10/2014

JULIAN J O REAR
07/10/2014

Product Quality Microbiology Review

3/28/2014

NDA 205834:

Drug Product Name
Proprietary:
Non-proprietary: ledipasvir/sofosbuvir, LDV/SOF

Review Number: 1

Dates of Submission(s) Covered by this Review

Submit	Received	Review Request	Assigned to Reviewer
2/08/2014	2/10/2014	2/10/2014	2/11/2014
3/25/2014	3/25/2014	N/A	N/A

Submission History (for 2nd Reviews or higher)
None

Applicant/Sponsor
Name: Gilead Sciences, Inc.
Address: 333 Lakeside Drive, Foster City, CA 94404
Representative: Michele Anderson, Regulatory Affairs
Telephone: (650) 522-5489

Name of Reviewer: Steven P. Donald, M.S.

Conclusion: Recommended for Approval

Product Quality Microbiology Data Sheet

A. 1. TYPE OF SUBMISSION: Original NDA submission

2. SUBMISSION PROVIDES FOR: Manufacturing and marketing of an oral solid drug product

3. MANUFACTURING SITE:

(b) (4)

Gilead Sciences Limited
IDA Business and Technology Park
Carrigtohill, Co. Cork
Ireland

4. DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY:

- Dosage Form: Tablet
- Route of Administration: Oral
- Strength/Potency: 90 mg/400 mg
- Container: High density polyethylene (HDPE) bottle and a (b) (4) child-resistant cap

5. METHOD(S) OF STERILIZATION: N/A

6. PHARMACOLOGICAL CATEGORY: Anti-viral (treatment of chronic hepatitis C in adults)

B. SUPPORTING/RELATED DOCUMENTS: None

C. REMARKS: An information request was sent to the sponsor on 2/28/2014; a response was received 3/25/2014 and is reviewed herein.

filename: N205834r1.doc

Executive Summary

I. Recommendations

A. Recommendation on Approvability - Recommended for Approval –

B. Recommendations on Phase 4 Commitments and/or Agreements, if Approvable – N/A

II. Summary of Microbiology Assessments

A. Brief Description of the Manufacturing Processes that relate to Product Quality Microbiology -
The tableted product is manufactured [redacted] (b) (4)

B. Brief Description of Microbiology Deficiencies –
No product quality microbiology deficiencies were identified based upon the information provided.

C. Assessment of Risk Due to Microbiology Deficiencies –
N/A

D. Contains Potential Precedent Decision(s)- ☐ **Yes** ☒ **No**

III. Administrative

A. Reviewer's Signature _____
Steven P. Donald, M.S.
Microbiology Reviewer

B. Endorsement Block _____
Stephen Langille, Ph.D.
Senior Microbiology Reviewer

C. CC Block
N/A

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/s/

STEVEN P DONALD
03/31/2014

STEPHEN E LANGILLE
03/31/2014

VIROLOGY FILING CHECKLIST FOR NDA or Supplement

NDA Number: 205834 **Applicant:** Gilead Sciences, Inc. **Stamp Date:** February 10, 2014

Drug Name: Ledipasvir/sofosbuvir FDC **NDA Type:** PDUFA V NME **PDUFA Goal Date:** October 10, 2014

On **initial** overview of the NDA application for filing:

	Content Parameter	Yes	No	Comments
1	Is the virology information (nonclinical and clinical) provided and described in different sections of the NDA organized in a manner to allow substantive review to begin?	X		
2	Is the virology information (nonclinical and clinical) indexed, paginated and/or linked in a manner to allow substantive review to begin?	X		
3	Is the virology information (nonclinical and clinical) legible so that substantive review can begin?	X		
4	On its face, has the applicant submitted cell culture data in necessary quantity, using necessary clinical and non-clinical strains/isolates, and using necessary numbers of approved current divisional standard of approvability of the submitted draft labeling?	X		
5	Has the applicant submitted any required animal model studies necessary for approvability of the product based on the submitted draft labeling?			N/A
6	Has the applicant submitted all special/critical studies/data requested by the Division during pre-submission discussions?	X		NGS data were submitted in accordance with previous agreement. 1,733 NGS fastq files were submitted.
7	Has the applicant submitted the clinical virology datasets in the appropriate format as described in the relevant guidance documents and are the datasets complete?	X		Baseline NGS data submitted for 1523 subjects, NGS data at time of failure submitted for 50 treatment failures.
8	Has the applicant used standardized or nonstandardized methods for virologic outcome measures? If nonstandardized methods were used, has the applicant included complete details of the method, the name of the laboratory where actual testing was done and performance characteristics of the assay in the laboratory where the actual testing was done?	X		Standard methods were used and NGS data submitted as agreed upon.
9	Has the applicant submitted draft labeling consistent with current regulation, divisional and Center policy, and the design of the development package?	X		

VIROLOGY FILING CHECKLIST FOR NDA or Supplement

	Content Parameter	Yes	No	Comments
10	Has the applicant submitted annotated microbiology draft labeling consistent with current divisional policy, and the design of the development package?	X		
11	Have all the study reports, published articles, and other references been included and cross-referenced in the annotated draft labeling or summary section of the submission?	X		
12	Are any study reports or published articles in a foreign language? If yes, has the translated version been included in the submission for review?		X	

IS THE VIROLOGY SECTION OF THE APPLICATION FILEABLE? The NGS portion of this NDA contains all of the data necessary for complete review and therefore, this application is fileable.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

There are currently no known review issues from the Clinical Virology perspective.

Eric F. Donaldson, Ph.D. Date
Clinical Virology Reviewer

Jules O'Rear, Ph.D. Date
Clinical Virology Team Leader

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/s/

ERIC F DONALDSON
03/11/2014

JULIAN J O REAR
03/11/2014

MICROBIOLOGY FILING CHECKLIST FOR NDA or Supplement

NDA Number: 205834 **Applicant: Gilead Sciences, Inc** **Stamp Date: October 10, 2014**

Drug Name: LDV/SOF **NDA Type: Original**

On **initial** overview of the NDA application for filing:

	Content Parameter	Yes	No	Comments
1	Is the virology information (nonclinical and clinical) provided and described in different sections of the NDA organized in a manner to allow substantive review to begin?	x		
2	Is the virology information (nonclinical and clinical) indexed, paginated and/or linked in a manner to allow substantive review to begin?	x		
3	Is the virology information (nonclinical and clinical) legible so that substantive review can begin?	x		
4	On its face, has the applicant submitted cell culture data in necessary quantity, using necessary clinical and non-clinical strains/isolates, and using necessary numbers of approved current divisional standard of approvability of the submitted draft labeling?	x		
5	Has the applicant submitted any required animal model studies necessary for approvability of the product based on the submitted draft labeling?	na		
6	Has the applicant submitted all special/critical studies/data requested by the Division during pre-submission discussions?	x		
7	Has the applicant submitted the clinical virology datasets in the appropriate format as described in the relevant guidance documents and are the datasets complete?	x		
8	Has the applicant used standardized or nonstandardized methods for virologic outcome measures? If nonstandardized methods were used, has the applicant included complete details of the method, the name of the laboratory where actual testing was done and performance characteristics of the assay in the laboratory where the actual testing was done?	x		standardized
9	Has the applicant submitted draft labeling consistent with current regulation, divisional and Center policy, and the design of the development package?	x		
10	Has the applicant submitted annotated microbiology draft labeling consistent with current divisional policy, and the design of the development package?	x		
11	Have all the study reports, published articles, and other references been included and cross-referenced in the	x		

MICROBIOLOGY FILING CHECKLIST FOR NDA or Supplement

	Content Parameter	Yes	No	Comments
	annotated draft labeling or summary section of the submission?			
12	Are any study reports or published articles in a foreign language? If yes, has the translated version been included in the submission for review?		x	

IS THE MICROBIOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

If the NDA is not fileable from the microbiology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Lisa K. Naeger — March 11, 2014
Reviewing Microbiologist — Date

Julian O' Rear — March 11, 2014
Microbiology Team Leader — Date

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/s/

LISA K NAEGER
03/11/2014

JULIAN J O REAR
03/11/2014

PRODUCT QUALITY MICROBIOLOGY FILING CHECKLIST

NDA Number: 205834 | **Applicant: Gilead Sciences, Inc.** | **Letter Date:** 2/07/2014

Drug Name: ledipasvir/sofosbuvir, LDV/SOF | **NDA Type**: 505(b)(1) | **Stamp Date:** 2/07/2014

The following are necessary to initiate a review of the NDA application:

	Content Parameter	Yes	No	Comments
1	Is the product quality microbiology information described in the NDA and organized in a manner to allow substantive review to begin? Is it legible, indexed, and/or paginated adequately?	x		
2	Has the applicant submitted an overall description of the manufacturing processes and microbiological controls used in the manufacture of the drug product?	x		(see manuf-process-and-controls.pdf, pg. 11-19)
3	Has the applicant submitted protocols and results of validation studies concerning microbiological control processes used in the manufacture of the drug product?	x		According to USP <61> and <62>
4	Are any study reports or published articles in a foreign language? If yes, has the translated version been included in the submission for review?		x	
5	Has the applicant submitted preservative effectiveness studies (if applicable) and container-closure integrity studies?		x	Not Applicable
6	Has the applicant submitted microbiological specifications for the drug product and a description of the test methods?		x	This information is partial. MLT is proposed (b) (4) [redacted]
7	Has the applicant submitted the results of analytical method verification studies?	x		Reference to USP
8	Has the applicant submitted all special/critical studies/data requested during pre-submission meetings and/or discussions?			N/A

	Content Parameter	Yes	No	Comments
9	If sterile, are extended post-constitution and/or post-dilution hold times in the draft labeling supported by microbiological data?			N/A; the drug product is not sterile
10	Is this NDA fileable? If not, then describe why.	x		

Additional Comments:
The proposal for reduced microbial limits testing appears to be reasonable but additional information will be requested during the review period to support this request.

Reviewing Microbiologist Date

Microbiology Secondary Reviewer/Team Leader Date

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/s/

STEVEN P DONALD
02/27/2014

STEPHEN E LANGILLE
02/27/2014