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APPLICATION NUMBER:

208341Orig1s000

MICROBIOLOGY/VIROLOGY REVIEW(S)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

NDA#: 208341

Serial #: 000

Reviewer's Name(s): Lisa K. Naeger, Ph.D.

Sponsor's Name and Address:

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Related/Supporting Documents: IND115670, IND106739, NDA204671

| Product Names | Sofosbuvir (GS-7977) | Velpatasvir (GS-5816) |
|--------------------------|--|---|
| Structures | (b) (4) | |
| 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 | (b) (4) |
| Molecular formula | C ₂₂ H ₂₉ FN ₃ O ₉ P | C ₄₉ H ₅₄ N ₈ O ₈ |
| Molecular weight | 529.46 | 883.00 |

Drug category: Antiviral

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

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Indication: Fixed-dose combination of velpatasvir, 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) (b) (4) infection

Dosage Form/Route of administration: Oral

Dispensed: Rx

Abbreviations: BVDV, bovine viral diarrhea virus; BL, baseline; DAA, direct acting antiviral; EC₅₀, effective concentration at 50%; FC, fold-change; FDA, Food and Drug Administration; FDC, fixed-dose combination; GT, genotype; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IC₅₀, inhibitory concentration at 50%; IFN, recombinant human interferon; LDV, ledipasvir; NGS, next generation sequencing; NRTIs, nucleoside reverse transcriptase inhibitors; PBL, peripheral blood lymphocytes; PDVF, protocol defined virologic failure; PI, NS3/4A protease inhibitor; P/R, pegylated interferon/ribavirin; RBV, ribavirin; RSV, respiratory syncytial virus; SDM, site-directed mutants; SOF, sofosbuvir; SVR, sustained virologic response; SVR12, sustained virologic response at 12 week after end of treatment; VEL, velpatasvir; WT, wild-type

TABLE OF CONTENTS

Executive Summary.....Page 3

1 *Recommendations*

1.1 *Recommendations on Approvability*.....Page 8

1.2 *Recommendation on Phase 4 Commitments*.....Page 8

2 *Summary of Virology Assessments*

2.1 *Non-Clinical Virology*.....Page9

2.2 *Clinical Virology*Page 10

3 *Administrative signatures*.....Page 13

4 *Virology Review*

4.1 *Important Milestones in Development*.....Page 14

4.2 *Methodology*.....Page 14

4.3 *Prior FDA Reviews*.....Page 15

4.4 *State of antivirals used for the indication*.....Page 15

4.5 *Non-Clinical Virology*Page17

4.6 *Clinical Studies*.....Page 43

4.7 *Clinical Virology*.....Page 47

5 *Conclusion*.....Page 66

6 *Package Insert*

6.1 *Applicant-Proposed*.....Page 68

6.2 *FDA-Proposed*Page 73

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

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EXECUTIVE SUMMARY

This NDA for a fixed-dose combination (FDC) of velpatasvir (VEL) and the approved NS5B nucleotide analog inhibitor sofosbuvir (SOF), seeks an indication with and without ribavirin (RBV) for the treatment of adult patients with chronic HCV infection. From a virology perspective, this application for SOF/VEL is approvable.

SOF/VEL is indicated for the treatment of GT1, 2, 3, 4, 5, and 6 HCV infections. The recommended treatment regimen for patients without cirrhosis and patients with compensated cirrhosis (Child-Pugh A) is 12 weeks of SOF/VEL. The recommended treatment regimen for patients with decompensated cirrhosis (Child-Pugh B and C) is 12 weeks of SOF/VEL + RBV. This virology review supports adding a footnote to the Dosage and Administration Table to consider adding RBV to 12 week SOF/VEL for GT3 subjects with compensated cirrhosis, because relapse rates were higher overall in this population and the consequences of failure with resistance to all NS5A inhibitors and potentially SOF for the cirrhotic population are significant. GT3 subjects with compensated cirrhosis treated with 12 weeks SOF/VEL had a relapse rate of 9% compared to 2% for GT3 subjects without cirrhosis. Importantly, relapse rates were much higher (33%) in GT3 compensated cirrhotic subjects who had baseline NS5A resistance-associated polymorphisms (RAPs). Furthermore, all the GT3 virologic failures with compensated cirrhosis had the Y93H NS5A resistance substitution at failure, which confers high-level resistance to all current NS5A inhibitors and may compromise future treatment options. Thus, it is important to optimize chances of virologic success for this advanced patient population. The data support adding RBV to 12 weeks SOF/VEL to optimize SVR12 rates in GT3 patients with compensated cirrhosis.

Sofosbuvir (SOF) is a uridine nucleotide analog inhibitor of the HCV NS5B RNA-dependent RNA polymerase, which is required for viral replication. Specifically, the SOF prodrug is hydrolyzed by cellular esterases to a uridine analog monophosphate that is subsequently converted by cellular kinases to uridine analog triphosphate. The uridine analog is incorporated into HCV RNA by the NS5B polymerase and acts as a chain terminator. Velpatasvir (VEL) is an inhibitor of the HCV NS5A protein, which is required for viral replication. Resistance selection experiments in cell culture and cross-resistance studies indicate velpatasvir targets NS5A as its mode of action.

SOF and VEL have antiviral activity against HCV genotypes (GT) 1, 2, 3, 4, 5, and 6. The EC₅₀ values for SOF range from 15 to 264 nM against laboratory replicons and the EC₅₀ values for VEL range from 0.004 to 0.130 nM. Against clinical isolates, median EC₅₀ values range from 29 - 102 nM and 0.002 – 0.024 nM for SOF and VEL, respectively.

Velpatasvir was not antagonistic in reducing HCV RNA levels in replicon cells when combined with sofosbuvir or IFN- α , RBV, a HCV NS3/4A protease inhibitor, the HCV NS5A inhibitor, ledipasvir, or HCV NS5B non-nucleoside inhibitors, GS-9190 or GS-9669.

In cell culture, HCV replicons with reduced susceptibility to sofosbuvir were selected in cell culture for genotypes 1b, 2a, 2b, 3a, 4a, 5a, and 6a. Reduced susceptibility to

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

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sofosbuvir was associated with the NS5B substitution S282T in all replicon genotypes examined. An M289L substitution developed along with the S282T substitution in genotype 2a, 5 and 6 replicons. Site-directed mutagenesis of the S282T substitution in replicons of genotypes 1 to 6 conferred 2- to 18-fold reduced susceptibility to sofosbuvir. HCV genotype 1a, 1b, 2a, 3a, 4a, 5a, and 6a replicon variants with reduced susceptibility to velpatasvir were also selected in cell culture. Selected viruses developed amino acid substitutions at NS5A resistance-associated positions 24, 28, 30, 31, 32, 58, 92, and 93. Phenotypic analysis of site-directed mutagenesis mutant replicons of the selected NS5A substitutions showed that single and double combinations of L31V and Y93H/N in genotype 1a, the combination of L31V +Y93H in genotype 1b, Y93H/S in genotype 3a, and L31V and P32A/L/Q/R in genotype 6 conferred greater than 100-fold reduction in velpatasvir susceptibility. In the genotype 2a replicon, the single mutants F28S and Y93H showed 91-fold and 46-fold reduced susceptibility to VEL, respectively. The single mutant Y93H conferred 3-fold reduced susceptibility to VEL in genotype 4a replicons. Combinations of these NS5A substitutions often showed greater reductions in susceptibility to velpatasvir than single substitutions alone.

Clinical Virology Assessment of ASTRAL Trials

For the FDA resistance analyses (see also the independent analysis of the next generation sequencing data by Virology Reviewer Eric Donaldson, Ph.D.), subjects who died, experienced an AE while serum HCV RNA was undetectable, or were lost to follow-up in the ASTRAL trials were removed from the analyses. Thus, 3 GT1a subjects in ASTRAL 1 and 16 GT3 subjects in ASTRAL 3 were censored for the FDA resistance analysis. The prevalence of baseline NS5A RAPs (any change at amino acid positions 24, 28, 30, 31, 58, 92 and 93) at a sensitivity threshold of 15% of the viral population was assessed in the ASTRAL trials. Analyses were performed to assess the effect of baseline NS5A RAPs and cirrhosis on relapse rates. In addition, the NS5A resistance-associated substitutions that emerged in virologic failures were examined.

ASTRAL 1 and 2

In the ASTRAL 1 and 2 studies of subjects with GT1, 2, 4, 5, and 6, the prevalence of baseline NS5A RAPs was 18% (38/211) in subjects with GT1a HCV infection and 31% (42/134) in subjects with GT1b HCV infection. The most prevalent NS5A RAPs in GT1a were at positions M28 (5%) and H58 (7%). The most prevalent NS5A RAPs in GT1b were at positions 30 (8%), 31 (7%), 58 (9%) and 93 (10%). The prevalence of baseline NS5A RAPs in subjects with GT2 HCV infection was 60% (233/387). The most prevalent GT2 NS5A RAPs were L31M (51%) and K24R/T/Q (17%). The prevalence of baseline NS5A RAPs in subjects with GT4, GT5, and GT6 infection was 63% (73/115), 9% (3/35), and 83% (35/42), respectively. The predominant polymorphisms were at positions 28, 30 and 58 in GT4 and at positions 24, 28, 30 and 58 in GT6.

There were only 2 GT1 virologic failures in ASTRAL 1 and there were no virologic failures in ASTRAL 2. Thus, for GT2, GT4, GT5 and GT6 subjects, SVR12 rates were 100% with or without the presence of baseline NS5A RAPs. Since there were only 2 GT1 virologic failures, the effect of baseline NS5A polymorphisms was not assessed for GT1 subjects in ASTRAL 1.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

GT1 Virologic Failures

There were 2 GT1 virologic failures who relapsed; one with GT1a and one with GT1c/h. The GT1a relapser had low level Q30R at baseline detectable with next generation sequencing (NGS) below the 15% threshold and had emergent Y93N at failure with an 805-fold reduced susceptibility to VEL. The GT1c/h relapse had cirrhosis and baseline NS5A RAPs Q30R, L31M and H58P (above 15% threshold). This subject had emergent L24M/T, L31I/V and Y93H substitutions with 763-fold reduced susceptibility to VEL. Neither subject had baseline or emergent NS5B nucleoside analog inhibitor resistance substitutions.

ASTRAL 3

In ASTRAL-3, a study of GT3 subjects both with and without compensated cirrhosis, the prevalence of NS5A RAPs at baseline was 21% (115/551) with the most prevalent NS5A RAPs at positions A30 (11%) and Y93H (6%).

The effect of the presence of baseline NS5A RAPs on relapse rates in subjects with GT3 HCV infection following 12-week SOF/VEL or 24-week SOF/RBV treatment were examined in ASTRAL 3. The overall relapse rate for the SOF/VEL 12 week treatment arm was 4% (11/275) compared to 15% (40/260) for the comparator SOF/RBV 24 weeks treatment arm. In the SOF/VEL arm, the relapse rate for subjects with baseline NS5A RAPs was 7% (4/56) compared to 3% (7/218) for subjects without RAPs. As expected, the presence of NS5A RAPs did not affect the relapse rates (15-16%) in the SOF/RBV arm because of the absence of an NS5A inhibitor in the treatment regimen.

Relapse rates were higher for subjects with cirrhosis in both treatment arms; 9% (7/80) for the SOF/VEL arm and 29% (23/78) for the SOF+RBV arm. For subjects without cirrhosis, relapse rates were 2% for both subjects with and without NS5A RAPs. However, for cirrhotic subjects treated with SOF/VEL for 12 weeks, relapse rates were higher for subjects with NS5A RAPs (33%; 3/9) than subjects without RAPs (6%; 4/71).

Four subjects (9%) in the SOF/VEL arm with 1 baseline NS5A RAP relapsed (1 with A30K and 3 with Y93H). Specifically, one non-cirrhotic subject with the Y93H polymorphism at baseline relapsed (8%; 1/13), but both cirrhotic subjects with the Y93H polymorphism relapsed (100%; 2/2). The fourth relapse subject in the SOF/VEL 12 Week arm had the A30K polymorphism at baseline. Thus overall, relapse rates in the SOF/VEL 12 Week arm were 20% (3/15) for GT3 subjects with the Y93H polymorphism and 4% (1/28) for subjects with an A30K polymorphism at baseline.

GT3 Virologic Failures with Compensated Cirrhosis

In ASTRAL 3, there were 11 GT3 virologic failures in the SOF/VEL 12 week arm compared to 38 relapsers in the SOF+ RBV 24 week arm. In the SOF/VEL arm, one failure subject (Subject 01069-62225) had GT3a HCV infection at screening but had GT1a HCV infection at virologic failure as determined by NS5B sequencing. This indicates reinfection and not a relapse of the original GT3a virus. This subject did not have NS5A or NS5B baseline polymorphisms or post-treatment substitutions. As stated above, 4 of the the relapsers in the SOF/VEL arm had baseline NS5A RAPs (3 had Y93H and 1 had A30K). Eight of the 11 relapsers had emergent NS5A resistance-associated substitutions; all 8 had emergent Y93H (1 from a mixture at baseline), 1 had emergent P58L at 2% frequency and 1 had emergent A30V at 12% frequency. In total,

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

10 of the 11 failures had Y93H at failure. If the subject who is suspected of being reinfected is removed from the analysis, then all 10 relapsers had Y93H at failure.

ASTRAL 4

In ASTRAL-4, a study in GT1, 2, 3, 4, 5, and 6 subjects with decompensated cirrhosis, 8 GT1 and 2 GT3a subjects were censored for the FDA resistance analysis, because they died or were lost to follow-up. The prevalence of NS5A RAPs at baseline was 24% (48/198), 60% (6/10), 11% (4/37), and 63% (5/8) in GT1, GT2, GT3, and GT4 HCV subjects, respectively. The prevalence of NS5A RAPs in GT1 subjects was balanced across the 3 treatment arms. There were no subjects with GT5 HCV infection and only 1 subject with GT6 infection in the SOF/VEL 24 Week arm who had a baseline NS5A RAP.

The effect of the presence of baseline NS5A RAPs on relapse rates in subjects with GT1 and GT3 HCV infection following 12-week SOF/VEL, 24-week SOF/VEL or 12-week SOF/VEL+RBV treatment were examined. Relapse rates were 0% for subjects with GT2, GT4 and GT6, so there was no effect of the presence of baseline NS5A RAPs in this study for these genotypes.

Genotype 1: Effect of Baseline NS5A RAPs on Relapse Rates

For GT1 subjects, the overall relapse rates were lower for the 12-week SOF/VEL + RBV arm (2%; 1/66) compared to 8% (5/65) and 4% (3/68) for the SOF/VEL 12-week and 24-week treatment arms, respectively. In the 12-week SOF/VEL + RBV arm, relapse rates were 0% (0/17) for subjects with NS5A RAPs compared to 2% (1/49) for subjects with no NS5A RAPs. In comparison, in the 12-week SOF/VEL arm, the relapse rate for subjects with baseline NS5A RAPs was 17% (2/12) compared to 6% (3/52) for subjects without RAPs. In the 24-week SOF/VEL arm, the relapse rate for subjects with NS5A RAPs was 11% (2/19) compared to 2% (1/48) for subjects without RAPs. Therefore in this patient population, the SOF/VEL + RBV for 12 weeks treatment option is more effective and reduces relapse rates compared to the other 2 tested treatments. This is especially seen for subjects with NS5A RAPs where relapse rates were 0% for the RBV containing arm compared to 17% and 11% for the 12-week and 24-week SOF/VEL regimens, respectively.

Genotype 3: Effect of Baseline NS5A RAPs on Relapse Rates

For GT3 subjects, the overall relapse rates were much higher than those seen with GT1 subjects. However, relapse rates were lower for GT3 subjects in the 12-week SOF/VEL + RBV arm (15%; 2/13) compared to 46% (6/13) and 45% (5/11) for the SOF/VEL 12-week and 24-week treatment arms, respectively. In the 12-week SOF/VEL arm, the relapse rate for subjects with NS5A RAPs was 33% (1/3) compared to 50% (5/10) for subjects without RAPs. In the 24-week SOF/VEL arm, the relapse rate for subjects with NS5A RAPs was 100% (1/1) compared to 40% (4/10) for subjects without RAPs. In the 12-week SOF/VEL + RBV arm, there were no subjects with NS5A RAPs, so no comparison could be made to the 15% (2/13) relapse rate for subjects without NS5A RAPs.

The data in ASTRAL 4 support the SOF/VEL + RBV 12 week regimen as the more effective treatment option for GT1 and GT3 decompensated subjects. These data also

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

inform us that treatment for GT3 compensated cirrhotic patients might be improved by the addition of RBV to the 12 Week SOF/VEL regimen.

ASTRAL 4 Virologic Failures with Decompensated Cirrhosis

There were 9 total GT1 virologic failures in all the arms; 5 in the SOF/VEL 12 week arm, 1 in the SOF/VEL 12 week + RBV arm and 3 in the SOF/VEL 24 week arm. Four of the GT1 relapsers had baseline NS5A RAPs (Q30Q/H + Y93Y/H, Y93Y/H, L31M, and R30Q + Y93Y/H). Six of the 9 relapsers had emergent NS5A resistance-associated substitutions; all 6 had Y93H or N at failure. Other emergent substitutions included Q30H/R, L31M/V and H58D. The one GT1 virologic failure in the 12 week SOF/VEL + RBV arm had no NS5A or NS5B resistance substitutions at baseline or failure.

There were 13 total GT3 virologic failures in all the arms; 6 in the SOF/VEL 12 week arm, 2 in the SOF/VEL 12 week + RBV arm and 5 in the SOF/VEL 24 week arm. Two of the GT3 relapsers had baseline NS5A RAPs (P58A or Y93H). Twelve of the 13 relapsers had emergent NS5A resistance-associated substitutions and all 13 had Y93H at failure. Other emergent substitutions included M28T/V, S38P/Y and H58T. The 2 GT3 virologic failures in the 12 week SOF/VEL + RBV arm had S38P + Y93H and M28V + Y93H emerge at failure.

Consideration for Adding RBV to SOF/VEL 12 Weeks for GT3 Compensated Cirrhotics

Because of the concern for the consequences of virologic failure with development of Y93H in all failures and loss of subsequent treatment options, we pushed for a consideration for adding RBV to 12 week SOF/VEL in the GT3 compensated cirrhotic population. Specifically we were concerned for those with baseline NS5A RAPs, but did not have enough data to support screening all patients for NS5A RAPs before treatment with SOF/VEL. Our virology proposal to the review team was to add a footnote to Table 1 in Section 2, Dosage and Administration stating "SOF/VEL + RBV for 12 weeks can be considered for GT3 patients with compensated cirrhosis [see Clinical 14 and Microbiology 12.4].

The benefits of our consideration included: 1) relapse rates could be reduced for GT3 patients with compensated cirrhosis who could take RBV. Based on a bridging assessment by the statistical reviewer, Karen Qi, Ph.D., relapse rates of 9% for GT3 cirrhotics could be reduced to 2-3% with the addition of RBV, 2) relapse rates are 33% (3/9) for compensated cirrhotic subjects with baseline NS5A RAPs, so adding RBV would be a better option for these subjects and it would not be necessary to screen for RAPs and 3) adding RBV could reduce failure with the Y93H resistance substitution. The presence of the Y93H resistance substitution has consequences for future treatment options with NS5A inhibitors. The cons of this approach are that 1) there are no phase 3 data for GT3 cirrhotics with RAPs in the 12 Week SOF/VEL + RBV arm, 2) there would be potentially unnecessary RBV use in some patients, and 3) GT/subtype at screening would be required to determine if a patient was GT3 even though SOF/VEL has efficacy against GTs 1-6.

The justification for adding the consideration footnote include:

- Phase 2 data showing the increase in SVR12 rate with RBV (88% (23/26) to 96% (25/26).

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

- Cirrhosis is a continuum from compensated to decompensated so results in decompensated can inform treatment for compensated.
- Results from ASTRAL 4 in decompensated patients show better SVR rates with RBV in both GT3 subjects (46% without compared to 15% with RBV) and GT1 subjects (8% without and 2% with RBV).

1. RECOMMENDATIONS

1.1. Recommendation and Conclusion on Approvability

This supplemental NDA for the fixed-dose combination of velpatasvir (VEL) and sofosbuvir (SOF) is approvable with respect to virology

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

The sponsor should submit the protocol and study results of the study (b) (4) of GT3 cirrhotic patients where SOF/VEL +/- RBV treatments are being compared.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

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2. SUMMARY OF OND VIROLOGY ASSESSMENTS

2.1 Non-Clinical Virology

Sofosbuvir (SOF) is a nucleotide prodrug of 2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate that is converted to the active uridine triphosphate form (GS-461203) within the hepatocyte. In a biochemical assay, GS-461203 inhibited the RNA polymerase activity of recombinant NS5B from HCV genotypes 1b, 2a, 3a and 4a with IC_{50} values ranging from 0.7 to 2.6 μ M. SOF had EC_{50} values ranging from 14-110 nM in stable full-length replicon cells of genotype 1a, 1b, 2a, 3a and 4a; and chimeric GT1b Con-1 replicons carrying NS5B coding sequences from genotypes 2b, 5a, or 6a. The median EC_{50} values of sofosbuvir against chimeric replicons encoding NS5B sequences from clinical isolates were 62 nM for genotype 1a (range 29-128 nM; N=67), 102 nM for genotype 1b (range 45-170 nM; N=29), 29 nM for genotype 2 (range 14-81 nM; N=15) and 81 nM for genotype 3a (range 24-181 nM; N=106). The EC_{50} values of sofosbuvir against genotype 1a and 2a viruses were 30 and 20 nM, respectively.

Velpatasvir (VEL; GS-5816) inhibits HCV replication by inhibiting NS5A protein activity. This is supported by resistance selection of substitutions in the NS5A protein in cell culture, the clinical resistance profile with NS5A resistance-associated substitutions emerging in virologic failures, cross-resistance studies with other NS5A inhibitors, and studies showing VEL does not inhibit HCV enzymes.

VEL demonstrated antiviral activity in multiple HCV replicon cell lines against major HCV genotypes/subtypes including 1a, 1b, 2a, 2b, 3a, 4a, 5a, 6a and 6e. In HCV replicon antiviral assays, VEL has EC_{50} values of 0.012 nM and 0.015 nM against GT1a and GT1b replicons, respectively, with no cytotoxicity observed at the highest concentrations tested (CC_{50} value >44,400 nM; selectivity indices of >3,000,000). VEL had EC_{50} values of 0.009 nM, 0.014 nM, 0.008 nM, 0.012 nM and 0.009 nM for genotype 2a (JFH-1), 2a (J6), 2b, 3a and 4a NS5A, respectively. The activity of VEL was not significantly different between L31 (JFH-1 strain) or M31 (J6 strain) forms of NS5A for GT2 HCV. VEL demonstrated antiviral activity against genotypes 5a, 6a and 6e with EC_{50} values of 0.075 nM, 0.006 nM, and 0.13 nM, respectively. Furthermore, VEL has activity against infectious HCV in cell culture with an EC_{50} value of 0.008 nM. In the presence of 40% human serum, VEL potency against the genotype 1a HCV replicon was reduced 13.3-fold. This serum binding shift was within the range of several other tested HCV inhibitors including the first generation NS5A inhibitor ledipasvir.

Cell-based analyses of an extensive collection of NS5A genetic polymorphisms (residues 28, 30, 31, 44, 56, 58, 62, 92, and 93 that play critical roles in determining susceptibility to NS5A inhibitors) showed that VEL is equally effective against the majority of polymorphisms ($\geq 89\%$) existing within genotypes 2a, 2b, 3a and 4a. VEL maintains consistent antiviral activity against a broad range of NS5A polymorphisms existing in these HCV genotypes/subtypes.

VEL does not have inhibitory activity against a related flavivirus, bovine viral diarrhea virus, or unrelated viruses including respiratory syncytial virus, hepatitis B virus and human immunodeficiency virus.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

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Virology Reviewer: Lisa K. Naeger, Ph.D.

The antiviral activity and cellular toxicity of VEL was tested in combination with IFN- α , RBV, and a panel of Gilead Sciences' clinical stage drug candidates including GS-9451 (an NS3/4A protease inhibitor), ledipasvir (GS-5885, an NS5A inhibitor), GS-9190 and GS-9669 (two non-nucleoside NS5B inhibitors), and GS-6620 and GS-7977 (two nucleotide analog NS5B inhibitors). There was no antiviral antagonism of VEL in combination with any of these tested compounds. Furthermore, no cellular toxicity was observed when VEL was combined with any of the inhibitors at concentrations tested in the antiviral combinations experiments.

Importantly, the replicon resistance selection studies conducted in GT1a and GT1b confirmed that VEL resistance maps to the NS5A protein, supporting the premise that NS5A is the antiviral target of VEL. In GT1a HCV replicon cells, NS5A substitutions L31V and Y93H were most frequently selected by VEL and conferred 129- and 1,004-fold resistance, respectively. Q30K, L31M and Y93N were selected less frequently and conferred 8-, 15-, and 2,926-fold, respectively, to VEL. Both Y93H and Y93N displayed >1,000-fold decreased susceptibility to VEL. Single substitutions Q30H and Q30R did not emerge in the cell culture resistance selection. Double mutants, including combinations of Q30H, Q30R or L31V with Y93H or Y93N, were observed at a low frequency.

In GT1b HCV replicon cells, only double substitutions were identified. Y93H was identified in each of the clones, but always emerged as a "double mutant" together with other NS5A substitutions, including L28M, L31F, L31M, L31V, or Q54H. The single Y93H substitution and other NS5A single substitutions showed less than 2-fold resistance to VEL. However, once these substitutions were combined with Y93H, increases in VEL resistance were observed. The L31V/Y93H double mutant, the most frequently selected in these studies, conferred 986-fold resistance to VEL. The other double mutants, L31M/Y93H, L31F/Y93H and L28M/Y93H, conferred 68-, 27- and 5-fold decreased VEL susceptibility, respectively.

Against a panel of clinically significant NS5A inhibitor resistant mutants in GT1, VEL showed less than or equal to 2-fold reduced antiviral activity against GT1a Q30H and Q30R mutants, as well as the GT1b Y93H resistant mutant. GT1a mutants M28T, L31M and Y93C showed 6- to 12-fold reduced susceptibility to VEL. VEL had less activity against GT1a mutant Y93H, with an EC₅₀ value of 6.7 nM. Colony reduction assays provided evidence that VEL has an improved resistance barrier across genotypes 1 to 4 relative to another NS5A inhibitor, daclatasvir (DCV).

2.2 Clinical Virology

Virology Assessment of ASTRAL Trials

For the FDA resistance analyses (see also the independent analysis of the next generation sequencing data by Virology Reviewer Eric Donaldson, Ph.D.), subjects who died, experienced an AE while undetectable, or were lost to follow-up in the ASTRAL trials were removed from the analyses. Thus, 3 GT1a subjects in ASTRAL 1 and 16 GT3 subjects in ASTRAL 3 were censored for the FDA resistance analysis. The prevalence of baseline NS5A RAPs (any change at amino acid positions 24, 28, 30, 31, 58, 92 and 93) at a sensitivity threshold of 15% of the viral population was assessed in the ASTRAL trials. Analyses were performed to assess the effect of baseline NS5A RAPs and

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

cirrhosis on relapse rates. In addition, the NS5A resistance-associated substitutions that emerged in virologic failures at relapse were examined.

ASTRAL 1 and 2

In the ASTRAL 1 and 2 studies of subjects with GT1, 2, 4, 5, and 6, the prevalence of baseline NS5A RAPs was 18% (38/211) in subjects with GT1a HCV infection and 31% (42/134) in subjects with GT1b HCV infection. The most prevalent NS5A RAPs in GT1a were at positions M28 (5%) and H58 (7%). The most prevalent NS5A RAPs in GT1b were at positions 30 (8%), 31 (7%), 58 (9%) and 93 (10%). The prevalence of baseline NS5A RAPs in subjects with GT2 HCV infection was 60% (233/387). The most prevalent GT2 NS5A RAPs were L31M (51%) and K24R/T/Q (17%). The prevalence of baseline NS5A RAPs in subjects with GT4, GT5, and GT6 infection was 63% (73/115), 9% (3/35), and 83% (35/42), respectively. The predominant polymorphisms were at positions 28, 30 and 58 in GT4 and at positions 24, 28, 30 and 58 in GT6.

There were only 2 GT1 virologic failures in ASTRAL 1 and there were no virologic failures in ASTRAL 2. Thus, for GT2, GT4, GT5 and GT6 subjects, SVR12 rates were 100% with or without the presence of baseline NS5A RAPs. Since there were only 2 GT1 virologic failures, the effect of baseline NS5A polymorphisms were not assessed for GT1 subjects in ASTRAL 1.

GT1 Virologic Failures

There were 2 GT1 virologic failures who relapsed; one with GT1a and one with GT1c/h. The GT1a relapse had low level Q30R detectable with next generation sequencing (NGS) below the 15% threshold and had emergent Y93N at failure with an 805-fold reduced susceptibility to VEL. The other GT1c/h subject had cirrhosis and baseline NS5A RAPs Q30R, L31M and H58P (above 15% threshold). This subject had emergent L24M/T, L31I/V and Y93H with 763-fold reduced susceptibility to VEL. Neither subject had baseline or emergent NS5B nucleoside analog inhibitor resistance substitutions.

ASTRAL 3

In ASTRAL-3, a study of GT3 subjects both with and without compensated cirrhosis, the prevalence of NS5A RAPs at baseline was 21% (115/551) with the most prevalent NS5A RAPs at positions A30 (11%) and Y93H (6%).

The effect of the presence of baseline NS5A RAPs on relapse rates in subjects with GT3 HCV infection following 12-week SOF/VEL or 24-week SOF/RBV treatment were examined in ASTRAL 3. The overall relapse rate for the SOF/VEL 12 week treatment arm was 4% (11/275) compared to 15% (40/260) for the SOF/RBV 24 weeks treatment arm. In the SOF/VEL arm, the relapse rate for subjects with baseline NS5A RAPs was 7% (4/56) compared to 3% (7/218) for subjects without RAPs. As expected, the presence of NS5A RAPs did not affect the relapse rates (15-16%) in the SOF/RBV arm because of the absence of an NS5A inhibitor in the treatment regimen.

Relapse rates were higher for subjects with cirrhosis in both treatment arms; 9% (7/80) for the SOF/VEL arm and 29% (23/78) for the SOF+RBV arm. For subjects without cirrhosis, relapse rates were 2% for both subjects with and without NS5A RAPs. For cirrhotic subjects treated with SOF/VEL for 12 weeks, relapse rates were higher for subjects with NS5A RAPs (33%; 3/9) than subjects without RAPs (6%; 4/71).

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

Four subjects (9%) in the SOF/VEL arm with 1 baseline NS5A RAP relapsed (1 with A30K and 3 with Y93H). Specifically, one non-cirrhotic subject with the Y93H polymorphism pretreatment relapsed (8%; 1/13), but both cirrhotic subjects with the Y93H polymorphism relapsed (100%; 2/2). The fourth relapse subject in the SOF/VEL 12 Week arm had the A30K polymorphism at baseline. Thus overall, relapse rates in the SOF/VEL 12 Week arm were 20% (3/15) for GT3 subjects with the Y93H polymorphism and 4% (1/28) for subjects with an A30K polymorphism at baseline.

GT3 Virologic Failures with Compensated Cirrhosis

In ASTRAL 3, there were 11 GT3 virologic failures in the SOF/VEL 12 week arm compared to 38 relapsers in the SOF+ RBV 24 week arm. In the SOF/VEL arm, one failure subject (Subject 01069-62225) had GT3a HCV infection at screening but had GT1a HCV infection at virologic failure as determined by NS5B sequencing (Note: in the review could not determine if confirmation was performed on more than one sample/ timepoint). This change in genotype indicates reinfection and not a relapse of the original GT3a virus. This subject did not have NS5A or NS5B baseline polymorphisms or post-treatment substitutions. Four of the the relapsers in the SOF/VEL arm had baseline NS5A RAPs (3 had Y93H and 1 had A30K). Eight of the 11 relapsers had emergent NS5A resistance-associated substitutions; all 8 had emergent Y93H (1 from a mixture at baseline), 1 had emergent P58L at 2% and 1 had emergent A30V at 12%. In total, 10 of the 11 failures had Y93H at failure. If the one subject who is suspected of being reinfected is removed, then all 10 relapsers had Y93H at failure.

ASTRAL 4

In ASTRAL 4, a study in GT1, 2, 3, 4, 5, and 6 subjects with decompensated cirrhosis, the prevalence of NS5A RAPs at baseline was 24% (48/198), 60% (6/10), 11% (4/37), and 63% (5/8) in GT1, GT2, GT3, and GT4 HCV subjects, respectively. The prevalence of NS5A RAPs in GT1 subjects was balanced across the treatment arms. There were no subjects with GT5 HCV infection and only 1 subject with GT6 infection in the SOF/VEL 24 Week arm who had a baseline NS5A RAP.

The effect of the presence of baseline NS5A RAPs on relapse rates in subjects with GT1 and GT3 HCV infection following 12-week SOF/VEL, 24-week SOF/VEL or 12-week SOF/VEL+RBV treatment were examined. Relapse rates were 0% for subjects with GT2, GT4 and GT6. There were no subjects with GT5 HCV infection in this trial.

Genotype 1: Effect of Baseline NS5A RAPs on Relapse Rates

For GT1 subjects, the overall relapse rates were lower for the 12-week SOF/VEL + RBV arm (2%; 1/66) compared to 8% (5/65) and 4% (3/68) for the SOF/VEL 12-week and 24-week treatment arms, respectively. In the 12-week SOF/VEL + RBV arm, relapse rates were 0% (0/17) for subjects with NS5A RAPs compared to 2% (1/49) for subjects with no NS5A RAPs. In comparison, in the 12-week SOF/VEL arm, the relapse rate for subjects with baseline NS5A RAPs was 17% (2/12) compared to 6% (3/52) for subjects without RAPs. In the 24-week SOF/VEL arm, the relapse rate for subjects with NS5A RAPs was 11% (2/19) compared to 2% (1/48) for subjects without RAPs. Therefore in this patient population, the SOF/VEL + RBV for 12 weeks treatment option is more effective and reduces relapse rates compared to the other 2 tested treatments. This is especially seen for subjects with NS5A RAPs where relapse rates were 0% for the RBV containing

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

4. VIROLOGY REVIEW

4.1 Important Milestones in Product Development

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

4.2 Methodology

SCREENING HCV GENOTYPE

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

HCV VIRAL LOAD ASSAY

Serum HCV RNA values were measured during the ASTRAL trials using the COBAS AmpliPrep/COBAS Taqman HCV test (v. 2.0) with a LLOQ of 15 IU/mL. SVR12 was defined as HCV RNA less than LLOQ at 12 weeks after stopping treatment.

RESISTANCE ANALYSIS

Deep nucleotide sequencing of full-length HCV NS3/4A, NS5A, and NS5B coding regions was performed by (b) (4) or the (b) (4) using reverse transcriptase polymerase chain reaction (RT-PCR) and then deep sequencing using the Illumina MiSeq deep sequencing platform. The NS3/4A, NS5A and NS5B sequences were utilized to confirm the results of HCV genotyping and subtyping by the INNO-LiPA assay (Innogenetics) performed at screening. All deep sequencing analyses of NS3/4A, NS5A, and NS5B regions were conducted with 1% and/or 15% cutoffs.

Table 1. Resistance-Associated NS5A Positions and Consensus Amino Acid Residues for HCV Genotypes 1 through 6

| HCV Genotype | HCV Reference Sequence | NS5A Amino Acid Position | | | | | | | | |
|--------------|------------------------|--------------------------|----|----|----|----|----|----|----|----|
| | | 24 | 28 | 30 | 31 | 32 | 38 | 58 | 92 | 93 |
| 1a | HCV1a_H77_NC_AF009606 | K | M | Q | L | P | S | H | A | Y |
| 1b | HCV1b_Con1_AJ238799 | Q | L | R | L | P | S | P | A | Y |
| 2a | HCV2a_JFH1_AB047639 | T | F | K | L | P | S | P | C | Y |
| 2b | HCV2b_MD2b10_AY232748 | S | L | K | L | P | S | P | C | Y |
| 3a | HCV3_S52_GU814263 | S | M | A | L | P | S | P | E | Y |
| 4a | HCV4_ED43_GU814265 | K | L | L | M | P | S | P | A | Y |
| 5a | HCV5a_SA13_AF064490 | Q | L | Q | L | P | S | P | A | T |
| 6a | HCV6a_EUHK2_Y12083 | Q | F | R | L | P | S | T | A | T |

NS5A RAPs were defined as any amino acid change from the corresponding HCV genotype specific reference at NS5A resistance-associated positions 24, 28, 30, 31, 32, 38, 58, 92, or 93 (Table 1; Phase 3 Integrated Virology Study Report, page 18). For

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

subjects with genotype 1 with non-1a/1b subtypes, the genotype 1a reference was used. For subjects with genotype 2 with non-2a/2b subtypes, the genotype 2a reference was used. Similarly, genotype 3a, 4a, 5a, and 6a references were used for subjects with all subtypes of genotype 4, 5, and 6, respectively. However, due to the presence of L31M in the reference sequence for genotype 4a and cell culture data showing reduced susceptibility of L31M to the first-generation NS5A inhibitors, the presence of L31M or any variant other than L at position 31 was considered a RAP across all genotypes. For this report, NS5A RAPs are presented at a 15% cutoff.

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. Dr. Lisa Naeger is the reviewer for IND-115670 and NDA-208341 covering the fixed-dose combination (VEL/SOF) of SOF and GS-5816 (velpatasvir, VEL).

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 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, and 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](#)].

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

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 1×10^{12} virions produced each day) ([Neumann et al., 1998](#)), which is error-prone because the HCV RNA-dependent RNA polymerase lacks a proofreading function. The error rate of the polymerase is approximately 10^{-4} /base/generation for a single mutation, which results in one mutation being introduced into every genome that is copied. New variants are constantly being generated, and it has been estimated that every possible point mutation along the HCV genome occurs at least once and probably many times each day ([Kieffer et al., 2010](#)). Treatment with DAA monotherapy can rapidly select for resistant variants in some patients, as has been demonstrated by the selection of variants with resistance to a number of DAAs, including NS3/4A protease inhibitors, NS5A inhibitors and NS5B polymerase inhibitors ([Sarrazin and Zeuzem, 2010](#)).

In November 2013, the FDA approved the protease inhibitor simeprevir (Olysio™) for the treatment of GT1 chronic hepatitis C virus infection in adult patients with compensated liver disease. Simeprevir has shown cure rates of up to 80% in GT1-infected patients. The once-daily treatment must be combined with pegylated recombinant human interferon α and ribavirin or with sofosbuvir. In December 2013, the FDA approved Gilead's sofosbuvir (Sovaldi™), a uridine nucleotide analog inhibitor of the HCV NS5B RNA polymerase enzyme. Sofosbuvir is a once-a-day treatment given in combination with ribavirin for the treatment of chronic hepatitis C virus infection in adult patients with genotype 2 or 3 virus. Sofosbuvir may also be given in combination with pegylated interferon and ribavirin for the treatment of chronic hepatitis C virus infection in treatment-naïve adult patients with GT1 and GT4 virus. In October 2014, the FDA approved Gilead's HARVONI™, an interferon-free combination therapy containing the NS5A inhibitor ledipasvir and sofosbuvir as a once-a-day treatment for GT1 infection and a 3 drug combination of AbbVie's Viekira Pak with or without ribavirin for GT1 infection. In November 2014, the FDA approved the combination therapy of sofosbuvir and simeprevir for use in GT1 patients. In July 2015, Bristol Myers Squibb Daklinza™ (daclatasvir), an NS5A inhibitor was approved for GT3 infection in combination with sofosbuvir.

Recommended treatments for adults with GT1 chronic hepatitis C (CHC) virus infection are treatment with HARVONI (LDV/SOF) for 12-weeks or 24 weeks (treatment-experienced with cirrhosis) or treatment with VIEKIRA PAK (dasabuvir/ombitasvir/paritaprevir/ritonavir) + RBV for 12 weeks (no cirrhosis) or 24 weeks (with cirrhosis) or treatment with ZEPATIER (elbasvir and grazoprevir) +/- RBV for 12 or 16 weeks. In the LDV/SOF registrational trials, 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 rs12979860 non-C/C alleles, HCV RNA >800,000 IU/mL and Metavir F3/F4 fibrosis was 71% (37/52). Additionally, physicians are prescribing SOF in combination with simeprevir both with and without RBV for 12 weeks for the treatment of HCV. In the phase 2 simeprevir study (COSMOS), overall SVR12 rates for 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). In the VIEKIRA PAK registrational trials, SVR12 rates were greater than 90% in both GT1a and GT1b subjects with RBV added to the dasabuvir/ombitasvir/paritaprevir/ritonavir regimen. In the ZEPATIER registrational trials, SVR12 rates were

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

94-95% for GT1 subjects treated with ZEPATIER for 12 weeks (90-92% for GT1a; 70% GT1a with baseline NS5A polymorphisms) and 97% (95% for GT1a; 100% (6/6) for GT1a with baseline NS5A polymorphisms) for subjects treated with ZEPATIER + RBV for 16 weeks.

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

Current treatment of GT4 HCV infection is HARVONI for 12-weeks or ZEPATIER +/- RBV for 12 or 16 weeks. Harvoni is also indicated for subjects with GT5 or GT6 infection.

4.5 NON-CLINICAL VIROLOGY

Sofosbuvir

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

Velpatasvir

MECHANISM OF ACTION

Velpatasvir (VEL) 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 VEL including resistance selection of substitutions in the NS5A protein in cell culture, the clinical resistance profile with NS5A resistance-associated substitutions emerging in virologic failures, cross-resistance studies with other NS5A inhibitors, and studies showing VEL does not inhibit HCV enzymes.

The ability of VEL to inhibit recombinant HCV enzymes and the HCV IRES was tested in biochemical assays (Table 2). HCV NS3 protease activity was monitored using a fluorescence resonance energy transfer (FRET) depsipeptide substrate (RET S1, ^{(b) (4)} based on the method of [Taliani et al, 1996](#). NS5B-dependent RNA elongation activity was determined using a heteropolymeric RNA template described previously [[Hung et al., 2002](#)].

VEL did not show significant inhibition of the NS3/4A-mediated proteolysis, NS5B-mediated polymerization or HCV IRES-mediated translation. The control compounds,

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

BILN-2061, (b) (4)

(b) (4) inhibited these targets at concentrations consistent with historical data. The results indicate that VEL does not target the NS3 or NS5B proteins or the HCV IRES.

Table 2. Lack of Inhibitory Activity of VEL (GS-5816) against HCV Recombinant Enzymes and the HCV IRES (PC-281-2010, page 10)

| Compound | Drug Target | IC ₅₀ (nM) ^{a,b} | | |
|------------|-------------|--------------------------------------|-----------------|-----------|
| | | NS3/4A Protease | NS5B Polymerase | HCV IRES |
| GS-5816 | NS5A | > 100,000 | > 100,000 | > 100,000 |
| GS-5885 | NS5A | > 100,000 | > 14,000 | > 100,000 |
| BMS-790052 | NS5A | > 100,000 | > 25,000 | > 100,000 |
| BILN-2061 | NS3/4A | 0.82 | 34,000 | 81,380 |

(b) (4)

a Values represent the geometric mean of at least two independent experiments

b IC₅₀ values preceded with ">" sign represent the highest soluble concentration of the compound observed in the assay

- = not tested

ANTIVIRAL ACTIVITY

Genotype 1

The selective anti-HCV activity of VEL was determined in genotype 1a (strain H77) and genotype 1b (strain Con-1) stable subgenomic replicon cells. VEL had an EC₅₀ value of 0.012 nM and 0.015 nM against the genotype 1a and 1b replicon, respectively with no cytotoxicity observed at the highest concentrations tested (CC₅₀ value > 44,400 nM) (Tables 3 and 4). The NS5A inhibitor GS-5885, Bristol-Myers Squibb NS5A inhibitor (BMS-790052), Boehringer Ingelheim NS3 protease inhibitor (BILN-2061), and nucleoside NS5B polymerase inhibitor (2'-C-Me-A) were tested as experimental controls and yielded EC₅₀ and CC₅₀ values that agree with historical data.

Table 3. Genotype 1a Replicon Activity and Selectivity for VEL (GS-5816) (Report PC-281-2007, page 15)

| Compound | Drug Target | EC ₅₀ nM ^a | CC ₅₀ nM ^a | SI ^b |
|------------|-------------|----------------------------------|----------------------------------|-----------------|
| GS-5816 | NS5A | 0.012 | > 44,400 | > 3,700,000 |
| GS-5885 | NS5A | 0.031 | > 44,400 | > 1,400,000 |
| BMS-790052 | NS5A | 0.063 | 25,800 | 410,000 |
| BILN-2061 | NS3/4A | 16.2 | 31,600 | 2000 |
| 2'-C-Me-A | NS5B | 344 | > 44,400 | > 130 |

a Values represent the geometric mean of at least three independent experiments in 384-well assays

b SI = selectivity index, calculated as CC₅₀ divided by EC₅₀

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

Table 4. Genotype 1b Replicon Activity and Selectivity for VEL (GS-5816) (Report PC-281-2007, page 15)

| Compound | Drug Target | EC ₅₀ nM ^a | CC ₅₀ nM ^a | SI ^b |
|------------|-------------|----------------------------------|----------------------------------|-----------------|
| GS-5816 | NS5A | 0.015 | > 44,400 | > 3,000,000 |
| GS-5885 | NS5A | 0.004 | > 44,400 | > 11,000,000 |
| BMS-790052 | NS5A | 0.016 | 31,300 | 2,000,000 |
| BILN-2061 | NS3/4A | 3.0 | 42,100 | 14,000 |
| 2'-C-Me-A | NS5B | 346 | > 44,400 | > 130 |

a Values represent the geometric mean of at least three independent experiments in 384-well assays

b SI = selectivity index, calculated as CC₅₀ divided by EC₅₀

Genotypes 2, 3, and 4

The antiviral activity of VEL was tested in genotype 2a JFH-1 replicon cells and genotype 2a (J6 strain), 2b (MD2b-1), 3a (S52) and 4a (consensus) NS5A chimeric HCV replicon cell lines. VEL had EC₅₀ values of 0.009 nM, 0.014 nM, 0.008 nM, 0.012 nM and 0.009 nM for genotype 2a (JFH-1), 2a (J6), 2b, 3a and 4a NS5A, respectively (Table 5).

The genotype 2a JFH-1 strain is a common lab strain that has leucine at amino acid position 31 (L31) in NS5A. The genotype 2a J6 strain has methionine at position 31 (M31) in NS5A, which is found in 85% of genotype 2a NS5A sequences in the EU HCV database. The activity of VEL was a little lower for replicons with L31 (JFH-1 strain) compared to M31 (J6 strain) in NS5A for genotype 2a HCV (0.009 nM vs 0.014 nM). In contrast, BMS-790052 showed 400-fold less activity against M31 in J6 (19.7 nM vs 0.047 nM with L31 in JFH-1). Ledipasvir (LDV; GS-5885) had lower activity against genotype 2 and 3 compared to its activity against genotypes 1 and 4. In contrast, VEL had comparable activity against each of the genotypes tested (GT1, GT2, GT3 and GT4) (Tables 3, 4, and 5).

Table 5. Genotype 2a (JFH-1) and Genotype 2a (J6), 2b, 3a and 4a NS5A Chimeric Replicon Antiviral Activity for VEL (GS-5816) (Report PC-281-2007, page 16)

| Compound | Drug Target | GT2a JFH-1 EC ₅₀ (nM) ^{a,b} | GT2a J6 NS5A EC ₅₀ (nM) ^{a,c} | GT2b NS5A EC ₅₀ (nM) ^{a,c} | GT3a NS5A EC ₅₀ (nM) ^{a,d} | GT4a NS5A EC ₅₀ (nM) ^{a,d} |
|------------|-------------|---|---|--|--|--|
| GS-5816 | NS5A | 0.009 | 0.014 | 0.008 | 0.012 | 0.009 |
| GS-5885 | NS5A | 21.2 | > 44.4 | > 44.4 | 35.1 | 0.11 |
| BMS-790052 | NS5A | 0.047 | 19.7 | 9.8 | 0.15 | 0.015 |
| 2'-C-Me-A | NS5B | 381 | 91.6 | 132 | 59.1 | 63.6 |

a Values represent the geometric mean of at least three independent experiments in 384-well assays

b GT2a (JFH-1) assay was performed in stable JFH-1 subgenomic replicon cells; The other were NS5A chimeric replicons assayed in transient transfection of Huh-Lunet cells

c NS5A chimeric replicons were based on genotype 2a JFH-1 Rluc-neo backbone

d NS5A chimeric replicons were based on genotype 1b Rluc-neo backbone

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

Clinical and cell culture data generated with ledipasvir (GS-5885) and daclatasvir (DCV; BMS-790052) revealed a number of amino acid substitutions in the N-terminal amphipathic helix linker and domain 1 of NS5A that confer resistance in genotype 1a and 1b, either individually or in combination. These resistance substitutions often occur at NS5A residues that are highly polymorphic within and across HCV genotypes. This high level of natural polymorphism has clearly impacted the efficacy of NS5A inhibitors in genotype 1 HCV patients as suboptimal responders have been observed during short-term monotherapy studies. In addition, the genotype 2a J6 strain that has methionine at position 31 (M31) in NS5A, was shown to have significantly reduced susceptibility to BMS-790052, compared to the genotype 2a JFH-1 strain that has leucine at position 31 (L31) in NS5A. Therefore, a broad panel of NS5A polymorphisms in the different HCV genotypes was assessed with VEL.

An NS5A genetic “fingerprint” or polymorph encompassing residues that play critical roles in determining susceptibility to NS5A inhibitors comprised of residues 28, 30, 31, 44, 56, 58, 62, 92, and 93. Co-variation of these residues across major HCV genotypes/subtypes was analyzed in all sequences available in the European HCV Database (<http://euhcldb.ibcp.fr/euHCVdb/>). HCV strains/sequences incorporated into NS5A chimeric replicons were selected to best represent full-length NS5A genetic variations based on phylogenetic analyses. Each chimeric replicon, encoding one of the NS5A fingerprints was made by synthesizing and cloning available NS5A clinical sequences into a corresponding genotype 2a JFH-1 RLuc-neo (for GT2a and GT2b) or genotype 1b RLuc-neo (for GT3a and GT4a) replicon backbone. Antiviral susceptibility was assessed following transient transfection of wild-type or chimeric replicons into Huh-Lunet cells. In instances where the NS5A chimeric replicons encoding clinical isolates did not replicate, site-directed mutagenesis was applied to create replicons that encoded the target NS5A fingerprint. Polymorphism analyses were completed for the major subtypes of genotypes 2, 3, and 4 (2b, 3a and 4a).

For genotype 2a, 8 NS5A chimeric replicons were assessed, which represent 7 distinct NS5A fingerprints and cumulatively cover 95% of the NS5A fingerprints observed in the EU; European Union database. VEL showed antiviral activity against all 7 NS5A fingerprints, with EC_{50} values ranging from 0.001 nM to 0.013 nM (Table 6). Daclatasvir had reduced antiviral activity (>200-fold) against 80% of the genotype 2a fingerprints, each of which expressed M31, with EC_{50} values ranging from 2.6 nM to 35.7 nM. The NS3 protease inhibitor grazoprevir (MK-5172) was tested as a control and showed consistent activity against all genotype 2a NS5A chimeras, since these replicons all contain the same genotype 2a JFH-1 NS3 sequence.

For genotype 2b, 8 NS5A chimeric replicons were established which represent 5 distinct NS5A fingerprints and cumulatively cover 92% of the NS5A fingerprints observed in the EU database. VEL had antiviral activity against all 5 NS5A fingerprints, with EC_{50} values ranging from 0.001 nM to 0.011 nM (Table 7). As with GT2a, daclatasvir (BMS-790052) had activity against NS5A fingerprints encoding L31 (EC_{50} values 0.001 - 0.003 nM), but reduced antiviral activity (EC_{50} values 6.5 – 27.9 nM) against fingerprints encoding M31 (66% of fingerprints in EU HCV database). Grazoprevir (MK-5172) showed consistent activity against all genotype 2b NS5A chimeras.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

Table 6. Antiviral Activity of VEL (GS-5816) against Genotype 2a NS5A Polymorphs (Report PC-281-2008, page 15)

| Finger print | 28/30/31/44/56/58/62/92/93 | Freq | Cum. Freq | No. Replicons | GS-5816 EC ₅₀ (nM) ^a | BMS-790052 EC ₅₀ (nM) | MK-5172 EC ₅₀ (nM) ^d |
|----------------|----------------------------|------|-----------|---------------|--|----------------------------------|--|
| 1 ^b | FKMKRPNCY | 50% | 50% | 2 | 0.007-0.013 ^c | 10.6-19.7 | 17.0-19.7 |
| 2 | FKMKRPNCY | 20% | 70% | 1 | 0.005 | 6.7 | 14.0 |
| 3 | FKMKRPVY | 5% | 75% | 1 | 0.010 | 35.7 | 11.7 |
| 4 | FKMKRPSY | 5% | 80% | 1 | 0.003 | 2.6 | 10.4 |
| 5 | FKLRPNCY | 5% | 85% | 1 | 0.002 | 0.007 | 10.5 |
| 6 | FRLRPNCY | 5% | 90% | 1 | 0.001 | 0.016 | 11.8 |
| 7 | FKLRPNCY | 5% | 95% | 1 | 0.005 | 0.043 | 10.9 |

a Values represent the geometric mean of at least two independent experiments in 384-well assays

b This fingerprint was represented in Gilead's standard genotype 2a EC₅₀ assay

c EC₅₀ values are presented as a range of the activity (low-high) against multiple HCV replicons with the fingerprint

d The NS3/4A sequence for each of these entries is based on genotype 2a JFH-1 backbone

Table 7. Antiviral Activity of VEL (GS-5816) against Genotype 2b NS5A Polymorphs (Report PC-281-2008, page 16)

| Finger print | 28/30/31/44/56/58/62/92/93 | Freq | Cum. Freq | No. Replicons | GS-5816 EC ₅₀ (nM) ^a | BMS-790052 EC ₅₀ (nM) | MK-5172 EC ₅₀ (nM) ^d |
|----------------|----------------------------|------|-----------|---------------|--|----------------------------------|--|
| 1 ^b | LKMRRPNCY | 54% | 54% | 3 | 0.004-0.009 ^c | 6.5-22.5 | 7.2-8.1 |
| 2 | LKLRRPNCY | 17% | 71% | 2 | 0.001-0.003 | 0.001-0.003 | 9.3-11.5 |
| 3 | LKMRRPNCY | 8% | 80% | 1 | 0.011 | 27.9 | 7.2 |
| 4 | LKLRRPSY | 8% | 88% | 1 | 0.002 | 0.001 | 9.9 |
| 5 | LKMRRSNCY | 4% | 92% | 1 | 0.010 | 7.9 | 11.3 |

a Values represent the geometric mean of at least two independent experiments in 384-well assays

b This fingerprint was represented in Gilead's standard genotype 2b EC₅₀ assay

c EC₅₀ values are presented as a range of the activity (low-high) against multiple HCV replicons with the fingerprint

d The NS3/4A sequence for each of these entries is based on genotype 2a JFH-1 backbone

For genotype 3a, 15 NS5A chimeric replicons were established representing 8 NS5A fingerprints, which cumulatively cover 91% of the NS5A fingerprints observed in the EU database. VEL showed antiviral activity against the top 6 NS5A fingerprints (representing 89% of polymorphs), with EC₅₀ values ranging from 0.005 nM to 0.018 nM (Table 8). Two minor NS5A polymorphs, A30K and Y93H (which combined represent about 2% of available sequences), showed reduced susceptibility (18- to 267-fold) to VEL, with EC₅₀ values of 0.21 nM and 3.2 nM, respectively (Table 8). Daclatasvir showed variable activity against this panel of genotype 3 NS5A chimeric replicons, with EC₅₀ values ranging from 0.021 nM to >44.4 nM. Grazoprevir (MK-5172) showed consistent activity against all genotype 3a NS5A chimeras, since these replicons all contain the same GT1b Con-1 NS3 sequence.

For genotype 4a, ten NS5A chimeric replicons were successfully established representing 8 NS5A fingerprints, which cumulatively cover 90% of the NS5A fingerprints observed in the EU database. VEL had antiviral activity against all 8

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

NS5A fingerprints, with EC₅₀ values ranging from 0.002 nM to 0.009 nM (Table 9). Daclatasvir had antiviral activity against genotype 4a polymorphs with the exception of those encoding L30R or L28V (fingerprints #5 and #8, respectively), which have reduced activity (7- to 17-fold) compared to the ED43 genotype 4a strain. Grazoprevir (MK-5172) has consistent activity against all genotype 4a NS5A chimeras.

Table 8. Antiviral Activity of VEL (GS-5816) against Genotype 3a NS5A Polymorphs (Report PC-281-2008, page 17)

| Finger print | 28/30/31/44/56/58/62/92/93 | Freq | Cum. Freq | No. Replicons | GS-5816 EC ₅₀ (nM) ^a | BMS-790052 EC ₅₀ (nM) | MK-5172 EC ₅₀ (nM) ^e |
|----------------|----------------------------|------|-----------|---------------|--|----------------------------------|--|
| 1 ^b | MALKRPSEY | 54% | 54% | 5 | 0.005-0.018 ^d | 0.038-0.35 | 0.14-0.42 |
| 2 | MALKRPTEY | 15% | 69% | 3 | 0.009-0.018 | 0.11-0.15 | 0.37-0.43 |
| 3 | MALKRPLEY | 9% | 77% | 2 | 0.009-0.012 | 0.077-0.26 | 0.16-0.32 |
| 4 | MTLKRPEY | 5% | 82% | 1 | 0.007 | 0.021 | 0.15 |
| 5 | MALKRSTEY | 4% | 86% | 1 | 0.010 | 0.28 | 0.15 |
| 6 | MALKRSSEY | 3% | 89% | 1 | 0.014 | 0.087 | 0.16 |
| 7 ^c | MKLKRPEY | 1% | 90% | 1 | 0.21 | 34.8 | 0.41 |
| 8 ^c | MALKRPSEH | 1% | 91% | 1 | 3.2 | > 44.4 | 0.25 |

- a Values represent the geometric mean of at least two independent experiments in 384-well assays
- b This fingerprint was represented in Gilead's standard genotype 3a EC₅₀ assay
- c Replicons were constructed by site-directed mutagenesis of the colored residues into the standard genotype 3a NS5A chimeric replicon. Others were established with clinical isolate NS5A genes from EU HCV database
- d EC₅₀ values are presented as a range of the activity (low-high) against multiple HCV replicons with the fingerprint
- e The NS3/4A sequence for each of these entries is based on genotype 1b Con-1 backbone

Table 9. Antiviral Activity of VEL (GS-5816) against Genotype 4a NS5A Polymorphs (Report PC-281-2008, page 18)

| Finger print | 28/30/31/44/56/58/62/92/93 | Freq | Cum. Freq | No. Replicons | GS-5816 EC ₅₀ (nM) ^a | BMS-790052 EC ₅₀ (nM) | MK-5172 EC ₅₀ (nM) ^e |
|----------------|----------------------------|------|-----------|---------------|--|----------------------------------|--|
| 1 ^b | LLMKTPEAY | 10% | 10% | 2 | 0.002-0.009 ^d | 0.006-0.009 | 0.08-0.09 |
| 2 | LLMKKPEAY | 20% | 30% | 2 | 0.002-0.005 | 0.005-0.008 | 0.03-0.14 |
| 3 ^c | MLMKTPEAY | 10% | 40% | 1 | 0.002 | 0.013 | 0.19 |
| 4 | LLMKTPEAY | 10% | 50% | 1 | 0.003 | 0.007 | 0.14 |
| 5 ^c | LRMKTPEAY | 10% | 60% | 1 | 0.003 | 0.034 | 0.13 |
| 6 ^c | LLMRTPPEAY | 10% | 70% | 1 | 0.002 | 0.007 | 0.11 |
| 7 | LLMRKPEAY | 10% | 80% | 1 | 0.002 | 0.005 | 0.11 |
| 8 ^c | VLMKTPEAY | 10% | 90% | 1 | 0.003 | 0.084 | 0.07 |

- a Values represent the geometric mean of at least two independent experiments in manual 96-well assays
- b This fingerprint was represented in Gilead's standard genotype 4a EC₅₀ assay
- c Replicons were constructed by site-directed mutagenesis of the colored residues into the standard genotype 4a NS5A chimeric replicon. Others were established with clinical isolate NS5A genes from EU HCV database
- d EC₅₀ values are presented as a range of the activity (low-high) against multiple HCV replicons with the fingerprint
- e The NS3/4A sequence for each of these entries is based on genotype 1b Con-1 backbone

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

Genotypes 5 and 6

The antiviral activity of VEL in cell culture against representative genotype 5a, 6a and 6e HCV strains was tested in genotype 1b chimeric replicons that encode NS5A amino acids 9-184 of genotype 5a (SA13 strain), 6a (HK6 strain) and 6e (D88 strain). Full length NS5A chimeric replicons were constructed, but lacked sufficient replication. In transient replicon inhibition assays, VEL demonstrated antiviral activity against genotypes 5a, 6a and 6e with EC₅₀ values of 0.075 nM, 0.006 nM, and 0.13 nM, respectively (Table 10).

Table 10. Genotype 5a, 6a and 6e NS5A Chimeric Replicon Antiviral Activity for VEL (GS-5816) (Report PC-281-2007, page 17)

| Compound | Drug Target | GT5a NS5A EC ₅₀ (nM) ^{a,b} | GT6a NS5A EC ₅₀ (nM) ^{a,b} | GT6e NS5A EC ₅₀ (nM) ^{a,b} |
|------------|-------------|--|--|--|
| GS-5816 | NS5A | 0.075 | 0.006 | 0.13 |
| GS-5885 | NS5A | 0.081 | 0.15 | > 44.4 |
| BMS-790052 | NS5A | 0.071 | 0.031 | > 28.5 |
| BILN-2061 | NS3/4A | 0.24 | 0.21 | 0.36 |

a Values represent the geometric mean of at least three independent experiments in 384-well assays

b Chimeric replicons were based on GT1b Rluc-neo backbone and evaluated in transient replicon assays

The antiviral activity of VEL was also tested using a tissue-culture adapted GT2a J6/JFH infectious virus (HCVcc). Treatment of CD81-Lunet cells infected with the GT2a J6/JFH virus at a multiplicity of infection (MOI) of 0.5 tissue culture infective dose (TCID₅₀) per cell yielded an EC₅₀ value of 0.008 nM for VEL. This result is in close agreement with the EC₅₀ value in corresponding GT2a JFH-1 replicon cells (EC₅₀= 0.009 nM), indicating that VEL has activity against infectious HCV in cell culture. The antiviral activities of GS-5885 (ledipasvir) and other control compounds BILN-2061, daclatasvir (BMS-790052) and 2'-C-Me-A were consistent with historical results. No cytotoxicity of VEL was observed at the highest concentration tested (CC₅₀ > 44,400 nM).

In a separate experiment, VEL was assessed for antiviral activity against a panel of HCV replicons representing NS5A sequences for genotypes 1-6 in standard 3-day cell-based replicon assays. VEL has EC₅₀ values of 0.014 nM and 0.016 nM in GT1a and GT1b replicon assays, respectively. VEL also exhibited antiviral activity ranging from 0.002 nM to 0.13 nM across genotypes 1-6 (Table 11). VEL demonstrated antiviral activity against full length HCV replicons of GT2a (J6), GT2b (J8), GT4d (QC382), GT5a (SA13) and GT6a (GSI6a-1) with EC₅₀ values of 0.005 nM, 0.004 nM, 0.004 nM, 0.021 nM, and 0.009 nM, respectively.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

Table 11. VEL Antiviral Activity against Genotype 1-6 HCV (Report PC-281-2026, page 12)

| Genotype | HCV Replicon Strain | Full length or NS5A chimera | VEL EC ₅₀ (nM) ^a | LDV EC ₅₀ (nM) ^a | SOF EC ₅₀ (nM) ^a |
|-------------------|-----------------------|-----------------------------|--|--|--|
| 1a ^b | H77 | Full length | 0.014 ^g | 0.031 ^h | 40 ⁱ |
| 1b ^b | Con1 | Full length | 0.016 ^g | 0.004 ^h | 110 ⁱ |
| 2a ^{b,d} | JFH-1 (L31 in NS5A) | Full length | 0.008 ^g | 21 ^h | 50 ⁱ |
| 2a ^{b,e} | J6 (M31 in NS5A) | Full length | 0.005 | 266 | 14.9 |
| 2a ^{c,e} | J6 (M31 in NS5A) | NS5A chimera | 0.016 ^g | 249 ^h | 15 ⁱ |
| 2b ^b | J8 (M31 in NS5A) | Full length | 0.004 | 369 | 8.6 ^c |
| 2b ^{c,e} | MD2b8-2 (L31 in NS5A) | NS5A chimera | 0.002 ^g | 16 ^h | 15 ⁱ |
| 2b ^{c,e} | MD2b-1 (M31 in NS5A) | NS5A chimera | 0.006 ^g | 530 ^h | NA |
| 3a ^c | S52 | Full length | 0.004 ^g | 168 ^h | 50 ⁱ |
| 4a ^b | ED43 | Full length | 0.009 ^g | 0.390 ^h | 40 ⁱ |
| 4d ^b | QC382 | Full length | 0.004 | 0.290 | 33.4 |
| 5a ^{c,e} | SA13 | NS5A chimera | 0.054 ^g | 0.150 ^h | 15 ⁱ |
| 5a ^f | SA13 | Full length | 0.021 | 0.257 | NA |
| 6a ^b | HK6a consensus | Full length | 0.006 ^g | 1.1 ^h | 14 ⁱ |
| 6a ^c | GSI6a-1 | Full length | 0.009 | 0.114 | 24.7 |
| 6e ^{c,e} | D88 | NS5A chimera | 0.130 ^g | 264 ^h | NA |

Not applicable = NA

- a Values represent the geometric mean from at least three independent experiments in either automated 96-well or 384-well format
- b Stable subgenomic Rluc-encoding replicon cell lines
- c Transiently transfected replicons in 1C cells
- d Transiently transfected JFH-1 (L31 in NS5A) yielded an EC₅₀ of 6.8 nM
- e NS5A chimeric replicons either encoding full-length NS5A (2a and 2b) or NS5A amino acids 9-184 (5a and 6e)
- f Stable subgenomic GT 5a replicon was tested in a NS3 activity assay
- g Values are from PC-281-2024
- h Values are from PC-256-2037
- i Values are from PC-334-2005

Activity of VEL against GT1 - 6 Clinical Isolates

The NS5A gene was amplified from individual subject plasma samples and cloned into the replicon-based shuttle vectors. This pooled population of molecules was utilized for transient replication assays. In total, NS5A from 261 clinical isolates were tested for their sensitivity to VEL. Of the 261 samples tested, 249 samples successfully replicated in cell culture. The 249 samples includes clinical isolates with GT/subtype 1a (n=23), 1b (n=34), 2a (n=8), 2b (n=16), 2c (n=3), 2i (n=3), 2j (n=1), 2k (n=2), 3a (n=38), 3b (n=2), 4a (n=5), 4b (n=1), 4c (n=1), 4d (n=9), 4f (n=1), 4k (n=1), 4n (n=3), 4o (n=2), 4r (n=7), 4t (n=1), 5a (n=35), 6a (n=26), 6e (n=10), 6h (n=2), 6k (n=1), 6l (n=5), 6m (n=1), 6n (n=2), and 6q (n=2). The median EC₅₀ values of VEL against GT1, GT2, GT3, GT4, GT5, and GT6 baseline subject isolates were 0.015 nM, 0.005 nM, 0.006 nM, 0.005 nM, 0.005 nM, and 0.014 nM, respectively (Table 12). Minimal variations in susceptibility among GT1, GT4 and GT5 baseline isolates were observed with 16.5, 9.9 and 8.1-fold differences between the 95th and 5th percentiles of EC₅₀ values respectively, compared to 574-, 111082- and 619-fold differences for GT2, GT3 and GT6 baseline isolates, respectively.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

The reductions in susceptibility observed for individual clinical isolates correlated with known NS5A resistance-associated polymorphisms present in those samples.

Table 12. VEL Activity against Treatment-Naïve GT 1-6 Clinical Isolates (Report PC-281-2029; page 13)

| VEL | GT1 (n=57) | GT2 (n=37) | GT3 (n=40) | GT4 (n=31) | GT5 (n=35) | GT6 (n=49) |
|---|---------------|----------------|----------------|----------------|----------------|---------------|
| Mean EC ₅₀ ± SD (nM) | 0.029 ± 0.068 | 0.027 ± 0.07 | 14.8 ± 65.1 | 0.005 ± 0.003 | 0.007 ± 0.004 | 0.11 ± 0.40 |
| Median | 0.015 | 0.005 | 0.006 | 0.005 | 0.005 | 0.014 |
| Range (Min, Max) | (0.005, 0.5) | (0.0003, 0.36) | (0.002, 319.1) | (0.001, 0.014) | (0.001, 0.019) | (0.0005, 2.6) |
| 5% Percentile | 0.005 | 0.0003 | 0.002 | 0.001 | 0.002 | 0.001 |
| 95% Percentile | 0.089 | 0.20 | 255.6 | 0.012 | 0.016 | 0.76 |
| Fold Change in the 95 th Percentile and the 5 th Percentile | 16.5 | 574.5 | 111082 | 9.9 | 8.1 | 619.1 |

Clinical isolates from 7 subtypes with GT2 infection were tested (2a, 2b, 2c, 2e, 2i, 2j, 2k). Median EC₅₀ values ranged from 0.002 nM to 0.11 nM (Table 13). The highest activity was observed against GT2b and the lowest potency against GT2i. L31M was observed in all GT2a isolates, 40% of the GT2b isolates (4 of 16), and the majority of the other GT2 subtypes. The presence of L31M coincided with a median of 6-fold reduced susceptibility to VEL against GT2a compared with GT2b.

For GT3 clinical isolates, GT3a isolates showed a median VEL EC₅₀ value of 0.005 nM (Table 14). There were only 2 GT3b isolates; both had NS5A polymorphisms A30K + L31M and strongly reduced susceptibility to VEL. The presence of single A30K and Y93H variants were also associated with reduced susceptibility in GT3a.

Table 13. Antiviral Activity of VEL against NS5A GT2 Subtypes (Report PC-281-2029; page 15)

| | GT2a (n=8) | GT2b (n=16) | GT2c (n=3) | GT2e (n=2) | GT2i (n=3) | GT2j (n=1) | GT2k (n=2) | GT2 (All) (n=37) ^a |
|----------------|--------------|---------------|---------------|--------------|-------------|------------|---------------|-------------------------------|
| Mean ± SD (nM) | 0.055 ± 0.13 | 0.002 ± 0.002 | 0.008 ± 0.009 | 0.093 ± 0.13 | 0.09 ± 0.08 | 0.007 | 0.003 ± 0.002 | 0.027 ± 0.07 |
| Median (nM) | 0.011 | 0.002 | 0.004 | 0.093 | 0.11 | 0.007 | 0.003 | 0.005 |

^a Two isolates with mix subtypes 2a/2b and 2j/2k included in GT 2 (All) analysis.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

Table 14. Antiviral Activity of VEL against NS5A GT3 Subtypes (Report PC-281-2029; page 16)

| | GT3a (n=38) | GT3b (n=2) | GT3 (All) (n=40) |
|----------------|-------------|-------------|------------------|
| Mean ± SD (nM) | 0.13 ± 0.39 | 294 ± 35.46 | 14.8 ± 65.1 |
| Median (nM) | 0.005 | 294 | 0.006 |

The activity of VEL was evaluated against 10 GT 4 subtypes: 4a, 4b, 4c, 4d, 4f, 4k, 4n, 4o, 4r, and 4t. Minimal differences were observed between the 10 subtypes and VEL susceptibility with median EC₅₀ values ranging between 0.002 nM to 0.008 nM against all subtypes tested. L28M, L30R and K24R were observed in a few of the GT4 isolates, but no effect on the antiviral activity of VEL was observed.

For GT5, only isolates with GT5a subtype were available. VEL showed activity against all GT5a isolates with a median EC₅₀ value of 0.005 nM.

When VEL was tested against 8 GT6 subtypes, the median EC₅₀ values ranged from 0.005 nM to 1.8 nM. The highest activity was observed for subtypes 6a, 6h, 6k, and 6l (median EC₅₀ value = 0.007 nM, 0.005 nM, 0.018 nM, 0.010 nM, respectively). The EC₅₀ values were highest in subtype 6n likely due to presence of double mutants F28V + T93S or F28M + T93S; however, there were only 1 or 2 subject isolates for the rare GT6 subtypes (h, k, m, n, q).

Activity of VEL against Rare Genotype 2-6 NS5A Clinical Isolates

In order to determine the susceptibilities of clinical isolates to NS5A inhibitors, the first 100 amino acids of the NS5A gene were amplified from individual patient samples and cloned into a NS5A GT1b shuttle vector. After DNA digestion and RNA transcription, the pooled population of viral genomes from each patient was used in a transient replication assays. NS5A variants from subject isolates from GT2 (subtypes c, j and r), GT3 (subtypes g and i), GT4 (subtypes d and k), GT5a and GT6 (subtypes e, f, i, j, n, o, and q), to VEL were characterized. The amino acids at positions 24, 28, 30, 31, 32, 38, 58, 92, and 93 in the rare subtype clinical isolates in genotype 1-6 infection are shown in Table 15.

All genotype 5 and 6a, 6e, 6f, 6i, 6j and 6o subject isolates have the Y93T substitution which confers 2.5- to 100-fold reductions in susceptibility to VEL in the GT1a and 1b replicons. At position Q30, GT2 and GT3 isolates have K, GT4 isolates have R, and GT6e, f, n, and q have S. The EC₅₀ values of VEL for GT4 and 5a isolates were similar to the EC₅₀ values of the wild-type GT4a or 5a (Table 16). For GT6 isolates, EC₅₀ values ranged from 0.0015 nM for the GT6o isolate to 6.1 nM for the GT6f isolate (>1000-fold of 6a consensus). VEL had approximately 50-fold decreased susceptibility for GT6j and GT6n compared to the GT6a consensus strain. VEL was less active against GT3g and 3i with EC₅₀ values of 14.4 nM and 4.2 nM, respectively (both >1000-fold of GT3a), likely due to the Q30K together with A92E.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

Table 15. Amino Acids at NS5A Resistance-Associated Positions (PC-281-2028, page 16-17)

| Genotype | Sample ^C | Amino Acid Position in GT1a | | | | | | | | |
|----------|------------------------|-----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| | | K24 | M28 | Q30 | L31 | P32 | S38 | H58 | A92 | Y93 |
| 1a | H77 ^a | K | M | Q | L | P | S | H | A | Y |
| 2a | JFH | T | F | K | | | | P | C | |
| 2c | isolate 1 | S | F | K | F | | | | C | |
| 2j | isolate 1 | S | F | K | M | | | | C | |
| | isolate 2 | S | F | K | M | | | | C | |
| 2q | isolate 1 | S | C | K | | | | | C | |
| 3a | S52 ^a | S | | A | | | | P | E | |
| 3g | isolate 1 | A | | K | | | | P | E | |
| 3i | isolate 1 | | | K | | | | P | E | |
| 4d | isolate 1 | | L | R | M | | | T | | |
| 4k | isolate 1 | | L | R | | | | P | | |
| 5a | isolate 1 | Q | L | | | | | P | | T |
| | isolate 2 | Q | L | | | | | P | | T |
| | isolate 3 | Q | L | | | | | P | | T |
| | isolate 4 | Q | L | | | | | P | | T |
| | isolate 5 | Q | L | | | | | P | | T |
| | isolate 6 | Q | L | | | | | P | | T |
| | 5a SA13 ^b | Q | L | | | | | P | | T |
| 6a | consensus ^a | | L | R | | | | T | | T |
| 6e | isolate 1 | R | | S | | | | P | | T |
| | isolate 2 | | | S | | | | P | | T |
| | isolate 3 | | V | S | | | | P | | T |
| | isolate 4 | | V | S | | | | P | | T |

| Genotype | Sample ^C | Amino Acid Position in GT1a | | | | | | | | |
|----------|------------------------|-----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| | | K24 | M28 | Q30 | L31 | P32 | S38 | H58 | A92 | Y93 |
| 6e | isolate 5 | | V | S | | | | | | T |
| | 6e D42 ^b | | | S | | | | P | | T |
| 6f | isolate 1 | | A | S | | | | A | | T |
| 6i | isolate 1 | | V | A | | | | | | T |
| 6j | isolate 1 | | | A | | | | A | | T |
| 6n | isolate 1 | | V | S | | | | T | | S |
| | isolate 2 | | V | S | | | | T | | S |
| | 6n D83/96 ^b | | V | S | | | | T | | S |
| 6o | isolate 1 | | L | A | | | | A | | T |
| 6q | isolate 1 | | V | S | | | | P | | S |

GT = genotype

a full-length replicon, wild-type lab strain

b Sequences obtained from data base

c All samples are chimeric replicons containing first 100 amino acid of NS5A in 1b backbone, except GT1a and 6a.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

Table 16. Susceptibility of Non-genotype 1 Patient Isolates to NS5A Inhibitors LDV and VEL (Report PC-281-2028, page 18-19)

| Genotype | Sample ^b | LDV | | VEL | |
|-----------------|----------------------|----------------------------------|----------|----------------------------------|----------|
| | | EC ₅₀ nM ^c | FC/1a WT | EC ₅₀ nM ^c | FC/1a WT |
| 1a ^a | H77 | 0.031 | | 0.014 | |
| 2a ^a | JFH | 20.6 | 664.5 | 0.008 | 0.6 |
| 2c | isolate 1 | 363.2 ± 2.7 | 11716.1 | 0.035 ± 0.014 | 2.5 |
| 2j | isolate 1 | 129.4 ± 9.2 | 4174.2 | 0.0076 ± 0.0024 | 0.54 |
| | isolate 2 | 182.5 ± 97.2 | 5887.1 | 0.0099 ± 0.003 | 0.71 |
| 2q | isolate 1 | 388.1 ± 3.5 | 12517.7 | 0.022 ± 0.0085 | 1.6 |
| 3a ^a | S52 | 168 | 5419.4 | 0.004 | 0.029 |
| 3g | isolate 1 | 1227 ± 62.2 | 39580.6 | 14.4 ± 2.7 | 1030.0 |
| 3i | isolate 1 | 1285.5 ± 51.6 | 41467.7 | 4.2 ± 0.5 | 300.7 |
| 4a ^a | ED43 | 0.39 | 12.6 | 0.009 | 0.6 |
| 4d | isolate 1 | 0.032 ± 0.008 | 1.0 | 0.0053 ± 0.0021 | 0.38 |
| 4k | isolate 1 | 0.005 ± 0.001 | 0.16 | 0.0037 ± 0.0012 | 0.26 |
| 5a | S13 | 0.15 | 4.8 | 0.054 | 3.9 |
| 5a | isolate 1 | 0.074 ± 0.006 | 2.4 | 0.0036 ± 0.0023 | 0.26 |
| | isolate 2 | 0.25 ± 0.16 | 8.1 | 0.0034 ± 0.0013 | 0.24 |
| | isolate 3 | 0.17 ± 0.07 | 5.5 | 0.0054 ± 0.0019 | 0.39 |
| | isolate 4 | 0.14 ± 0.04 | 4.5 | 0.0054 ± 0.0019 | 0.39 |
| | isolate 5 | 0.17 ± 0.08 | 5.5 | 0.0053 ± 0.0029 | 0.38 |
| | isolate 6 | 0.24 ± 0.1 | 7.7 | 0.003 ± 0.0016 | 0.21 |
| | 5a SA13 ^b | 0.11 ± 0.05 | 3.5 | 0.0069 ± 0.004 | 0.49 |
| 6a ^a | consensus | 1.1 | 35.5 | 0.006 | 0.4 |
| 6e | isolate 1 | 830.1 ± 394.5 | 26775.8 | 0.0049 ± 0.0003 | 0.35 |
| | isolate 2 | 242.0 ± 78.4 | 7804.8 | 0.0094 ± 0.0033 | 0.67 |
| | isolate 3 | 85.4 ± 23.8 | 2754.2 | 0.012 ± 0.01 | 0.86 |
| | isolate 4 | 125.0 ± 9.6 | 4030.6 | 0.024 ± 0.023 | 1.7 |

| Genotype | Sample ^b | LDV | | VEL | |
|----------|------------------------|----------------------------------|----------|----------------------------------|----------|
| | | EC ₅₀ nM ^c | FC/1a WT | EC ₅₀ nM ^c | FC/1a WT |
| | 6e D42 ^b | 17.0 ± 0.62 | 548.7 | 0.0054 ± 0.0044 | 0.39 |
| 6f | isolate 1 | 422.6 ± 21.3 | 13630.6 | 6.1 ± 0.91 | 432.1 |
| 6i | isolate 1 | 2.1 ± 0.02 | 68.7 | 0.0051 ± 0.002 | 0.36 |
| 6j | isolate 1 | 157.4 ± 1.1 | 5075.8 | 0.20 ± 0.14 | 14.2 |
| 6n | isolate 1 | 152 ± 50.6 | 4903.2 | 0.28 ± 0.072 | 20.0 |
| | isolate 2 | 27.2 ± 5.2 | 877.4 | 0.16 ± 0.06 | 11.4 |
| | 6n D83/96 ^b | 38.2 ± 5.1 | 1231.9 | 0.067 ± 0.025 | 4.8 |
| 6o | isolate 1 | 0.7 ± 0.3 | 22.6 | 0.0015 ± 0.0004 | 0.11 |
| 6q | isolate 1 | 1298.5 ± 1313.1 | 41887.1 | 0.024 ± 0.016 | 1.7 |

a full-length replicon, wild-type lab strain

b Sequences obtained from data base

c All samples are chimeric replicons containing first 100 amino acid of NS5A in 1b backbone, except GT1a and 6a.

d Values from two or more independent experiments

Twenty substitutions in genotype 3g, 5a, 6e, 6f, 6i, 6o and 6q that are different from those in genotype 1a were selected and reverted back to the genotype 1a wild-type

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

amino acid (Table 17). Five amino acid substitutions at positions 24, 27, 30, 37 and 92 in genotype 3g were changed to the wild-type amino acids of genotype 1a; A24K, K30Q, E92A, and I27L and I37F. VEL had an EC₅₀ value of 16.1 nM against GT3g (WT). The single reversion mutants K30Q restored VEL susceptibility (0.0084 nM) comparable to the GT1a wild-type (0.014 nM) (Table 18). Reversion mutant E92A partially restored VEL susceptibility to EC₅₀ value of 0.21 nM, while the double mutant K30Q+E92A fully restored VEL susceptibility with an EC₅₀ value of 0.0015 nM. For VEL, it appears that reversion of K30Q alone is sufficient to restore full susceptibility in this genotype 3g isolate.

Table 17. Substitutions in Genotype 3g, 5a, 6e, 6f, 6i, 6o and 6q Reverted Back to 1a Wild-type Amino Acid (PC-281-2028, page 19)

| GT | Substitutions at GT1a Resistance Associated Positions | | | | | | Substitutions at GT1a Other Positions | | | |
|-------|---|----|----|----|----|----|---------------------------------------|----|----|----|
| | 24 | 28 | 30 | 58 | 92 | 93 | 7 | 27 | 37 | 83 |
| 1a WT | K | M | Q | H | A | Y | D | L | F | M |
| 1b WT | Q | L | R | | | | | | F | T |
| 3g | A | | K | | E | | | I | I | |
| 5a | | L | | | | T | A | | | |
| 6e | | V | S | | | T | | | | V |
| 6f | | A | | A | | T | | | L | |
| 6i | | | A | | | | | | | |
| 6o | | L | | | | | | | | |
| 6q | | | | | | S | | | | I |

Wild-type = WT; Genotype = GT
Marked in bold are all amino acids that are different from GT 1a

Table 18. Susceptibility of Genotype 3g Revertants to VEL (PC-281-2028, page 21)

| Genotype | Mutation | Amino Acid Changes from GT1a | LDV EC ₅₀ nM ^c | Fold Change from Genotype 1a WT | VEL EC ₅₀ nM ^c | Fold Change from Genotype 1a WT |
|-----------------|-----------|------------------------------|--------------------------------------|---------------------------------|--------------------------------------|---------------------------------|
| 1a ^a | WT | K24, L27, Q30, F37, A92 | 0.031 | | 0.014 | |
| 3a ^a | WT | S24, I27, A30, I37, E92 | 168 | 5419.4 | 0.028 | 2 |
| 3g ^b | WT | A24, I27, K30, I37, E92 | 854 ± 760.7 | 27548.4 ± 24538.7 | 16.1 ± 5.32 | 1147.9 ± 380 |
| | A24K | I27, K30, I37, E92 | 803.3 ± 696.1 | 25912.9 ± 22454.8 | 21.78 ± 7.8 | 1555.7 ± 557.1 |
| | I27L | A24, K30, I37, E92 | 1404.0 ± 206.6 | 45290.3 ± 6664.5 | 21.62 ± 8.65 | 1544.3 ± 617.9 |
| | K30Q | A24, I27, I37, E92 | 216.7 ± 42.7 | 6990.3 ± 1377.4 | 0.0084 ± 0.0051 | 0.6 ± 0.36 |
| | I37F | A24, I27, K30, E92 | 849.0 ± 735.4 | 27387.1 ± 23722.6 | 10.4 ± 4.3 | 742.9 ± 307.1 |
| | E92A | A24, I27, K30, I37 | 473.5 ± 83.4 | 15274.2 ± 2690.3 | 0.21 ± 0.04 | 15 ± 2.9 |
| | K30Q+E92A | A24, I27, I37 | 0.49 ± 0.11 | 15.8 ± 3.5 | 0.0015 ± 0.0004 | 0.11 ± 0.029 |

Wild-type = WT; GT = genotype
a full-length replicon, WT lab strain
b a clone of chimeric replicon with first 100 amino acid of NSSA in 1b backbone
c Values from two or more independent experiments

Likewise, 6 amino acids at positions 28, 30, 37, 58, 83 and 93 in GT6 isolates were changed to the wild-type amino acids in GT1a. In GT6e, substitutions V28M and S30Q restored the susceptibility to VEL with 4.7- and 2.2-fold changes from the 1a wild-type replicon. In GT6f, both A28M and T93Y restored VEL activity (fold changes in EC₅₀

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

value were 0.01 and 0.039, respectively) (Table 19). L28M in genotype 6o was less susceptible (37-fold) to VEL than the wild-type.

Table 19. Susceptibility of Genotype 6 Revertants to VEL (PC-281-2028, page 23)

| Genotype | Mutation | Amino Acid Changes from GT1a | LDV EC ₅₀ nM ^c | Fold Change from Genotype 1a WT | VEL EC ₅₀ nM ^c | Fold Change from Genotype 1a WT |
|-----------------|----------|------------------------------|--------------------------------------|---------------------------------|--------------------------------------|---------------------------------|
| 1a ^a | WT | M28, Q30, H58, M83, Y93 | 0.031 | | 0.014 | |
| 6a ^a | WT | L28, R30, T58, V83, T93 | 1.1 ± 0.25 | 35.5 ± 8.1 | 0.006 | 0.43 |
| 6e ^b | WT | V28, S30, V83, T93 | 13.7 ± 0.32 | 441.9 ± 10.3 | 0.42 ± 0.0059 | 30 ± 0.42 |
| | V28M | S30, V83, T93 | 11.7 ± 3.7 | 377.4 ± 119.4 | 0.066 ± 0.017 | 4.7 ± 1.2 |
| | S30Q | V28, V83, T93 | 2.4 ± 0.4 | 77.4 ± 12.9 | 0.031 ± 0.018 | 2.2 ± 1 |
| | V83M | V28, S30, T93 | 11.1 ± 3.2 | 358.1 ± 103.2 | 0.3 ± 0.05 | 21.4 ± 4 |
| | T93Y | V28, S30, V83 | n.r | | n.r | |
| 6f ^b | WT | A28, S30, L37, A58, T93 | 636.3 ± 54.4 | 20525.8 ± 1754.8 | 19.08 ± 7.7 | 1362.9 ± 550 |
| | A28M | S30, L37, A58, T93 | 86.0 ± 6.5 | 2774.2 ± 209.7 | 0.011 ± 0.0039 | 0.79 ± 0.28 |
| | L37F | A28, S30, A58, T93 | 1081.8 ± 242.1 | 34896.8 ± 7809.7 | 23.7 ± 2.9 | 1692.9 ± 207.14 |
| | A58H | A28, S30, L37, T93 | 342.0 ± 37.0 | 11032.3 ± 1193.5 | 18.9 ± 0.078 | 1350 ± 5.6 |
| | T93Y | A28, S30, L37, A58 | 6.4 ± 1.2 | 206.5 ± 38.7 | 0.039 ± 0.0016 | 2.8 ± 0.11 |
| 6i ^b | WT | V28, A30, T93 | 0.70 ± 0.1 | 22.6 ± 3.2 | 0.003 ± 0.0002 | 0.21 ± 0.01 |
| | A30Q | V28, T93 | 0.16 ± 0.0087 | 5.2 ± 0.3 | 0.003 ± 0.0011 | 0.21 ± 0.08 |
| 6o ^b | WT | L28, A30, A58, T93 | 0.21 ± 0.036 | 6.8 ± 1.2 | 0.00091 ± 0.00019 | 0.07 ± 0.01 |
| | L28M | A30, A58, T93 | 35.8 ± 10.6 | 1154.8 ± 341.9 | 0.034 ± 0.0045 | 2.4 ± 0.32 |
| 6q ^b | WT | V28, S30, I83, S93 | 3806.0 ± 3406.8 | 122774.2 ± 109896.8 | 0.057 ± 0.033 | 4.1 ± 2.4 |
| | I83M | V28, S30, S93 | 1620.0 ± 435.6 | 52258.1 ± 14051.6 | 0.116 ± 0.028 | 8.3 ± 2 |
| | S93Y | V28, S30, I83 | n.r | | n.r | |

GT = genotype; n.r = no replication

a full-length replicon, WT lab strain

b a clone of chimeric replicon with first 100 amino acid of NS5A in 1b backbone

c Values from two or more independent experiments

ANTIVIRAL ACTIVITY IN THE PRESENCE OF HUMAN SERUM

To determine the effect of plasma protein binding on the activity of VEL, stable GT1a HCV replicon cells were treated with VEL in complete cell culture medium (CCM) containing 10% fetal bovine serum (FBS) plus 40% human serum (HS). VEL showed a 13.3-fold reduction in activity against the GT1a HCV replicon in the presence of 40% human serum which is comparable to ledipasvir (GS-5885) (11.6-fold) but higher than daclatasvir (BMS-790052) (2.5-fold) (Table 20). Other compounds tested, including BILN-2061 and 2'-CMe-A, yielded fold shifts similar to historical results.

Table 20. Effect of 40% Human Serum on Genotype, 1a Replicon Activity of VEL (GS-5816) (Report PC-281-2007 page 18)

| Compound | Drug Target | EC ₅₀ (nM) ^a (CCM) | EC ₅₀ (nM) ^a (CCM + 40% HS) | EC ₅₀ Fold Shift |
|------------|-------------|---|--|-----------------------------|
| GS-5816 | NS5A | 0.012 | 0.16 | 13.3 |
| GS-5885 | NS5A | 0.031 | 0.36 | 11.6 |
| BMS-790052 | NS5A | 0.063 | 0.16 | 2.5 |
| BILN-2061 | NS3/4A | 16.2 | 327 | 20.2 |
| 2'-C-Me-A | NS5B | 344 | 1054 | 3.1 |

a Values represent the geometric mean of at least three independent experiments in 384-well assays

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

ANTIVIRAL ACTIVITY AGAINST OTHER VIRUSES

The antiviral activity of VEL was tested against HCV and other viruses, including bovine viral diarrhea virus (BVDV), respiratory syncytial virus (RSV), hepatitis B virus (HBV) and human immunodeficiency virus (HIV) in their respective cell-based assay systems. In contrast to its antiviral activity against HCV (EC₅₀ value = 0.000012 – 0.00013 μM for genotype 1-6 replicons; PC-281-2007), VEL showed no selective antiviral activity against the related flavivirus BVDV (EC₅₀ value = 9.9 μM), or unrelated viruses including RSV, HBV and HIV as EC₅₀ values were similar to CC₅₀ values (Table 21). Known inhibitors were included in each assay and showed antiviral activities that agree with historical data.

Table 21. Antiviral Activity and Cytotoxicity of VEL (GS-5816) against HCV, BVDV, RSV, HBV and HIV (Report PC-281-2009, page 9)

| Virus | Description | Cell line | Positive Control | | GS-5816 ^{a,b} | |
|----------------------------|---|-----------|------------------|---------------------|------------------------|---------------------|
| | | | Compounds | EC ₅₀ μM | EC ₅₀ μM | CC ₅₀ μM |
| HCV Replicon (genotype 1a) | Hepacivirus (positive ssRNA) | Huh-7 | GS-5885 | 0.000031 | 0.000012 | > 44.4 |
| BVDV Replicon | Pestivirus (positive ssRNA) | Huh-7 | 2-C-Me-A | 0.17 | 9.9 | > 44.4 |
| RSV | Paramyxovirus (negative ssRNA) | Hep-2 | YM-53404 | 0.26 | > 100 | > 100 |
| HBV | Hepadnavirus (DNA reverse-transcribing) | AD-38 | TFV | 19 | > 50 | > 50 |
| HIV | Lentivirus (RNA reverse transcribing) | MT-4 | TDF | 6.3 | > 47 | > 47 |

a Values represent the geometric mean of at least two independent experiments

b Sign ">" indicates that the value is above the highest compound concentration tested in the assay

ANTIVIRAL ACTIVITY OF VEL IN COMBINATION WITH OTHER HCV AGENTS IN CELL CULTURE

The antiviral effects of VEL in combination with other anti-HCV compounds were evaluated in genotype 1a HCV replicon cells. The resulting data were analyzed using MacSynergy II. Using 95% confidence intervals, the mean synergy and antagonism volumes were between 25 and –25 μM²% when VEL was combined with IFN-α, RBV, vedroprevir (GS-9451), ledipasvir (GS-5885), GS-9669 or SOF (GS-7977), indicative of additive interaction with those compounds (Table 22). VEL showed synergy volumes in the range of 25 to 50 μM²% when combined with GS-6620, suggesting a minor synergistic interaction. Furthermore, VEL showed an average synergy volume of 86 μM²% when combined with GS-9190, suggesting a moderately synergistic interaction. No significant antagonism was observed for any combination in these experiments. In all combination experiments, the cell viability was higher than 75% at the highest concentrations tested indicating that the results of the antiviral combination experiments are not driven by any cytotoxic effects.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

Table 22. Quantification of Antiviral Synergy or Antagonism for Drug Combinations with VEL (GS-5816) (Report PC-281-2012, page 10)

| Drug Used in Combination with GS-5816 | Drug Target | Synergy Volume ($\mu\text{M}^2\%$) ^a | Antagonism Volume ($\mu\text{M}^2\%$) ^a | Interaction |
|---------------------------------------|-------------|---|--|------------------|
| GS-9451 | NS3/4A | 20 ± 16 | -4 ± 5 | Additive effect |
| GS-5885 | NS5A | 24 ± 8 | -1 ± 1 | Additive effect |
| GS-9190 | NonNuc NS5B | 86 ± 33 | -1 ± 1 | Moderate synergy |
| GS-9669 | NonNuc NS5B | 10 ± 8 | -1 ± 1 | Additive effect |
| GS-6620 | Nuc NS5B | 46 ± 21 | -13 ± 5 | Minor synergy |
| GS-7977 | Nuc NS5B | 3 ± 3 | -4 ± 3 | Additive effect |
| IFN- α | Host | 8 ± 6 | -1 ± 1 | Additive effect |
| RBV | Host | 9 ± 12 | -6 ± 7 | Additive effect |

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

RESISTANCE SELECTION

Genotype 1a

To determine the resistance profile in cell culture, drug-resistant colonies were selected with VEL in HCV genotype 1a and 1b replicon cells. To select GT1a replicons resistant to VEL, stable 1a H77 replicon cells were treated with 0.1 nM, 0.5 nM, 2.5 nM, or 12.5 nM VEL (8, 40, 200, and 1000 × EC₅₀ value, respectively) for three weeks, which resulted in selection of resistant colonies at each drug concentration. The number of selected colonies significantly decreased as the drug concentration increased. At a drug concentration of 12.5 nM, only one replicon clone survived from 1 × 10⁶ replicon cells. A total of thirty individual resistant colonies were isolated, expanded, and confirmed to have reduced susceptibility to VEL. The remaining colonies were pooled. All individual colonies and the pools were analyzed for substitutions in the NS5A gene. Most GT1a resistant clones encoded a single substitution L31V (15/29 clones; 52%) or Y93H (11/29 clones; 38%) (Table 23). Other single substitutions included Q30K (1/9 clones), L31M (2/9 clones) and Y93N (1/10 clones), although they occurred at a lower frequency. Double substitutions, Q30H/Y93H (1/9 clones) or L31V/Y93H/N (2/19 clones), were also observed in a small number of clones resistant to VEL.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

Table 23. NS5A Substitutions Observed in HCV Genotype 1a Replicon Cells after VEL Selection (PC-281-2013, page 12)

| Drug Concentration (Fold EC ₅₀) | No. of Colonies Selected from 1 x 10 ⁶ Cells | Clonal NS5A Mutations ^{a,b} | Frequency ^c | NS5A Mutations in Pool ^d |
|---|---|--------------------------------------|------------------------|-------------------------------------|
| 0.1 nM (8x) | > 500 | L31V | 4/9 | N.A. |
| | | L31M | 2/9 | |
| | | Q30K | 1/9 | |
| | | Y93H | 1/9 | |
| | | L31V + Y93H/N | 1/9 | |
| 0.5 nM (40x) | 300 | L31V | 5/10 | L31V, Y93H |
| | | Y93H | 3/10 | |
| | | Y93N | 1/10 | |
| | | L31V + Y93H/N | 1/10 | |
| 2.5 nM (200x) | 135 | L31V | 5/10 | L31V, Y93H |
| | | Y93H | 4/10 | |
| | | Q30H + Y93H | 1/10 | |
| 12.5 nM (1000x) | 1 | K24E + Q30R + Y93H | 1/1 | N.A. |

a Genotypic analyses were performed by population sequencing. The amino acid changes outside the NS5A domain I and the linker region are not listed

b Mutations listed within single rows were present in the same virus genome

c Frequency was presented as the number of resistant clones with the indicated NS5A mutations/ the total number of clones genotyped at each drug concentration

d Mutations were identified by population sequencing but it is unknown whether they were present in the same virus genome. N.A. indicates "not available"

To define the role of VEL-selected substitutions in conferring resistance to GT1a HCV, identified substitutions were introduced into a GT1a replicon by site-directed mutagenesis either individually or in combination as identified in the selected colonies. Results from transient replicon replication assays indicated that the NS5A substitutions L31V and Y93H conferred decreased susceptibility to VEL, with EC₅₀ value shifts of 129- and 1004-fold, respectively (Table 24). Both L31V and Y93H conferred larger shifts in susceptibility to ledipasvir (664- and 8341-fold, respectively) and daclatasvir (764- and 1325-fold, respectively). Single substitutions Q30K, L31M and Y93N, conferred 8-, 15-, and 2,926-fold resistance to VEL, respectively. These substitutions (Q30K, L31M, and Y93N) conferred a greater shift in susceptibility against ledipasvir (631-, 394- and 25,957-fold, respectively) and daclatasvir (745-, 161- and >15,221-fold, respectively). Q30H and Q30R, which are observed as frequent resistance substitutions selected by ledipasvir and daclatasvir in patients, were not selected as single resistance substitutions by VEL, but were found only in combination with Y93H. Q30H and Q30R alone conferred a 2-fold reduced susceptibility to VEL compared to >100-fold reduced susceptibility to ledipasvir and daclatasvir. Double mutants conferred greater reduced susceptibility to VEL than single mutants. Q30H/Y93H conferred 2248-fold resistance and Q30R/Y93H conferred 6452-fold resistance. The greatest resistance to VEL was observed when the resistance substitution L31V was selected in combination with either Y93H or Y93N (>89,660-fold for both). High-fold resistance was also seen for both ledipasvir and daclatasvir for these mutants. Deep sequencing analysis of patient samples from ledipasvir clinical studies indicated double mutants L31V + Y93H/N pre-exist at low frequency (< 0.01%).

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

Table 24. Phenotypic Analyses of Selected Genotype 1a Substitutions against VEL in Transient Replicon Replication Assays (PC-281-2013, page 14)

| | NS5A Mutation | GS-5816 | GS-5885 | BMS-790052 | MK-5172 | BILN-2061 | 2'-C-Me-A |
|------------------------------------|---------------|---------|---------|------------|---------|-----------|-----------|
| EC ₅₀ (nM) ^a | wild type | 0.005 | 0.010 | 0.029 | 0.69 | 21 | 61 |
| Fold Resistance ^b | Q30H | 2.1 | 96 | 167 | 1.6 | 1.1 | 1.3 |
| | Q30K | 7.6 | 631 | 745 | 1.3 | 1.2 | 1.0 |
| | Q30R | 2.5 | 167 | 105 | 1.4 | 1.1 | 1.2 |
| | L31M | 15 | 394 | 161 | 1.2 | 0.9 | 1.2 |
| | L31V | 129 | 664 | 764 | 1.5 | 1.4 | 1.6 |
| | Y93H | 1004 | 8341 | 1325 | 2.1 | 1.2 | 1.0 |
| | Y93N | 2926 | 25957 | >15221 | 2.7 | 1.7 | 1.1 |
| | Q30H+Y93H | 2248 | 9546 | 12708 | 1.7 | 1.1 | 0.7 |
| | Q30R+Y93H | 6452 | 17029 | 8348 | 1.4 | 0.6 | 0.7 |
| | L31V+Y93H | >89660 | >44609 | >15221 | 2.2 | 1.4 | 1.1 |
| | L31V+Y93N | >89660 | >44609 | >15221 | 2.5 | 1.2 | 1.3 |

a Values represent a geometric mean of two independent experiments in transient-transfection 384-well assays

b Fold resistance is calculated as the ratio of mutant EC₅₀ to wild-type EC₅₀

Genotype 1b

To select GT1b replicons resistant to VEL, stable 1b Con1 replicon cells were treated with 0.03 nM, 0.06 nM, 0.1 nM, 0.2 nM, or 0.5 nM VEL (2, 4, 6.6, 13, and 33 × EC₅₀ value, respectively) for three weeks, which resulted in the selection of resistant colonies at each drug concentration, though the number of selected colonies were fewer compared to the GT1a selections. Twenty-five individual VEL-resistant colonies (five colonies at each drug concentration) were isolated, expanded, and confirmed to have reduced susceptibility to VEL. The remaining colonies were pooled. Genotypic analyses revealed at least two NS5A amino acid substitutions in all of the resistant clones and pools (Table 25). Y93H was identified in each of the clones and pools and was never selected as a single substitution and instead always emerged in combination with one additional residue change in NS5A, including L28M, L31F, L31M, L31V, or Q54H (Table 25). The most frequent NS5A substitutions selected by VEL were L31V (15/25 clones; 60%) and Y93H (25/25 clones).

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

Table 25. NS5A Substitutions Observed in HCV Genotype 1b Replicon Cells after VEL Selection (PC-281-2013, page 13)

| Drug Concentration (x EC ₅₀) | No. of Colonies Selected from 1 x 10 ⁶ Cells | Clonal NS5A Mutations ^{a,b} | Frequency ^c | NS5A Mutations in Pool ^d |
|--|---|--------------------------------------|------------------------|-------------------------------------|
| 0.03 nM (2x) | 50 | L28M + Y93H | 2/5 | L31M/V, Q54H, Y93H |
| | | L31V + Y93H | 2/5 | |
| | | L31F + Y93H | 1/5 | |
| 0.06 nM (4x) | 39 | L31F + Y93H | 2/5 | L31F/V/M, Y93H |
| | | L31V + Y93H | 2/5 | |
| | | L31V + Y93H + Q54H | 1/5 | |
| 0.1 nM (7x) | 33 | L31F + Y93H | 3/5 | L31F/V/M, Y93H |
| | | L31V + Y93H | 2/5 | |
| 0.2 nM (13x) | 30 | L31F + Y93H | 2/5 | L31F/V/M, Y93H |
| | | L31V + Y93H | 2/5 | |
| | | L31V + Y93H + Q54H | 1/5 | |
| 0.5 nM (33x) | 18 | L31V + Y93H | 5/5 | L31V/M, Y93H |

- a Genotypic analyses were performed by population sequencing. The amino acid changes outside the NS5A domain I and the linker region are not listed
- b Mutations listed within single rows were present in the same virus genome
- c Frequency was presented as the number of resistant clones with the indicated NS5A mutations/ the total number of clones genotyped at each drug concentration
- d Mutations were identified by population sequencing but Y93H mutation represents 100% signal at residue position 93 in the sequencing chromatogram

To define the role of VEL-selected substitutions in genotype GT1b resistance, the observed substitutions were introduced into the GT1b replicon either individually or in combination as detected in the resistant replicon clones. In transient replicon assays, the single Y93H substitution in GT1b conferred a 2-fold decrease in VEL susceptibility, but had 27- and 1187-fold decreased susceptibility to daclatasvir and ledipasvir, respectively (Table 26). Single Y93H mutants were not selected in GT1b replicon cells at any of the tested drug concentrations. Y93H was only selected in combination with other NS5A substitutions as double mutants. Similarly, the other single NS5A substitutions selected in GT1b selections (L28M, L31F, L31M, L31V and Q54H) conferred ≤2-fold decreased susceptibility to VEL; however, when combined with Y93H, significant increases in VEL resistance were observed. The most commonly selected double mutant, L31V/Y93H, (present in all clones analyzed at the highest drug concentration; 0.5 nM), conferred 986-fold decreased susceptibility to VEL in GT1b. The second most common double mutant identified, L31F/Y93H, was selected at drug concentrations up to 13x (but not 33x EC₅₀ value) and showed a 27-fold decreased VEL susceptibility. Similarly, the double mutants L28M/Y93H (only selected at 2x EC₅₀ value) and L31M/Y93H (identified in resistance colony pools up to 33x EC₅₀ value) conferred 5- and 68-fold decreased susceptibility to VEL, respectively. All the double mutants showed >2,000-fold resistance to ledipasvir and daclatasvir except for L28M/Y93H with 226-fold to daclatasvir. The NS5A triple mutant, L31V/Q54H/Y93H, was observed during GT1b selections, but appeared to confer less resistance relative to L31V/Y93H (263-fold vs. 986-fold, respectively; Table 26).

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

Table 26. Phenotypic Analyses of Selected Genotype 1b Substitutions against VEL in Transient Replicon Replication Assays (PC-281-2013, page 16)

| | NS5A Mutation | GS-5816 | GS-5885 | BMS-790052 | MK-5172 | BILN-2061 | 2'-C-Me-A |
|------------------------------------|---------------|---------|---------|------------|---------|-----------|-----------|
| EC ₅₀ (nM) ^a | wild type | 0.016 | 0.006 | 0.027 | 0.48 | 1.2 | 75 |
| Fold Resistance ^b | L28M | 0.7 | 2.2 | 0.8 | 0.7 | 0.7 | 0.7 |
| | L31F | 0.7 | 2.4 | 2.1 | 1.0 | 1.1 | 0.7 |
| | L31M | 1.1 | 5.3 | 1.9 | 1.2 | 2.1 | 0.6 |
| | L31V | 0.6 | 33 | 3.7 | 0.4 | 0.6 | 0.6 |
| | Q54H | 1.0 | 1.2 | 1.1 | 1.1 | 2.1 | 0.8 |
| | Y93H | 2.0 | 1187 | 27 | 1.0 | 0.7 | 0.7 |
| | L28M+Y93H | 5.0 | 21736 | 225 | 1.3 | 0.9 | 1.3 |
| | L31F+Y93H | 27 | 16915 | 2956 | 1.2 | 0.7 | 0.8 |
| | L31M+Y93H | 68 | 27433 | 2068 | 1.5 | 1.2 | 1.2 |
| | L31V+Y93H | 986 | >70547 | 7662 | 1.6 | 1.3 | 1.5 |
| L31V+Q54H+Y93H | 263 | 51958 | 2387 | 0.9 | 1.1 | 0.6 | |

a Values represent a geometric mean of two independent experiments in transient-transfection 384-well assays

b Fold resistance is calculated as the ratio of mutant EC₅₀ to wild-type EC₅₀

Genotypes 2-6

To determine the resistance profile in cell culture, drug-resistant colonies were selected with VEL in HCV genotypes 2a (JFH), 2a (J6), 3a (S52), 4a (ED43), 5a (SA13) and 6a (GSI6a-1). Genotypic analysis of the NS5A coding region by deep sequencing and subsequent phenotypic analyses of resistance-associated substitutions that emerged in VEL-resistance replicon cells were performed (Tables 27 and 28).

Table 27. Resistance-Associated Substitutions Selected by VEL in Genotypes 2-6 (Report PC-281-2027, page 10)

| | 24 | 28 | 30 | 31 | 32 | 58 | 92 | 93 |
|-----------------|------|------|----|----------|------------|------|------|--------|
| GT 2a (JFH) | T24A | F28S | | | | | C92R | Y93D |
| GT 2a (J6) | T24A | F28S | | | | | | Y93H |
| GT 3a (S52) | | M28T | | L31I/F/V | | | E92K | Y93H/S |
| GT 4a (ED43) | | | | M31I | | P58L | | Y93H |
| GT 5a (SA13) | | | | L31P | | | | |
| GT 6a (GSI6a-1) | | | | L31V | P32A/L/Q/R | | | |

GT = genotype

Two stable full length GT2 replicon cells that have different amino acids at position 31 were treated with VEL; JFH that have L at position 31 and J6 that have M at position 31. In GT2a (JFH) cells, the pooled cells and individual clone selected variants at positions 24, 92, and 93 (Table 27) and C92R (99.7%) and Y93D (99.7%) were the dominant variants observed in the clone. In addition, F28S (50.2%) was observed in the pooled cells only. Linkage analysis by deep sequencing was evaluated for double mutants at

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

the observed positions and the following double mutants were observed: T24A + Y93D (28.2%), C92R + Y93D (21.4%), F28S + Y93D (4.4%), T24A + C92R (3.1%), and F28S + C92R (1.9%). Phenotypic analyses showed that both the pooled cells and the clone had reduced susceptibility to VEL (1,000- and 1,578.2-fold, respectively). In the replicon assay, T24A did not show reduced susceptibility to VEL, Y93D did not replicate, and C92R showed low levels of resistance to VEL (Table 28).

Table 28. Susceptibility of VEL against Site-Directed Mutants in Replicon Assay
(Report PC-281-2027, page 15)

| Genotype | SDM | Replication Capacity | VEL EC ₅₀ FC from WT |
|--------------|------|----------------------|---------------------------------|
| 2a (JFH) | T24A | 68.3 ± 62.3 | 0.5 ± 0.4 |
| | F28L | 153.3 ± 59.2 | 0.1 ± 0.04 |
| | F28S | 30.2 ± 2.5 | 91.1 ± 4.9 |
| | L31M | 130.1 ± 42.8 | 1.5 ± 0.7 |
| | C92R | 19.0 ± 9.7 | 4.4 ^a |
| | Y93D | 0.1 ± 0.02 | Replication too low |
| | Y93H | 53.0 ± 12.6 | 45.7 ± 8.7 |
| 3a (S52) | M28T | 1.9 ^a | Replication too low |
| | L31F | 39.6 ± 8.9 | 30.8 ± 0.5 |
| | L31I | 12.3 ± 13.7 | Replication too low |
| | L31V | 204.7 ^a | 0.3 ^a |
| | E92K | 1.5 ± 0.1 | 0.7 ± 0.1 |
| | Y93H | 30.5 ± 2.7 | 723.5 ± 129.7 |
| | Y93S | 3.8 ± 0.02 | 750.6 ± 347.5 |
| 4a (E43D) | M31I | 28.6 ^a | 1.7 ^a |
| | P58L | 54.6 ^a | 1.2 ^a |
| | Y93H | 55.1 ± 26.7 | 2.9 ± 0.01 |
| 5a (SA13) | L31P | 1.4 ± 0.8 | Replication too low |
| 6a (GSI6a-1) | L31V | 114.6 ± 59.0 | > 100 ^a |
| | P32A | 40.8 ± 19.7 | 105.1 ± 110.8 |
| | P32L | 73.1 ± 47.8 | > 100 ^a |
| | P32Q | 56.2 ± 45.9 | 284.7 ± 38.9 |
| | P32R | 79.3 ± 49.3 | 148.0 ± 54.4 |

^a Data from one experiment

Similarly, stable GT2a (J6) cells selected variants at positions 24 and 93 (Table 27). As expected, L31M was observed in the control DMSO, pooled cells and clone 1 at >99% of the viral population. F28S was selected in both the pooled cells (89.9%) and 3 clones in frequencies of >99% of the viral population. The double mutant T24A + F28S was observed at a frequency of 3.1% of the viral population. The single mutant F28S showed 91-fold reduced susceptibility to VEL in the GT2a replicon assay (Table 28).

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

Phenotypic analysis of the pooled GT2a (J6) cells and one clone showed high levels of resistance to VEL (>500-fold).

Two GT3a VEL-resistant colonies were isolated; one clone had E92K (84.0%) and Y93S (15.6%), and the other clone had Y93H (99.6%). The pooled cells were observed to have 6 variants in the viral population: M28T (at 2.3%), L31F (at 31%), L31I (at 2.1%), L31V (at 16.9%), E92K (at 1.2%) and Y93H (at 44.7%). The double mutant L31F + Y93H was observed at a frequency of 2.9% of the viral population. The pooled cells and the clones showed >1,000-fold reduced susceptibility to VEL. Results from the GT3a replicon phenotypic assay showed that the single mutants E92K and L31V did not confer reduced susceptibility to VEL, while M28T and L31I did not replicate, or did not have enough replication to calculate an EC₅₀ value. However, Y93H and Y93S were associated with high levels of reduced susceptibility to VEL (723.5- and 750.6-fold, respectively, Table 28).

In GT4a, the pooled cells selected variants P58L and Y93H at low levels of the viral population (21.8% and 10.7%, respectively). The double mutant P58L + Y93H was observed in the pooled cells at a frequency of 7.5% of the viral population. M31I was selected in one clone at 99.6% of the viral population. Phenotypic analysis showed low levels of resistance (<2.0-fold) for the clone with M31I and the pooled cells. All single mutants conferred low levels of resistance to VEL in the transient GT4 replicon phenotypic assay (1.7-, 1.2- and 2.9-fold, for M31I, P58L and Y93H, respectively) (Table 28).

In GT5a cells, L31P was selected in both the pooled cells at 5.7% of the viral population and 2 clones at 33% and 9%. No reduced antiviral activity of VEL was observed for the pool or the clones. The L31P existed in very low levels of the viral population which may explain the lack of a change in susceptibility of the cells to VEL. The replication of L31P in GT5a replicon was too low to calculate an EC₅₀ value (Table 28).

In GT6a cells, the pooled cells contained a mixture of P32A/Q/L/R and L31V. No double mutants in frequencies of >1.5% of the viral population were observed. L31V was identified in one clone at 99% and P32L was identified in another clone at 99.7%. Phenotypic analysis showed both clones were resistant to VEL. The transient GT6a replicon assay also showed that the single mutants conferred >100-fold reduced susceptibility to VEL (Table 28).

In summary, Y93H appears to confer reduced susceptibility in all genotypes. The degree of resistance varies between different genotypes; low levels were observed in GT4a, mid-levels in GT2a and high levels in GT3a. Some variants confer a high level of resistance in one genotype, but did not impact the susceptibility in other genotypes (for example, L31V did not confer resistance in genotype 3a, but had high levels of resistance in genotype 6a). In the GT2a replicon, F28S that was selected in both J6 and JFH strains, showed 91-fold resistance to VEL in the JFH replicon. Y93H demonstrated 45.7-fold resistance to VEL, C92R showed low levels of resistance (4.4-fold), and T24A, F28L and L31M as single mutants did not confer reduced susceptibility to VEL. In GT3a, Y93H and Y93S showed >720-fold resistance, L31F had 31-fold reduced susceptibility, and L31V and E92K showed no reduction in susceptibility. In GT4a, all tested variants that were selected with VEL (M31I, P58L and Y93H) demonstrated <3-fold reduction in

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

susceptibility. In GT5, L31P did not replicate in the replicon assay. In GT6a, high levels of resistance were observed with L31V and P32A/L/Q/R.

CROSS-RESISTANCE

The activity of VEL was determined in transient replicon transfection assays against a panel of clinically significant GT1a and 1b NS5A inhibitor resistant mutants (M28T, Q30H, Q30R, L31M, Y93C and Y93H for GT1a and Y93H for GT1b). VEL had ≤ 2 -fold shift in EC₅₀ values against GT1a Q30H and Q30R and GT1b Y93H (Table 29) compared to >100-fold shifts in EC₅₀ values for ledipasvir (GS-5885) and daclatasvir (BMS-790052). GT1a mutants M28T, L31M and Y93C showed 6- to 12-fold decreased susceptibility to VEL, compared to 35- to 618-fold and 112- to 273-fold for ledipasvir and daclatasvir, respectively. VEL had less activity against mutant Y93H in GT1a with an EC₅₀ value of 6.7 nM; Y93H also had EC₅₀ values >100 nM for both ledipasvir and daclatasvir. The Merck NS3 protease inhibitor grazoprevir (MK-5172) and the nucleoside NS5B inhibitor 2'-C-Me-A did not exhibit any change in susceptibility against any of the NS5A substitutions.

Table 29. Antiviral Activity of VEL against Clinically Significant Genotype 1a and 1b NS5A Inhibitor Resistance Mutants^a (Report PC-281-2015, page 12)

| Compound | GT1a wt EC ₅₀ (nM) | Fold Shift | | | | | | GT1b wt EC ₅₀ (nM) ^b | Fold Shift GT1b Y93H ^b |
|---------------------------|-------------------------------------|--------------|--------------|--------------|--------------|--------------|---------------------------|--|---|
| | | GT1a M28T | GT1a Q30R | GT1a Q30H | GT1a L31M | GT1a Y93C | GT1a Y93H ^b | | |
| | | GS-5816 | 0.011 | 5.9 | 1.7 | 2.2 | 12 | | |
| GS-5885 | 0.034 | 35 | 141 | 321 | 274 | 618 | 3209 | 0.004 | 925 |
| BMS-790052 | 0.079 | 200 | 179 | 106 | 112 | 273 | 1329 | 0.015 | 27 |
| MK-5172 ^{c, d} | 3.1 | 1.0 | 1.2 | 1.0 | 1.0 | 1.0 | - | - | - |
| 2'-C-Me-A ^{c, d} | 625 | - | - | - | - | - | 0.8 | 349 | 1.4 |

^a Values represent the geometric mean of at least three independent experiments in 384-well assays

^b Assays were performed in stably replicating replicon cells; the others were in transient-transfection replicon cells.

^c MK-5172 is a Merck HCV protease inhibitor, and 2'-C-Me-A is a Merck HCV NS5B polymerase inhibitor

^d "-" indicates "not tested"

Additionally, the activity of VEL, as well as ledipasvir and daclatasvir, was determined in a transient transfection HCV replicon assay against a panel of NS5A replicon variants that consisted of variants that were commonly observed in treatment-naïve subjects, variants that emerged in subjects with genotype 1-6 infection who failed NS5A inhibitor treatment, and substitutions that were observed to confer resistance to NS5A inhibitors in GT1a and GT1b replicons that were also tested in genotype 2a, 2b, 3a, 4a, 5a, and 6a replicons. Variants were engineered by site-directed mutagenesis and their susceptibility to VEL, ledipasvir, and daclatasvir was tested. A total of 376 replicons were cloned, including 288 single mutants and 88 double mutants. Of the 376 mutants, 328 mutants replicated (87%), while 40 single and 8 double mutants showed low replication signal or had no signal at all. The 328 single and double mutants were grouped into 3 categories based on their level of resistance to each NS5A inhibitor and categorized as non-resistant (≤ 2.5 -fold EC₅₀ value change), low to moderate-level resistance (2.5- to

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

100-fold EC₅₀ value change), or high resistance (≥100-fold EC₅₀ value change) compared to the wild-type replicons.

In GT1a, 26 single mutants showed no resistance to VEL, ledipasvir, or daclatasvir. These variants included K24Q/S/T, A25T, M28I/L/V, Q30C/V, S38T, H58C/P/Q/R/Y, E62D/G/R, R81K/W, I90V, A92V, P97S, L138I, H340Y, and S366L (Table 30). L31F/I showed mid-level resistance to all 3 compounds, while M28G, A92K, and Y93H/N demonstrated high levels of resistance to all 3 compounds. VEL displayed high resistance to 6 single mutants (M28G, A92K, Y93H/N/R/W) (Table 30).

In GT1b, less than 2.5-fold resistance to VEL was observed for 24 single mutants (Table 30). Substitutions L28M/V, R30K/L/Q/T, P58Q/R/S, A92E/P/T/V, and Y93F/L showed no resistance to VEL, ledipasvir, or daclatasvir. For all 3 drugs, mid-levels of resistance were observed for substitutions L31F/I and Y93T; however, while ledipasvir and daclatasvir showed mid or high levels of resistance for L31M/V, P58D, and Y93C/S, VEL displayed less than 2.5-fold reduced susceptibility for the same substitutions. All 3 drugs demonstrated high levels of resistance to A92K.

Table 30. Levels of Resistance Conferred by Genotype 1a and Genotype 1b NS5A Single Mutants (PC-281-2030, page 16)

| Genotype | Levels of resistance | VEL | LDV | DCV |
|----------|----------------------|---|---|---|
| GT 1a | <2.5 | K24A, K24E, K24G, K24N, K24Q, K24R, K24S, K24T, K26E, A25T, M28I, M28L, M28V, Q30C, Q30H, Q30I, Q30L, Q30R, Q30S, Q30T, Q30V, Q30Y, S38F, S38T, H54Y, H58C, H58L, H58N, H58P, H58Q, H58R, H58Y, E62D, E62G, E62R, R81K, R81W, I90V, A92P, A92T, A92V, Y93F, P97S, L138I, H340Y, S366L | K24Q, K24S, K24T, A25T, M28I, M28L, M28V, Q30C, Q30V, S38T, H54Y, H58C, H58P, H58Q, H58R, H58Y, E62D, E62G, E62R, R81K, R81W, I90V, A92V, P97S, L138I, H340Y, S366L, A92P | K24A, K24E, K24N, K24Q, K24R, K24S, K24T, A25T, K26E, M28I, M28L, M28V, Q30C, Q30I, Q30L, Q30V, S38T, H58C, H58L, H58N, H58P, H58Q, H58R, H58Y, E62D, E62G, E62R, R81K, R81W, I90V, A92T, A92V, P97S, L138I, H340Y, S366L |
| | 2.5 - 100 | M28A, M28T, Q30E, Q30G, Q30K, L31F, L31I, L31M, L31V, P32L, H58D, Y93C, Y93L, Y93S, Y93T | K24A, K24E, K24G, K24N, K24R, K26E, M28T, Q30I, Q30L, Q30S, Q30T, L31F, S38F, H58L, H58N, A92T, Y93F | K24G, Q30S, Q30T, L31F, L31I, S38F, A92P, Y93F |
| | >100 | M28G, A92K, Y93H, Y93N, Y93R, Y93W | M28A, M28G, Q30E, Q30G, Q30H, Q30K, Q30R, Q30Y, L31I, L31M, L31V, P32L, H58D, A92K, Y93C, Y93H, Y93L, Y93N, Y93R, Y93S, Y93T, Y93W | M28A, M28G, M28T, Q30E, Q30G, Q30H, Q30K, Q30R, Q30Y, L31M, L31V, P32L, H58D, Y93C, Y93S, Y93T, A92K, Y93H, Y93N |
| GT 1b | <2.5 | L28M, L28V, R30H, R30K, R30L, R30Q, R30S, R30T, L31M, L31V, P32L, Q54H, P58D, P58Q, P58R, P58S, A92E, A92P, A92T, A92V, Y93C, Y93F, Y93L, Y93S | Q24K, L28M, L28V, R30H, R30K, R30L, R30Q, R30S, R30T, Q54H, P58Q, P58R, P58S, P58T, A92E, A92P, A92T, A92V, Y93F, Y93L | Q24K, L28M, L28V, R30K, R30L, R30Q, R30T, P32L, P58Q, P58R, P58S, P58T, A92E, A92P, A92T, A92V, Y93F, Y93L |
| | 2.5 - 100 | Q24K, L31F, L31I, P58T, Y93H, Y93N, Y93T | L31F, L31I, L31M, L31V, P32L, Y93T, Y93C | R30H, R30S, L31M, L31V, L31F, L31I, P58D, Y93H, Y93N, Y93S, Y93T |
| | >100 | A92K | P58D, Y93H, Y93N, Y93S, A92K | A92K |

Of the 50 double GT1a mutants tested, 13 (26%), 15 (30%), and 22 (44%) had low-, mid-, and high-levels of resistance to VEL (Table 31). The same 22 mutants with high levels of resistance to VEL demonstrated high levels of resistance to ledipasvir.

For GT1b double mutants, of the 13 double mutants tested, low-, mid-, and high-levels of resistance were observed for 4, 6, and 3 double mutants for VEL. The 3 double mutants with high levels of resistance to VEL included L31M + Y93N, L31V + Y93H, and L31V + Y93N; all other double mutants displayed <100-fold change in EC₅₀ values to VEL.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

Table 31. Levels of Resistance Conferred by Genotype 1a and Genotype 1b NS5A Double Mutants (PC-281-2030, page 17)

| Genotype | Levels of resistance | VEL | LDV |
|----------|----------------------|--|--|
| GT 1a | <2.5 | K24Q + H58D, K24Q + Q30R, K24R + H58P, K24R + M28V, A25T + Q30R, A25T + H58P, M28A + H58R, M28L + Q30R, M28V + Q30H, M28V + Q30R, M28V + H58R, M28V + R81W, Q30R + H58R | A25T + H58P, M28L + Q30R, M28V + H58R, M28V + R81W |
| | 2.5 - 100 | K24R + H58D, K24R + L31M, K24R + M28T, K24R + Q30H, K24R + Q30R, K24R + Y93C, K24R + Y93F, L31M + E62G, L31M + R81K, M28T + Q30R, Q30H + Y93C, Q30H + Y93F, Q30L + Y93H, Q30R + Y93F, H58P + Y93C | K24R + H58P, K24R + M28V, K24R + M28T, A25T + Q30R, M28A + H58R, M28V + Q30H, M28V + Q30R, Q30R + H58R |
| | >100 | M28A + Q30L, M28A + Q30R, M28G + H58R, M28T + Q30H, M28T + H58D, M28T + Y93C, M28V + Y93N, Q30E + Y93C, Q30H + L31M, Q30H + H58D, Q30H + Y93H, Q30R + L31M, Q30R + H58D, Q30R + Y93C, Q30R + Y93H, Q30R + Y93L, Q30Y + Y93H, L31M + H58D, L31M + Y93C, L31V + H58D, L31V + Y93C, H58D + Y93N | K24Q + H58D, K24Q + Q30R, K24R + H58D, K24R + L31M, K24R + Q30H, K24R + Q30R, K24R + Y93C, K24R + Y93F, L31M + E62G, L31M + R81K, M28A + Q30L, M28A + Q30R, M28G + H58R, M28T + H58D, M28T + Q30H, M28T + Q30R, M28T + Y93C, M28V + Y93N, Q30E + Y93C, Q30H + H58D, Q30H + L31M, Q30H + Y93C, Q30H + Y93F, Q30H + Y93H, Q30L + Y93H, Q30R + H58D, Q30R + L31M, Q30R + Y93C, Q30R + Y93F, Q30R + Y93H, Q30R + Y93L, Q30Y + Y93H, L31M + H58D, L31M + Y93C, L31V + H58D, L31V + Y93C, H58D + Y93N, H58P + Y93C |
| GT 1b | <2.5 | Q24R + R30Q, R30Q + L31M, R30Q + L31V, R30Q + Y93H | Q24R + R30Q |
| | 2.5 - 100 | Q24R + Y93H, L28M + Y93H, L31I + Y93H, L31M + Y93C, L31M + Y93H, L31V + Y93C | Q24R + Y93H, R30Q + L31M, R30Q + L31V |
| | >100 | L31M + Y93N, L31V + Y93H, L31V + Y93N | L28M + Y93H, R30Q + Y93H, L31I + Y93H, L31M + Y93C, L31M + Y93H, L31M + Y93N, L31V + Y93C, L31V + Y93H, L31V + Y93N |

The majority of the single mutants in genotypes 2a, 2b, 3a, 4a, and 5a displayed low levels of resistance to VEL. None of the genotype 2a, 4a, or 5a single mutants displayed high levels of resistance to VEL (Table 32). In GT2a, 5 single mutants (F28S, L31V, C92R, Y93H/N) and 1 double mutant (L31M + P58S) displayed 2.5- to 100-fold reductions in susceptibility to VEL. All other single mutants, including all substitutions tested at positions 24, 58, 30, F28C/L/V, L31M, and Y93C/F/L/S/T, demonstrated less than 2.5-fold EC₅₀ value change to VEL (Table 32). In GT2b, 3 single mutants (C92T, Y93H/N) and 1 double mutant (M31V+Y93H) displayed >100-fold level of resistance to VEL. All substitutions tested at positions 24 (S24A/T/Y), 30 (K30H/M/N/R/S), and 31 (M31I/L/V) showed no reduction in susceptibility to VEL (Table 32). In GT3a, A30H/K, L31F/M, and P58G displayed mid-level resistance to VEL; while Y93H displayed >100-fold shift in EC₅₀ value. No decreased susceptibility was observed for S24A/K/T, M28V/L, A30Q/R/S/V, L31V, P58A/H/S, and Y93F. Most double mutants showed >100-fold resistance and included A30K/S/T + Y93H/N or A30K + L31I/M (Table 32). In GT4a, L28T and Y93H/N/S demonstrated mid-levels (2.5 -100) of resistance to VEL. None of the single GT4a mutants tested showed high levels of resistance to VEL. No resistance was observed for K24G/R, L28M/V, L30H/R/S/T/V, M31I/L, P58L/S/T, or Y93C (Table 32). When L30H was combined with other mutants, it displayed mid- to high-levels of resistance including L30H + Y93H and L30H + Y93S with >200-fold reduced susceptibility to VEL. In GT5a, except for L31I with low-level resistance (4.4-fold), all single mutants, including Q30H/K/L/R/S/T, L31F/M/V, P32L, P58S, and T93C/F/H/L/N/S, demonstrated <2.5-fold change in EC₅₀ value to VEL (Table 32). In GT6a, L31V and P32A/L/Q/R showed >100-fold reduced susceptibility to VEL; low- to mid-levels of resistance were observed for F28M/V, L28M, L31I/M, T58H/G, A92T, T93A/H/N/S, and no resistance was observed for all substitutions at position 24, and 30 as well as L28F, T58A/P/S, or T93C/F/L/Y (Table 32).

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

Table 32. Levels of Resistance to Velpatasvir Conferred by Genotypes 2-6 NS5A Single and Double Mutants (PC-281-2030, page 18)

| Levels of resistance | RAVs | GT 2a | GT 2b | GT 3a | GT 4a | GT 5a | GT 6a |
|----------------------|--------|--|--|--|--|--|--|
| <2.5 | Single | T24A, T24P, T24S, F28C, F28L, F28V, K30A, K30H, K30Q, K30R, K30S, K30T, L31M, P58A, P58T, C92A, C92K, C92N, C92S, C92T, Y93C, Y93F, Y93L, Y93S, Y93T | S24A, S24T, S24Y, K30H, K30M, K30N, K30R, K30S, M31I, M31L, M31V, S38F, P58S, P58T, C92A | S24A, S24K, S24T, M28L, M28V, A30Q, A30R, A30S, A30V, L31V, S38Y, P58A, P58H, P58S, E92K, Y93F | K24G, K24R, L28M, L28V, L30H, L30R, L30S, L30T, L30V, M31I, M31L, P58L, P58S, P58T, D62E, Y93C | Q30H, Q30K, Q30L, Q30R, Q30S, Q30T, L31F, L31M, L31V, P32L, P58S, T93C, T93F, T93H, T93L, T93N, T93S | Q24K, Q24R, Q24T, F28A, L28F, R30A, R30H, R30K, R30N, R30Q, R30S, R30T, T58A, T58P, T58S, A92P, T93C, T93F, T93L, T93Y |
| | | F28S, L31V, C92R, Y93H, Y93N | L28F, P58A, C92S, Y93F | A30H, A30K, L31F, L31M, P58G | L28T, Y93H, Y93N, Y93S | L31I | F28M, F28V, L28M, L31I, L31M, T58G, T58H, A92T, T93A, T93H, T93N, T93S |
| | | None | C92T, Y93H, Y93N | Y93H | None | None | L31V, P32A, P32L, P32Q, P32R |
| >100 | None | C92T, Y93H, Y93N | Y93H | None | None | L31V, P32A, P32L, P32Q, P32R | |
| <2.5 | Double | NA | M31I + Y93H | M28T + A30V | L30H + M31V, L30H + P32L, M31V + P32L | ND | Q24R + R30S |
| 2.5 - 100 | | L31M + P58S | NA | A30V + Y93H | L30H + Y93H, L30H + Y93S | ND | NA |
| >100 | | NA | M31V + Y93H | A30K + L31I, A30K + L31M, A30K + Y93H, A30K + Y93N, A30S + Y93H, A30T + Y93H, L31P + Y93H | None | ND | NA |

ND = not done; NA = not applicable

Colony reduction assays were performed in stably replicating HCV replicon cells. Due to the high error rate of HCV RNA polymerase, stable HCV replicon cell lines are genetically heterogeneous systems that mimic the viral quasispecies in HCV patients and include pre-existing variants. Cells stably replicating genotype 1a, 1b, 3a or 4a subgenomic replicons (genotype 3a and 4a were novel subgenomic replicons established for this study) or genotype 2a (J6) and 2b NS5A chimeric replicons were plated and treated with VEL (GS-5816) at a series of concentrations in the presence of neomycin (G418). Cells harboring replicons with reduced susceptibility to the test compound at the indicated drug concentrations survive G418-mediated cell killing and grow into colonies by the end of treatment period; in contrast, cells harboring replicons susceptible at the applied inhibitor concentration are killed and disappear. Therefore, fewer colonies indicates a higher resistance barrier at any given drug concentration. daclatasvir (BMS-790052) was evaluated in parallel using the same replicon cells.

At a drug concentration as low as 0.1 nM, VEL suppressed nearly all resistant variants in genotypes 1b, 3a and 4a (Figure 1). To achieve the same results, daclatasvir required 25- to 125-fold higher drug concentrations. For GT1a, a number of colonies were selected by 0.1 nM VEL, but 0.5 nM VEL effectively suppressed the emergence of resistance mutants with ~ 10 colonies selected, whereas daclatasvir achieved the same effect at 62.5 nM.

There were more resistant GT2a (J6) and GT2b colonies (67 – 99 at 0.5 nM and 2.5 nM) selected by VEL, indicating the resistance barrier of VEL is lower in these two GT2 subtypes compared to the others tested genotypes. While in GT2b cells, 183 colonies were selected at 62.5 nM concentration of daclatasvir, the number of GT2a cells

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

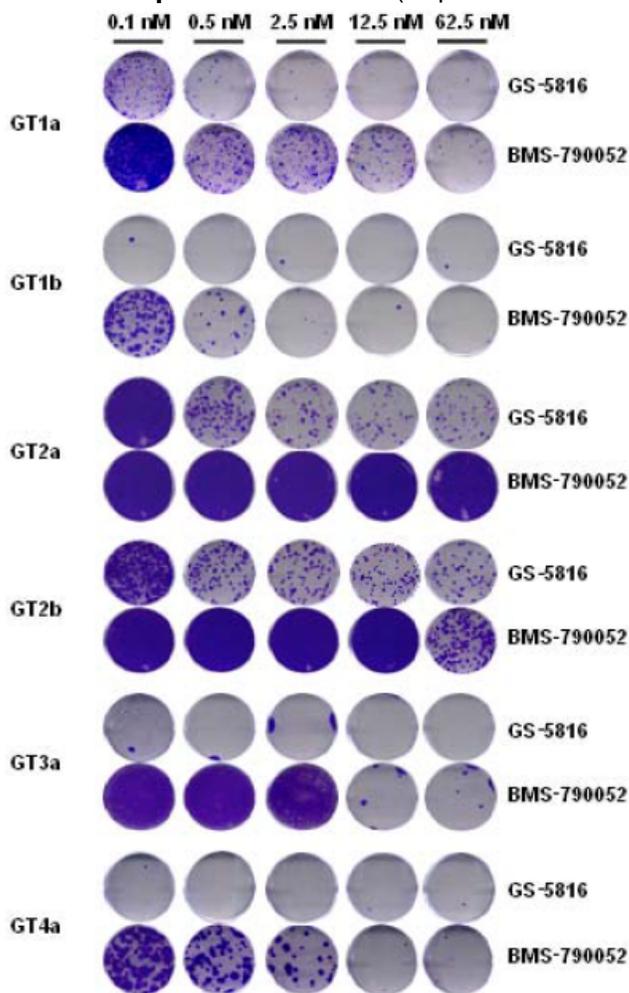
NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

surviving was higher than could be counted at all lower concentrations of this compound. Taken together, the data suggest that VEL has higher resistance barrier for GT1-4 compared to daclatasvir.

Figure 1. Colony Reduction Assays of VEL and Daclatasvir in Stable Genotype 1a, 1b, 3a and 4a Subgenomic Replicon Cell Lines or Genotype 2a and 2b NS5A Chimeric Replicon Cell Lines (Report PC-281-2015, page 14)



4.6 CLINICAL STUDIES

Study GS-US-342-1138 (ASTRAL-1) is a Phase 3, randomized, double-blind, placebo-controlled, multicenter, international study to assess the antiviral efficacy, safety, and tolerability of 12 weeks of SOF/VEL treatment compared with 12 weeks of placebo treatment in subjects with chronic genotype 1, 2, 4, 5, or 6 HCV infection. A total of 740 subjects were enrolled and received study drug in a double-blind manner in the following 2 treatment groups:

- SOF/VEL 12 Week group: SOF/VEL FDC (400/100 mg) once daily for 12 weeks (N = 624)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

- Placebo 12 Week group: SOF/VEL placebo once daily for 12 weeks (N = 116)
No subject in the SOF/VEL 12 Week group had on-treatment virologic failure. In the SOF/VEL 12 Week group, 6 of 624 subjects (1%) did not achieve SVR12. Two subjects had virologic failure due to relapse at the post-treatment Week 4 visit (1 GT1a treatment-naive without cirrhosis; 1 GTb by LiPA/TRUGENE (GT1c/1h by sequencing-based BLAST) treatment-experienced with cirrhosis) (Table 33). Four additional subjects (categorized as “other”) did not achieve SVR12. No GT2, GT4, GT5, or GT6 subjects experienced virologic failure (Table 34).

Table 33. GS-US-342-1138: Virologic Outcomes (SOF/VEL 12 Week Group; All Genotypes, Genotype 1 Subgroups [1a and 1b], and Genotype 1 Total) (by LiPA/TRUGENE)

| n/N (%) | SOF/VEL 12 Weeks | | | |
|--------------------------------|---------------------------------------|-------------------|-------------------|---------------------------|
| | Total (All Genotypes) (N = 624) | GT1a (N = 210) | GT1b (N = 118) | GT1 Total (N = 328) |
| SVR12 | 618/624 (99.0%) | 206/210 (98.1%) | 117/118 (99.2%) | 323/328 (98.5%) |
| Overall Virologic Failure | 2/624 (0.3%) | 1/210 (0.5%) | 1/118 (0.8%) | 2/328 (0.6%) |
| Relapse | 2/623 (0.3%) | 1/209 (0.5%) | 1/118 (0.8%) | 2/327 (0.6%) |
| On-Treatment Virologic Failure | 0/624 | 0/210 | 0/118 | 0/328 |
| Other | 4/624 (0.6%) | 3/210 (1.4%) | 0/118 | 3/328 (0.9%) |

Other = subject who did not achieve SVR12 and did not meet virologic failure criteria.)

Source: CSR GS-US-342-1138

Table 34. GS-US-342-1138: Virologic Outcomes (SOF/VEL 12 Week Group; Genotypes 2, 4, 5, and 6) (by LiPA/TRUGENE; Full Analysis Set)

| n/N (%) | SOF/VEL 12 Weeks | | | |
|--------------------------------|------------------|------------------|-----------------|-----------------|
| | GT2 (N = 104) | GT4 (N = 116) | GT5 (N = 35) | GT6 (N = 41) |
| SVR12 | 104/104 (100.0%) | 116/116 (100.0%) | 34/35 (97.1%) | 41/41 (100.0%) |
| Overall Virologic Failure | 0/104 | 0/116 | 0/35 | 0/41 |
| Relapse | 0/104 | 0/116 | 0/35 | 0/41 |
| On-Treatment Virologic Failure | 0/104 | 0/116 | 0/35 | 0/41 |
| Other | 0/104 | 0/116 | 1/35 (2.9%) | 0/41 |

Other = subject who did not achieve SVR12 and did not meet virologic failure criteria.

Source: CSR GS-US-342-1138

Study GS-US-342-1139 (ASTRAL-2) is a Phase 3, randomized, open-label multicenter study to assess the antiviral efficacy, safety, and tolerability of 12 weeks of SOF/VEL treatment compared with 12 weeks of SOF+RBV treatment in subjects with chronic genotype 2 HCV infection. A total of 266 subjects were randomized in a 1:1 ratio into 1 of the following 2 treatment groups:

- SOF/VEL 12 Week group (Group 1): SOF/VEL FDC (400/100 mg) once daily for 12 weeks (N = 134)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

- SOF+RBV 12 Week group (Group 2): SOF (400 mg) once daily + RBV (1000 or 1200 mg/day divided twice daily) for 12 weeks (N = 132)

No subject in either treatment group had on-treatment virologic failure and no subject had virologic failure due to relapse in the SOF/VEL 12 Week group. In the SOF+RBV 12 Week group, 8 of 132 subjects (6%) did not achieve SVR12. Six subjects (4.5%) had virologic failure due to relapse.

Study GS-US-342-1140 (ASTRAL-3) is a Phase 3, randomized, open-label multicenter study to assess the antiviral efficacy, safety, and tolerability of 12 weeks of SOF/VEL treatment compared with 24 weeks of SOF+RBV treatment in subjects with chronic GT3 HCV infection. A total of 552 subjects were randomized in a 1:1 ratio study into 1 of the following 2 treatment groups:

- SOF/VEL 12 Week group (Group 1): SOF/VEL fixed-dose combination (FDC; 400/100 mg) Tablet once daily for 12 weeks (N = 277)
- SOF+RBV 24 Week group (Group 2): SOF (400 mg) Tablet once daily + RBV (1000 or 1200 mg/day divided twice daily) Tablets for 24 weeks (N = 275)

In the SOF/VEL 12 Week group, 13 of 277 subjects (4.7%) did not achieve SVR12. Of these, no subject had on-treatment virologic failure, 11 subjects had virologic failure due to relapse, and 2 additional subjects (categorized as “other”) did not achieve SVR12 (Table 35). In the SOF+RBV 24 Week group, 54 of 275 subjects (19.6%) did not achieve SVR12. Of these, 1 subject (2%) had on-treatment virologic failure (nonresponsive), 38 subjects (70%) had virologic failure due to relapse, and 15 additional subjects (28%) (categorized as “other”) did not achieve SVR12.

Table 35. GS-US-342-1140: Virologic Outcomes (by LiPA/TRUGENE)

| n/N (%) | SOF/VEL 12 Weeks (N = 277) | SOF+RBV 24 Weeks (N = 275) |
|--------------------------------|----------------------------------|----------------------------------|
| SVR12 | 264/277 (95.3%) | 221/275 (80.4%) |
| Overall Virologic Failure | 11/277 (4.0%) | 39/275 (14.2%) |
| Relapse | 11/276 (4.0%) | 38/272 (14.0%) |
| On-Treatment Virologic Failure | 0/277 | 1/275 (0.4%) |
| Other | 2/277 (0.7%) | 15/275 (5.5%) |

Other = subject who did not achieve SVR12 and did not meet virologic failure criteria,
Source: CSR GS-US-342-1140

Study GS-US-342-1137 (ASTRAL-4) is an ongoing Phase 3, randomized, open-label multicenter study to assess the antiviral efficacy, safety, and tolerability of SOF/VEL±RBV for 12 weeks and SOF/VEL for 24 weeks in subjects with chronic HCV infection and Child-Pugh-Turcotte class B cirrhosis.

A total of 267 subjects were randomized (1:1:1) to 1 of the following 3 treatment groups:

- SOF/VEL 12 Week group (Group 1): SOF/VEL FDC (400/100 mg) once daily for 12 weeks (N = 90)
- SOF/VEL+RBV 12 Week group (Group 2): SOF/VEL FDC once daily + RBV (1000 or 1200 mg/day divided twice daily) Tablets for 12 weeks (N = 87)
- SOF/VEL 24 Week group (Group 3): SOF/VEL FDC once daily for 24 weeks (N = 90)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

The overall virologic failure rates by treatment group in this population with decompensated cirrhosis were as follows:

- SOF/VEL 12 Week group: 12% of subjects (11 of 90) did not achieve SVR12
- SOF/VEL+RBV 12 Week group: 3.4% of subjects (3 of 87) did not achieve SVR12
- SOF/VEL 24 Week group: 8.9% of subjects (8 of 90) did not achieve SVR12

11 additional subjects (categorized as “other”) did not achieve SVR12. Table 36 shows the proportion of subjects who achieved SVR12 by the 3 treatment groups and Genotype/subtype.

Table 36. GS-US-342-1137: Virologic Outcomes by Genotype/Subtype and Treatment Groups

| n/N (%) | Total (All Genotypes) | GT-1a | GT-1b | GT-1 Total | GT-2 | GT-3 | GT-4 | GT-6 |
|-------------------------------------|-----------------------|---------------|----------------|---------------|--------------|---------------|--------------|--------------|
| SOF/VEL 12 Week Group, n | 90 | 50 | 18 | 68 | 4 | 14 | 4 | 0 |
| SVR12 | 75/90 (83.3%) | 44/50 (88.0%) | 16/18 (88.9%) | 60/68 (88.2%) | 4/4 (100.0%) | 7/14 (50.0%) | 4/4 (100.0%) | 0 |
| Overall Virologic Failure | 11/90 (12.2%) | 3/50 (6.0%) | 2/18 (11.1%) | 5/68 (7.4%) | 0/4 | 6/14 (42.9%) | 0/4 | 0 |
| Relapse | 11/90 (12.2%) | 3/50 (6.0%) | 2/18 (11.1%) | 5/68 (7.4%) | 0/4 | 6/14 (42.9%) | 0/4 | 0 |
| On-Treatment Virologic Failure | 0/90 | 0/50 | 0/18 | 0/68 | 0/4 | 0/14 | 0/4 | 0 |
| Other | 4/90 (4.4%) | 3/50 (6.0%) | 0/18 | 3/68 (4.4%) | 0/4 | 1/14 (7.1%) | 0/4 | 0 |
| SOF/VEL+RBV 12 Week Group, n | 87 | 54 | 14 | 68 | 4 | 13 | 2 | 0 |
| SVR12 | 82/87 (94.3%) | 51/54 (94.4%) | 14/14 (100.0%) | 65/68 (95.6%) | 4/4 (100.0%) | 11/13 (84.6%) | 2/2 (100.0%) | 0 |
| Overall Virologic Failure | 3/87 (3.4%) | 1/54 (1.9%) | 0/14 | 1/68 (1.5%) | 0/4 | 2/13 (15.4%) | 0/2 | 0 |
| Relapse | 2/85 (2.4%) | 1/53 (1.9%) | 0/14 | 1/67 (1.5%) | 0/4 | 1/12 (8.3%) | 0/2 | 0 |
| On-Treatment Virologic Failure | 1/87 (1.1%) | 0/54 | 0/14 | 0/68 | 0/4 | 1/13 (7.7%) | 0/2 | 0 |
| Other | 2/87 (2.3%) | 2/54 (3.7%) | 0/14 | 2/68 (2.9%) | 0/4 | 0/13 | 0/2 | 0 |
| SOF/VEL 24 Week Group, n | 90 | 55 | 16 | 71 | 4 | 12 | 2 | 1 |
| SVR12 | 77/90 (85.6%) | 51/55 (92.7%) | 14/16 (87.5%) | 65/71 (91.5%) | 3/4 (75.0%) | 6/12 (50.0%) | 2/2 (100.0%) | 1/1 (100.0%) |
| Overall Virologic Failure | 8/90 (8.9%) | 2/55 (3.6%) | 1/16 (6.3%) | 3/71 (4.2%) | 0/4 | 5/12 (41.7%) | 0/2 | 0/1 |
| Relapse | 7/88 (8.0%) | 2/55 (3.6%) | 1/16 (6.3%) | 3/71 (4.2%) | 0/4 | 4/10 (40.0%) | 0/2 | 0/1 |
| On-Treatment Virologic Failure | 1/90 (1.1%) | 0/55 | 0/16 | 0/71 | 0/4 | 1/12 (8.3%) | 0/2 | 0/1 |
| Other | 5/90 (5.6%) | 2/55 (3.6%) | 1/16 (6.3%) | 3/71 (4.2%) | 1/4 (25.0%) | 1/12 (8.3%) | 0/2 | 0/1 |

GT = genotype

Other = subject who did not achieve SVR12 and did not meet virologic failure criteria.

Source: CSR GS-US-342-1137

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

4.7 CLINICAL VIROLOGY

PROPORTION OF SUBTYPES IN ASTRAL 1, 2 and 3 TRIALS

Screening of Genotype/Subtype was determined by LiPA/TRUGENE. Subsequently, basic local alignment search tool (BLAST) analysis of baseline amino acid sequences was used to provide sequence-based HCV genotype/subtype. The screening and analysis results of genotype/subtype are shown in Table 37 (FDA analysis). Sequencing-based BLAST analyses of NS5A and/or NS5B sequences provided a correction of HCV genotype/subtype for 8 subjects who were initially determined as another genotype at screening by LiPA or TRUGENE assay (shown bold italicized in Table 37). Two subjects were determined by LiPA/TRUGENE as GT1b but were determined by BLAST analysis to be genotype 6I. In addition, 2 subjects determined at screening as GT2 were found by BLAST analysis to be GT1b; 2 subjects determined as GT2b at screening were GT1a; 1 subject determined at screening as GT3a was GT1a; and 1 subject determined as GT4f was GT1e. All of these misclassified subjects achieved SVR12, so the inaccurate determination of genotype at screening did not affect the overall efficacy. One GT1 subject who relapsed was determined as GT1b at screening, but was refined to GT1c/1h by BLAST analysis.

For GT3, the predominant subtype at screening was GT3a (514/551; 93%). Almost all were confirmed as GT3a by subsequent sequencing analysis. One subject screened as GT3a was found to be GT1a by sequencing analysis. Other GT3 subtypes present in this study included 3b, 3g, 3h, 3i, and 3k (Table 37).

For GT4, most subjects were determined as GT4 or GT4a/4c/4d at screening. Confirmation by sequencing showed that most GT4 subjects had GT4a (n=74; 54%) followed by GT4d (n=31; 22%) (Table 37). Other GT6 subtypes as determined by sequencing included 4b, 4c, 4f, 4k, 4n, 4o, 4r, and 4t. Multiple subjects were determined to have mixtures of GT4 subtypes by sequencing. One subject screened as GT4f was later determined to be GT1e by nucleotide sequence analysis.

All subjects with GT5 infection had GT5a with the screening LiPA/TRUGENE assay, which was confirmed by nucleotide sequence analysis.

For GT6, most of the subjects were determined as GT6a/6b or GT6c-I at screening with the LiPA/TRUGENE assay and 56% (27/48) were confirmed GT6a by sequencing analysis (Table 37). The remaining GT6 subjects had a wide range of subtypes including 6e (n=10), 6h (n=2), 6k (n=1), 6l (n=3), 6m (n=1), 6n (n=2), and 6q (n=2).

Overall, SOF/VEL for 12 weeks produced 99.4% to 100% SVR12 rates across highly diverse subtypes in subjects with genotype 1, 2, 4, 5, 6, or 7 HCV infection.

Table 37. Proportion of GT/Subtypes in ASTRAL1, 2, 3 Studies

| Screening GT/Subtype | Number | Analysis GT/Subtype | Number |
|----------------------|--------|---------------------|--------|
| GT1a | 256 | | |
| | | 1a | 253 |

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

| | | | |
|-------------|-----|-----------|----------|
| | | 1a/1e | 1 |
| | | 1e | 1 |
| | | 1b | 1 |
| GT1b | 137 | 1b | 134 |
| | | 1c/1h | 1 |
| | | 6l | 2 |
| GT2 | 388 | | |
| 2 | 62 | 1b | 2 |
| | | 2 | 1 |
| | | 2a | 23 |
| | | 2b | 12 |
| | | 2a/2b | 1 |
| | | 2c | 11 |
| | | 2c/2d | 1 |
| | | 2d | 1 |
| | | 2e | 1 |
| | | 2e/2j | 1 |
| | | 2i | 3 |
| | | 2j | 1 |
| | | 2k | 6 |
| 2a | 9 | 2a | 7 |
| 2a/2c | 64 | 2a/2c | 63 |
| | | 2b | 1 |
| 2b | 253 | 2b | 249 |
| | | 1a | 2 |
| | | 2a/2b | 1 |
| | | 2b/2c | 1 |
| GT3 | 551 | | |
| 3 | 27 | 3a | 27 |
| 3a | 514 | 3a | 511 |
| | | 1a | 1 |
| | | 3g | 1 |
| | | 3i | 1 |
| 3b | 7 | 3b | 7 |
| 3h | 2 | 3h | 2 |
| 3k | 1 | 3k | 1 |
| | | | |
| GT4 | 138 | | |
| 4 | 62 | 4 | 1 |
| | | 4a | 35 |
| | | 4a/4d | 1 |
| | | 4a/4k | 1 |
| | | 4a/4l/4v | 1 |
| | | 4d | 15 |
| | | 4g/4k | 1 |
| | | 4k | 1 |
| | | 4n | 3 |

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

| | | | |
|-------------|----|-----------|----------|
| | | 4o | 1 |
| | | 4p/4t | 1 |
| | | 4r | 1 |
| 4a | 3 | 4a | 1 |
| | | 4d/4r | 1 |
| | | 4r | 1 |
| 4a/4c/4d | 58 | 4a | 33 |
| | | 4b | 2 |
| | | 4c | 2 |
| | | 4d | 16 |
| | | 4n | 4 |
| | | 4t | 1 |
| 4e | 5 | 4o | 5 |
| 4f | 3 | 4f | 2 |
| | | 1e | 1 |
| 4g | 1 | 4o | 1 |
| 4h | 2 | 4a | 2 |
| 4l | 3 | 4a | 3 |
| 4r | 1 | 4r | 1 |
| | | | |
| GT5a | 35 | 5a | 35 |
| GT6 | 48 | | |
| 6 | 1 | 6e | 1 |
| 6a | 2 | 6a | 2 |
| 6a/6b | 24 | 6a | 24 |
| 6c-l | 21 | 6a | 1 |
| | | 6e | 9 |
| | | 6h | 2 |
| | | 6k | 1 |
| | | 6l | 3 |
| | | 6m | 1 |
| | | 6n | 2 |
| | | 6q | 2 |

In ASTRAL 4, there were 159 subjects with GT1a and 48 subjects with GT1b at screening and confirmation BLAST sequence analysis (Table 38; FDA analysis). For GT2, 10 of 12 were GT2b and the other 2 were GT2a. All 39 GT3 subjects were GT3a. Half the subjects with GT4 HCV had GT4a by sequencing analysis with the other subjects having 4d, 4l, 4n, or 4r. There were no GT5 subjects and only one GT6 (6d) subject.

Table 38. Proportion of GT/Subtypes in ASTRAL 4

| Screening GT/Subtype | Number | Analysis GT/Subtype | Number |
|----------------------|--------|---------------------|--------|
| GT1 | | | |
| GT1a | 159 | GT1a | 159 |
| GT1b | 48 | GT1b | 48 |

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

| | | | |
|---------------|----|----|----|
| GT2 | | | |
| 2a/2c | 2 | 2a | 2 |
| 2b | 10 | 2b | 10 |
| GT3 | 39 | | |
| 3a | 37 | 3a | 39 |
| 3 | 2 | | |
| GT4 | 8 | | |
| 4 | 5 | 4a | 4 |
| 4e | 1 | 4d | 1 |
| 4a/c/d | 2 | 4l | 1 |
| | | 4n | 1 |
| | | 4r | 1 |
| GT5 | 0 | - | |
| GT6c-l | 1 | 6d | 1 |

PREVALENCE OF NS5A RAPS

The prevalence of baseline NS5A RAPS (any change at amino acid positions 24, 28, 30, 31, 58, 92 and 93) at a sensitivity threshold of 15% of the viral population was assessed in the ASTRAL trials. In the ASTRAL 1 and 2 studies, the prevalence of baseline NS5A RAPS was 18% (38/211) in subjects with GT1a HCV infection and 31% (42/134) in subjects with GT1b HCV infection and 60% (233/387) in GT2 HCV infection (Table 39; FDA analysis). The most prevalent NS5A RAPS in GT1a were at amino acid positions M28 (5%) and H58 (7%). The most prevalent NS5A RAPS in GT1b were at positions 30 (8%), 31 (7%), 58 (9%) and 93 (10%).

Table 39. Presence of Baseline NS5A RAPS in GT1 Subjects in ASTRAL 1 and ASTRAL 2 (Any change at amino acid positions 24, 28, 30, 31, 58, 92, and 93) (15% sensitivity threshold)

| | GT1a (n=211) | GT1b | GT1 (n=345) |
|---------------------|---------------------|--------------|--------------------|
| None | 82% (173/211) | 69% (92/134) | 77% (264/345) |
| Any | 18% (38/211) | 31% (42/134) | 23% (80/345) |
| 24K/R/T/Q | 6 (3%) | 1 (0.7%) | 7 (2%) |
| 28M/I/V | 11 (5%) | 2 (1.5%) | 13 (4%) |
| 30Q/R/L/H | 5 (2%) | 11 (8%) | 16 (5%) |
| L31M/I | 5 (2%) | 9 (7%) | 14 (4%) |
| H58P/R/D | 15 (7%) | 12 (9%) | 27 (8%) |
| A92T | 1 (0.5%) | 6 (4%) | 7 (2%) |
| Y93H/N/C/L/F | 4 (2%) | 14 (10%) | 18 (5%) |
| 32 | none | none | none |
| 38 | none | none | none |

The prevalence of baseline NS5A RAPS was 60% (233/387) in subjects with GT2 HCV infection (Table 40; FDA analysis). The most prevalent NS5A RAPS in GT2 were L31M (51%) and K24R/T/Q (17%). The prevalence of baseline NS5A RAPS in subjects with

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

GT4, GT5, and GT6 infection was 63% (73/115), 9% (3/35), and 83% (35/42), respectively. The predominant polymorphisms were at positions 28, 30 and 58 in GT4 and at positions 24, 28, 30 and 58 in GT6.

Table 40. Presence of Baseline NS5A RAPS in GT2, 4, 5, 6 Subjects in ASTRAL 1 and ASTRAL 2 (Any change at amino acid positions 24, 28, 30, 31, 58, 92, and 93) (15% sensitivity threshold)

| | GT2 (n=387) | GT4 (n=115) | GT5 (n=35) | GT6 (n=42) |
|----------------------|---------------|--------------|-------------|-------------|
| None | 40% (153/387) | 37% (42/115) | 91% (32/35) | 17% (7/42) |
| Any | 60% (233/387) | 63% (73/115) | 9% (3/35) | 83% (35/42) |
| 24K/R/T/Q | 66 (17%) | 3 (3%) | none | 23 (55%) |
| 28M/I/V/L/F | 29 (7%) | 22 (19%) | none | 34 (81%) |
| 30Q/R/L/H/S/A | 10 (3%) | 57 (50%) | 2 (6%) | 19 (45%) |
| 31M/I/L | 196 (51%) | 5 (4%) | none | 1 (2%) |
| 58P/R/D/T/S | 22 (6%) | 20 (17%) | 1 (3%) | 17 (40%) |
| A92T | 8 (2%) | none | none | none |
| 93H/N/C/L/F/S | 1 (0.3%) | 2 (2%) | none | 4 (10%) |
| 32 | none | none | none | none |
| 38 | none | none | none | none |

In ASTRAL-3, the prevalence of NS5A RAPS at baseline was 21% (115/551) with the most prevalent NS5A RAPS at positions A30 (11%) and Y93H (6%) (Table 41; FDA analysis).

Table 41. Presence of Baseline NS5A RAPS in GT3 Subjects in ASTRAL 3 (Any change at 24, 28, 30, 31, 58, 92, and 93) (15% sensitivity threshold)

| | GT3 (n=551) |
|-------------------|---------------|
| None | 79% (434/551) |
| Any | 21% (115/551) |
| 24A | 4 (1%) |
| 28L/V | 7 (1%) |
| A30K/V/T/S | 61 (11%) |
| 31M | 9 (2%) |
| 58P/R/D | 23 (4%) |
| 92T | 2 (0.4%) |
| Y93H | 32 (6%) |
| 32 | none |
| 38 | none |

In ASTRAL-4, the prevalence of NS5A RAPS at baseline was 24% (48/198), 60% (6/10), 11% (4/37), and 63% (5/8) in GT1, GT2, GT3, and GT4 HCV subjects, respectively (Table 42; FDA analysis). The prevalence of NS5A RAPS in GT1 subjects was balanced across the treatment arms. There were no subjects with GT5 HCV infection and only 1 subject with GT6 infection in the SOF/VEL 24 Week arm who had a baseline RAP.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

Table 42. Prevalence of Baseline NS5A RAPS in GT-1, -2, -3, -4, and -6 in ASTRAL 4 (Any change at 24, 28, 30, 31, 58, 92, and 93) (15% sensitivity threshold)

| | GT1 N=198 | GT2 N=10 | GT3 N=37 | GT4 N=8 | GT6 N=1 |
|--------------------------|----------------------|---------------------|---------------------|--------------------|--------------------|
| No RAPs | 149 (75%) | 4 (40%) | 33 (89%) | 3 (37%) | |
| Any RAPs | 48 (24%) | 6 (60%) | 4 (11%) | 5 (63%) | 1 (100%) |
| SOF/VEL 12 WK | 65* | 4 | 13 | 4 | |
| No RAPs | 49 (75%) | 3 (75%) | 10 (77%) | 1 (25%) | |
| Any RAPs | 12 (18%) | 1 (25%) | 3 (23%) | 3 (75%) | |
| Y93H | | | 2 (5%) | | |
| SOF/VEL +RBV 12WK | 66 | 4 | 13 | 2 | |
| No RAPs | 49 (74%) | 1 (25%) | 13 (100%) | 1 (50%) | |
| Any RAPs | 17 (26%) | 3 (75%) | 0 | 1 (50%) | |
| SOF/VEL 24 WK | 67 | 2 | 11 | 2 | 1 |
| No RAPs | 48 (72%) | 0 | 10 (91%) | 1 (50%) | |
| Any RAPs | 19 (28%) | 2 (100%) | 0 | 1 (50%) | 1 (100%) |

*4 missing sequence data

CENSORING SUBJECTS FOR FDA ANALYSIS

For the FDA resistance analyses, subjects who died, experienced an AE while undetectable, or were lost to follow-up in the ASTRAL trials were removed from the analysis. Thus, 3 GT1a and 16 GT3 were censored for the FDA resistance analysis (Table 43 and 44; FDA analysis).

Table 43. CENSORED SUBJECTS in ASTRAL1 (n=3)

| PID | GT | ARM | | |
|----------------------------|-----------|---------------------------------|-------------------|-------------------|
| GS-US-342-1138-00472-63493 | 1a | SOF/VEL once daily for 12 weeks | Adverse Event | Withdrew Consent |
| GS-US-342-1138-01305-63384 | 1a | SOF/VEL once daily for 12 weeks | | Lost to Follow-Up |
| GS-US-342-1138-05283-63398 | 1a | SOF/VEL once daily for 12 weeks | Lost to Follow-Up | Lost to Follow-Up |

Table 44. CENSORED SUBJECTS in ASTRAL 3 (n=16)

| PID | ARM | | |
|----------------------------|------------------------|-------------------|-------------------|
| GS-US-342-1140-00532-62204 | SOF + RBV for 24 weeks | | Lost to Follow-Up |
| GS-US-342-1140-00595-62494 | SOF + RBV for 24 weeks | Adverse Event | |
| GS-US-342-1140-00972-62135 | SOF + RBV for 24 weeks | Adverse Event | Adverse Event |
| GS-US-342-1140-00972-62159 | SOF + RBV for 24 weeks | Adverse Event | Adverse Event |
| GS-US-342-1140-01081-62466 | SOF + RBV for 24 weeks | Lost to Follow-Up | Lost to Follow-Up |
| GS-US-342-1140-01154-62556 | SOF + RBV for 24 weeks | Death | Death |
| GS-US-342-1140- | SOF + RBV for 24 | | |

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

| | | | |
|----------------------------|---------------------------------|--------------------------------|-------------------|
| 01257-62499 | weeks | | |
| GS-US-342-1140-01305-62507 | SOF + RBV for 24 weeks | Adverse Event | Adverse Event |
| GS-US-342-1140-01305-62530 | SOF + RBV for 24 weeks | Lost to Follow-Up | Lost to Follow-Up |
| GS-US-342-1140-01543-62024 | SOF + RBV for 24 weeks | Withdrew Consent | Withdrew Consent |
| GS-US-342-1140-01589-62546 | SOF + RBV for 24 weeks | Withdrew Consent | Withdrew Consent |
| GS-US-342-1140-01815-62170 | SOF + RBV for 24 weeks | Adverse Event | Adverse Event |
| GS-US-342-1140-03060-62441 | SOF + RBV for 24 weeks | Lost to Follow-Up | Lost to Follow-Up |
| GS-US-342-1140-04262-62067 | SOF + RBV for 24 weeks | Death | Death |
| GS-US-342-1140-00071-62458 | SOF/VEL once daily for 12 weeks | | Lost to Follow-Up |
| GS-US-342-1140-02075-62491 | SOF/VEL once daily for 12 weeks | Non-Compliance with Study Drug | Lost to Follow-Up |

In ASTRAL-4, 10 GT1a and GT3a subjects were censored for the FDA resistance analysis, because they died or were lost to follow-up (Table 45; FDA analysis).

Table 45. CENSORED SUBJECTS in ASTRAL 4 (n=10)

| PID | GT | ARM | | |
|-------------|----|----------------------------|--------------------------------|-------------------|
| 01039-64143 | 1a | SOF/VEL for 12 weeks | | Lost to Follow-Up |
| 04421-64014 | 1a | SOF/VEL for 12 weeks | | Death |
| 07275-64103 | 1a | SOF/VEL for 12 weeks | | Death |
| 03060-64241 | 1a | SOF/VEL + RBV for 12 weeks | Adverse Event | Death |
| 06991-64042 | 1a | SOF/VEL + RBV for 12 weeks | | Death |
| 01039-64171 | 1a | SOF/VEL for 24 weeks | Non-Compliance with Study Drug | Lost to Follow-Up |
| 01516-64077 | 1a | SOF/VEL for 24 weeks | | Lost to Follow-Up |
| 01589-64150 | 1b | SOF/VEL for 24 weeks | | Lost to Follow-Up |
| 01249-64047 | 3a | SOF/VEL for 12 weeks | | Death |
| 03060-64200 | 3a | SOF/VEL for 24 weeks | | Death |

ANALYSIS OF RESPONSE BY BASELINE NS5A RESISTANCE-ASSOCIATED POLYMORPHISMS (RAPs)

ASTRAL 3

The effect of the presence of baseline NS5A RAPs on relapse rates in subjects with GT3 HCV infection following 12-week SOF/VEL or 24-week SOF/RBV treatment was examined in ASTRAL 3. The overall relapse rate for the SOF/VEL 12 week treatment arm was 4% (11/275) compared to 15% (40/260) for the SOF/RBV 24 weeks treatment arm (Table 46; FDA analysis). In the SOF/VEL arm, the relapse rate for subjects with baseline NS5A RAPs was 7% (4/56) compared to 3% (7/218) for subjects without RAPs. As expected, the presence of NS5A RAPs did not affect the relapse rates (15-16%) in

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

the SOF/RBV arm because of the absence of an NS5A inhibitor in the treatment regimen.

Relapse rates were higher for subjects with cirrhosis in both treatment arms; 9% (7/80) for the SOF/VEL arm and 29% (23/78) for the SOF+RBV arm. For subjects without cirrhosis, relapse rates were 2% for both subjects with and without NS5A RAPs. For cirrhotic subjects treated with SOF/VEL for 12 weeks, relapse rates were higher for subjects with NS5A RAPs (33%; 3/9) than subjects without RAPs (6%; 4/71) (Table 46 and Fig. 2; FDA analysis).

Of the 10 subjects in the SOF/VEL arm with 2 NS5A RAPs, none relapsed. However, 4 subjects (9%) with 1 baseline NS5A RAP relapsed (1 with A30K and 3 with Y93H). Specifically, one non-cirrhotic subject with the Y93H polymorphism pretreatment relapsed (8%; 1/13), but both cirrhotic subjects with the Y93H polymorphism relapsed (100%; 2/2). The fourth relapse subject in the SOF/VEL 12 Week arm had the A30K polymorphism at baseline. Thus, relapse rates were 20% (3/15) for GT3 subjects with the Y93H polymorphism in the SOF/VEL 12 Week arm and relapse rates for subjects with an A30K polymorphism at baseline were 4% (1/28).

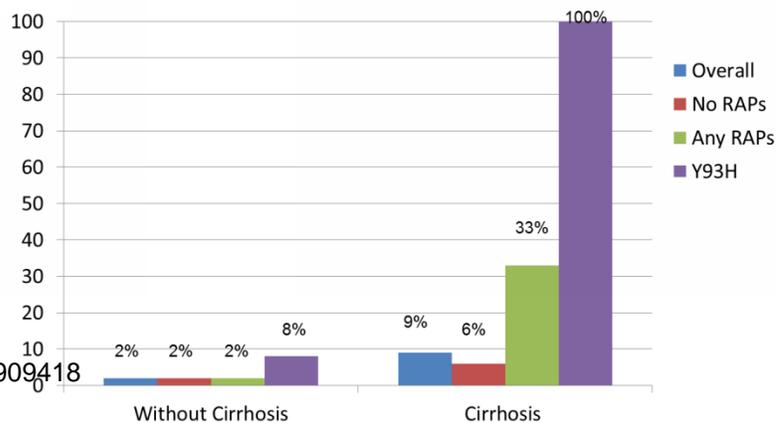
Table 46. Relapse Rates of GT3 Subjects by NS5A Polymorphisms (RAPs) and Cirrhosis in ASTRAL-3

| | SOF +VEL 12 Weeks N=275 | SOF + RBV 24 Weeks N=260 |
|-------------------|----------------------------|-----------------------------|
| Overall | 4% (11/275) | 15% (40/260) |
| Without cirrhosis | 2% (4/195) | 9% (16/178) |
| With cirrhosis | 9% (7/80) | 29% (23/78) |
| No RAPs | 3% (7/218) | 15% (31/202) |
| Without cirrhosis | 2% (3/147) | 10% (14/137) |
| With cirrhosis | 6% (4/71) | 27% (17/62) |
| Any RAPs | 7% (4/56) | 16% (9/57) |
| Without cirrhosis | 2% (1/47) ^a | 5% (2/41) |
| With cirrhosis | 33% (3/9) ^b | 40% (6/15) |
| 1 RAP | 9% (4/46) | 19% (9/48) |
| 2 or more RAPs | 0% (0/10) | 0% (0/9) |
| A30K | 4% (1/28) | 9% (3/33) |
| Y93H | 20% (3/15) | 35% (6/17) |
| Without cirrhosis | 8% (1/13) | 11% (1/9) |
| With cirrhosis | 100% (2/2) | 63% (5/8) |

^a 93Y/H

^b 2 with Y93H and 1 with 30K

Figure 2. GT3 Subjects with Cirrhosis in ASTRAL 3



DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

In ASTRAL-4, the effect of the presence of baseline NS5A RAPs on relapse rates in subjects with GT1 and GT3 HCV infection following 12-week SOF/VEL, 24-week SOF/VEL or 12-week SOF/VEL+RBV treatment were examined. Relapse rates were 0% for subjects with GT2, GT4 and GT6. There were no subjects with GT5 HCV infection in this trial.

Genotype 1

For GT1 subjects, the overall relapse rates were lower for the 12-week SOF/VEL + RBV arm (2%; 1/66) compared to 8% (5/65) and 4% (3/68) for the SOF/VEL 12-week and 24-week treatment arms, respectively (Table 47; FDA analysis) (Fig. 3; FDA analysis). In the 12-week SOF/VEL arm, the relapse rate for subjects with baseline NS5A RAPs was 17% (2/12) compared to 6% (3/52) for subjects without RAPs. In the 24-week SOF/VEL arm, the relapse rate for subjects with NS5A RAPs was 11% (2/19) compared to 2% (1/48) for subjects without RAPs. However, in the 12-week SOF/VEL + RBV arm, relapse rates were 0% (0/17) for subjects with NS5A RAPs compared to 2% (1/49) for subjects with no NS5A RAPs. Therefore in this patient population, the SOF/VEL + RBV for 12 weeks treatment option is more effective and reduces relapse rates compared to the other 2 tested treatments. This is especially seen for subjects with NS5A RAPs were relapse rates were 0% for the RBV containing arm compared to 17% and 11% for the 12-week and 24-week SOF/VEL regimens, respectively.

Table 47. Relapse Rate by Presence of Baseline RAPs (amino acid positions 24, 28, 30, 31, 58, 92, and 93) in ASTRAL 4

| | GT1 | GT2 | GT3 | GT4 | GT6 |
|--------------------------|------------|-----------------------|-------------|-----------------------|----------|
| Overall | 5% (9/198) | 0% (0/10) | 35% (13/37) | 0% (0/8) | 0% (0/1) |
| No RAPs | 3% (5/149) | 0% (0/4) | 33% (11/33) | 0% (0/3) | |
| Any RAPs | 8% (4/48) | 0% (0/6) ^a | 50% (2/4) | 0% (0/5) ^b | |
| SOF/VEL 12 WK | 8% (5/65) | 0% (0/4) | 46% (6/13) | 0% (0/4) | |
| No RAPs | 6% (3/52) | | 50% (5/10) | 0% (0/1) | |
| Any RAPs | 17% (2/12) | | 33% (1/3) | 0% (0/3) | |
| SOF/VEL +RBV 12WK | 2% (1/66) | 0% (0/4) | 15% (2/13) | 0% (0/2) | |
| No RAPs | 2% (1/49) | | 15% (2/13) | 0% (0/1) | |
| Any RAPs | 0% (0/17) | | 0 | 0% (0/1) | |
| SOF/VEL 24 WK | 4% (3/68) | 0% (0/2) | 45% (5/11) | 0% (0/2) | 0% (0/1) |
| No RAPs | 2% (1/49) | | 40% (4/10) | 0% (0/1) | |
| Any RAPs | 11% (2/19) | | 100% (1/1) | 0% (0/1) | |

^a5 of 6 had L31M^bK24N**Genotype 3**

For GT3 subjects, the overall relapse rates were much higher than those seen with GT1 subjects. However, relapse rates were lower for GT3 subjects in the 12-week SOF/VEL + RBV arm (15%; 2/13) compared to 46% (6/13) and 45% (5/11) for the SOF/VEL 12-week and 24-week treatment arms, respectively (Table 47; FDA analysis) (Fig. 4; FDA analysis). In the 12-week SOF/VEL arm, the relapse rate for subjects with NS5A RAPs was 33% (1/3) compared to 50% (5/10) for subjects without RAPs. In the 24-week SOF/VEL arm, the relapse rate for subjects with NS5A RAPs was 100% (1/1) compared to 40% (4/10) for subjects without RAPs. In the 12-week SOF/VEL + RBV arm, there

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

were no subjects with NS5A RAPs, so no comparison could be made to the 15% (2/13) relapse rate for subjects without NS5A RAPs.

The ASTRAL 4 data support the SOF/VEL + RBV 12 week regimen as the more effective treatment option for GT1 and GT3 decompensated subjects. These data also inform us that treatment for GT3 compensated cirrhotic patients could be improved by the addition of RBV to the 12 Week SOF/VEL regimen.

Figure 3. Effect of Baseline NS5A RAPs in GT1 Decompensated Cirrhotics: ASTRAL 4

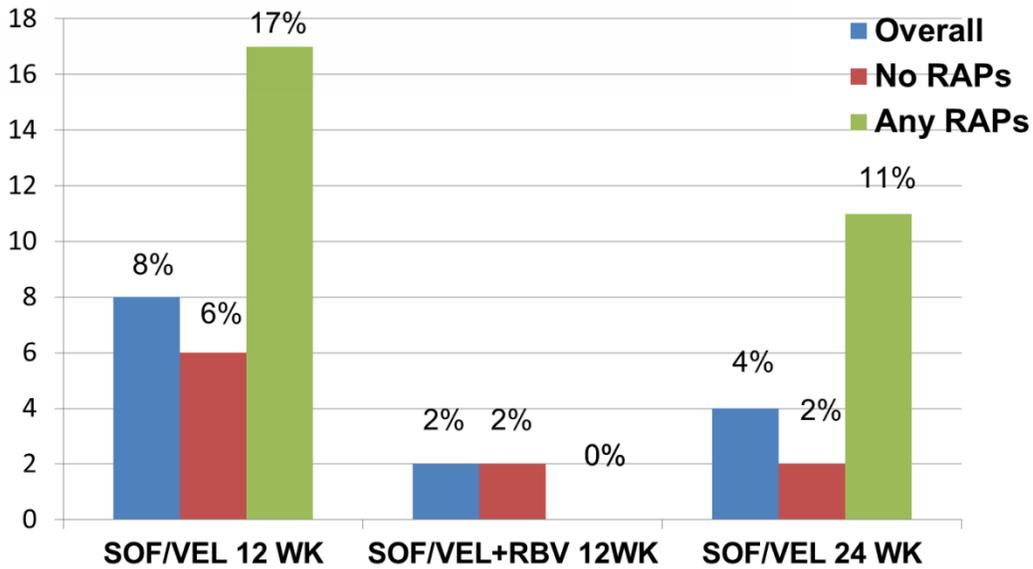
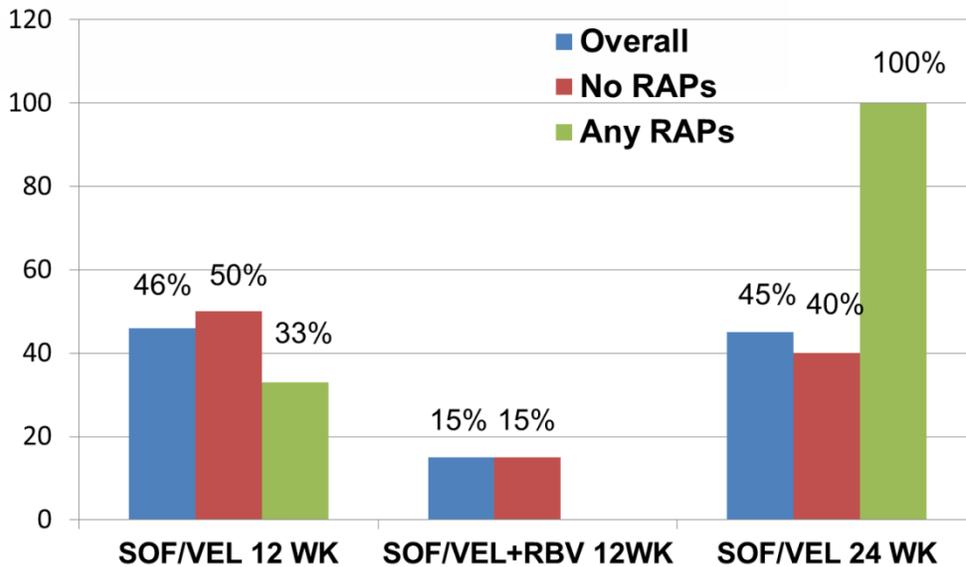


Figure 4. Effect of Baseline NS5A RAPs in GT3 Decompensated Cirrhotics: ASTRAL 4



DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

Combining the ASTRAL 1, 3 and 4 results

The the GT1 results from ASTRAL 1 and ASTRAL 4 and GT3 results from ASTRAL 3 and 4 show that relapse rates are higher for both GT1 and GT3 subjects with cirrhosis compared to non-cirrhotics (Table 48 and 49; FDA analysis). Additionally, relapse rates are higher for subjects with cirrhosis and baseline RAPs. When all the results are combined, relapse rates were 33% (4/12) for GT3 cirrhotic subjects with baseline RAPs compared to 11% (9/81) for cirrhotic subjects without RAPs (Table 49). In particular, relapse rates were 24% (4/17) for GT3 subjects with the Y93H polymorphism at baseline. Of the 4 relapse subjects with Y93H, 75% (3/4) had cirrhosis.

Table 48. Relapse Rate by Presence of Baseline RAPs (amino acid positions 24, 28, 30, 31, 58, 92, and 93) and Cirrhosis with 12 Weeks SOF/VEL (unless noted otherwise)

| | Overall | No RAPs (15%) | Any RAPs |
|----------------------------|--------------|---------------|------------|
| ASTRAL 1 | | | |
| GT1a | 0.5% (1/207) | 0.6% (1/171) | 0% (0/36) |
| Without cirrhosis | 1% (1/158) | 1% (1/128) | 0% (0/30) |
| With cirrhosis | 0/49 | 0% (0/43) | 0% (0/6) |
| GT1b | 1% (1/116) | 0% (0/78) | 3% (1/38) |
| Without cirrhosis | 0/92 | 0% (0/60) | 0% (0/32) |
| With cirrhosis | 4% (1/24) | 0% (0/18) | 17% (1/6) |
| GT1 | 1% (2/323) | 0.4% (1/249) | 1% (1/74) |
| Without cirrhosis | 0.4% (1/250) | 0.5% (1/188) | 0% (0/62) |
| With cirrhosis | 1% (1/73) | 0% (0/61) | 8% (1/12) |
| ASTRAL-4 | | | |
| GT1 With Cirrhosis | 5% (9/198) | 3% (5/149) | 8% (4/48) |
| All arms | | | |
| 12 Weeks | 8% (5/65) | 6% (3/52) | 17% (2/12) |
| SOF/VEL+RBV 12 Weeks | 2% (1/66) | 2% (1/49) | 0% (0/17) |
| 24 Weeks | 4% (3/67) | 2% (1/48) | 11% (2/19) |
| GT1a With Cirrhosis | 4% (6/151) | 4% (5/120) | 3% (1/30) |
| All arms | | | |
| 12 Weeks | 6% (3/47) | 7% (3/41) | 0% (0/5) |
| SOF/VEL+RBV 12 Weeks | 2% (1/52) | 2% (1/43) | 0% (0/9) |
| 24 Weeks | 4% (2/52) | 3% (1/36) | 6% (1/16) |
| ASTRAL-3 | | | |
| GT3 | 4% (11/275) | 3% (7/218) | 7% (4/56) |
| Without cirrhosis | 2% (4/195) | 2% (3/147) | 2% (1/47) |
| With cirrhosis | 9% (7/80) | 6% (4/71) | 33% (3/9) |
| ASTRAL-4 | | | |
| GT3 with cirrhosis | | | |
| 12 Weeks | 46% (6/13) | 50% (5/10) | 33% (1/3) |
| SOF/VEL+RBV 12 Weeks | 15% (2/13) | 15% (2/13) | 0 |
| 24 Weeks | 45% (5/11) | 40% (4/10) | 100% (1/1) |

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

Table 49. ASTRAL 1, 3 and 4 Data

| NS5A Polymorphisms | Relapse Rates after 12 Weeks of Treatment with SOF+VEL | |
|--|--|------------------------------|
| | With NS5A Polymorphism(s) | Without NS5A Polymorphism(s) |
| | % (n/N) | % (n/N) |
| HCV genotype 1-infected subjects: Any RAP | 3% (3/86) | 1% (4/301) |
| Without cirrhosis | 0% (0/62) | 0.5% (1/188) |
| With cirrhosis | 13% (3/24) | 3% (3/113) |
| HCV genotype 3-infected subjects: Any RAP | 8% (5/59) | 5% (12/228) |
| Without cirrhosis | 2% (1/47) | 2% (3/147) |
| With cirrhosis | 33% (4/12) | 11% (9/81) |
| HCV genotype 3-infected subjects: Y93H | 24% (4/17) | |
| Without cirrhosis | 8% (1/13) | |
| With cirrhosis | 75% (3/4) | |

TREATMENT-EMERGENT NS5A AND NS5B RESISTANCE-ASSOCIATED SUBSTITUTIONS

ASTRAL-1 VIROLOGIC FAILURES

In ASTRAL 1, there were 2 GT1 virologic failures who relapsed; one with GT1a and one with GT1c/h (Table 50; FDA analysis). The GT1a relapse had low level Q30R detectable with NGS below the 15% threshold and had emergent Y93N at failure with an 805-fold reduced susceptibility to VEL. The other GT1c/h subject had cirrhosis and baseline NS5A RAPs Q30R, L31M and H58P (above 15% threshold). This subject had emergent L24M/T, L31I/V and Y93H with a 763-fold reduced susceptibility to VEL. Neither subject had baseline or emergent NS5B nucleoside analog inhibitor resistance substitutions.

No subjects with GT2, 4, 5, or 6 HCV infection experienced virologic failure.

Table 50. Treatment-Emergent NS5A and NS5B Resistance-Associated Substitutions in ASTRAL 1

| Subject | GT | IL28 | Baseline NS5A RAPs | Emergent NS5A Resistance Substitutions | Change in Phenotype from Reference at Failure (from BL) | Baseline NS5B RAPs | Emergent NS5B Resistance Substitutions |
|------------|----|------|--------------------|--|---|--------------------|--|
| GS-US-342- | 1a | CT | Q30R (2.6%) | Y93N | 805 | none | none |

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

| | | | | | | | |
|-----------------------------|-------------------|----|----------------------|---|-----|------|------|
| 1138-00529-63184 | | | | | | | |
| GS-US-342-1138-05294-63312* | 1b (1c/ 1h) | TT | Q30R L31M H58P | Y93H L31I (2.8%) L31V (8.6%) K24M (1.5%) K24T (1.3%) | 763 | none | none |

*Subject had cirrhosis; screened as GT1b, but found by sequencing/BLAST analysis to be GT1c/1h.

ASTRAL-2

There were no virologic failures in ASTRAL 2.

ASTRAL-3 VIROLOGIC FAILURES

In ASTRAL 3, there were 11 GT3 virologic failures in the SOF/VEL 12 week arm compared to 38 relapsers in the SOF+ RBV 24 week arm. In the SOF/VEL arm, one failure subject (Subject 01069-62225) had GT3a HCV infection at screening but had GT1a HCV infection at virologic failure as determined by NS5B sequencing, suggesting reinfection and not a relapse of the original GT3a virus. This subject did not have NS5A or NS5B baseline polymorphisms or post-treatment substitutions. Four of the the relapsers in the SOF/VEL arm had baseline NS5A RAPs, 3 had Y93H and 1 had A30K (Table 51; FDA analysis). Eight of the 11 relapsers had emergent NS5A resistance-associated substitutions; all 8 had emergent Y93H (1 from a mixture at baseline), 1 had emergent P58L at 2% and 1 had emergent A30V at 12%. In total, 10 of the 11 failures had Y93H at failure. If the one subject who is suspected of being reinfected is removed, then all 10 relapsers had Y93H at failure.

In the SOF/VEL 12 week arm, the cirrhotic subjects with NS5A RAPs had relapse rates of 33% (3/9) and the 2 subjects with Y93H at baseline relapsed.

Table 51. GT3 Virologic Failures# in ASTRAL-3 in SOF/VEL 12 Week Arm (n=11)

| PID | GT | Prior TRT | Cirr | Baseline NS5A RAPs | Emergent NS5A Resistance Substitutions | BL NS5B RAPs | Emergent NS5B Resistance Substitutions |
|--------------|-----------|----------------------------------|------|--------------------|--|--------------|--|
| 00472-62512 | 3a | NAIVE-ALL | Y | Y93Y/H | Y93H A30V (12%) | none | none |
| 00529-62069 | 3a | P/R NULL RESPONDER | Y | | Y93H | none | none |
| 00529-62147 | 3a | NAIVE-ALL | Y | Y93H | | none | none |
| 01065-62502 | 3a | P/R BREAKTHROUGH OR P/R RELAPSER | N | | Y93H | none | none |
| 01069-62225* | 3a/ 1a | P/R BREAKTHROUGH OR P/R | N | | | none | none |

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

| | | | | | | | |
|-------------|----|----------------------------------|---|------|-------------------|-------|------|
| | | RELAPSER | | | | | |
| 01589-62011 | 3a | NAIVE-ALL | N | Y93H | | none | none |
| 02080-62118 | 3a | P/R BREAKTHROUGH OR P/R RELAPSER | Y | | Y93H | none | none |
| 03314-62107 | 3a | P/R BREAKTHROUGH OR P/R RELAPSER | N | | Y93H | N142S | |
| 04472-62202 | 3a | P/R NULL RESPONDER | Y | A30K | P58L (2%) Y93H | none | none |
| 05730-62185 | 3a | NAIVE-ALL | Y | | Y93H | none | none |
| 05873-62186 | 3a | P/R NULL RESPONDER | Y | | Y93H | none | none |

#all relapsers except 01069-62225*

*This subject had GT1a at virologic failure (by NS5B sequencing) indicating reinfection and not relapse of baseline virus.

In ASTRAL 4, there were 9 total GT1 virologic failures in all the arms; 5 in the SOF/VEL 12 week arm, 1 in the SOF/VEL 12 week + RBV arm and 3 in the SOF/VEL 24 week arm (Table 52; FDA analysis). Four of the GT1 relapsers had baseline NS5A RAPs (Q30Q/H + Y93Y/H, Y93Y/H, L31M, and R30Q + Y93Y/H) (Table 52). Six of the 9 relapsers had emergent NS5A resistance-associated substitutions; all 6 had Y93H or N at failure (Fig. 5 and Table 53; FDA analysis). Other emergent substitutions included Q30H/R, L31M/V and H58D. The one GT1 virologic failure in the 12 week SOF/VEL + RBV arm had no NS5A or NS5B resistance substitutions at baseline or failure.

Table 52. Virologic Failures in ASTRAL-4 (n = 22)

| Subject | GT | ARM | Prior TRT | Cirr | Baseline NS5A RAPs | Emergent NS5A Resistance Substitutions | Baseline NS5B RAPs | Emergent NS5B Resistance Substitutions |
|--------------------|----|-----------|----------------------|------|--------------------|--|--------------------|--|
| 00380-64032 | 1a | 12 WK | NAIVE-ALL | yes | | | | |
| 01039-64172 | 1a | 12 WK RBV | P/R PARTIAL | yes | | | | |
| 01651-64225 | 1a | 12 WK | P/R NULL or PARTIAL | yes | | | | |
| 02127-64221 | 1a | 24 WK | NAIVE-ALL | yes | Q30Q/H Y93Y/H | Q30H Y93H | | L159F (96%) S282T (3%) |
| 07275-64065 | 1a | 12 WK | NO category suitable | yes | | Y93N | | |
| 08734-64091 | 1a | 24 WK | NAIVE-ALL | yes | | Q30R 46A H58D Y93N (4%) | | |
| 02127- | 1b | 12 | P/R NULL | yes | Y93Y/H | L31M/V | | |

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

| | | | | | | | | |
|--------------------|-----------|------------------|--|------------|----------------|------------------------------------|--|--------------------------------------|
| 64167 | | WK | | | | Y93H | | |
| 05969-64193 | 1b | 24 WK | P/R+DAA BT OR P/R+DAA RELAPSE | yes | L31M | 46I Y93H | | |
| 08224-64040 | 1b | 12 WK | NAIVE-ALL | yes | R30Q Y93Y/H | L31M L31V (10%) Y93H | | L159F (14%) S282T (3.6%) |
| 00331-64095 | 3a | 12 WK | P/R | yes | Y93H | S38Y (1.1%) P58T (1.3%) | | S96Y (1%) L320I (1.1%) |
| 00407-64262 | 3a | 24 WK | IFN+RBV | yes | | 83M/I Y93H | | |
| 00585-64263 | 3a | 12 WK | P/R | yes | | 64A Y93H | | |
| 00619-64062 | 3a | 24 WK | IFN+RBV | yes | | Y93H | | |
| 01249-64072 | 3a | 12 WK | P/R | yes | | Y93H | | |
| 01657-64105 | 3a | 24 WK | P/R | yes | | Y93H | | |
| 02127-64256 | 3a | 12 WK | NAIVE-ALL | yes | | Y93H | | |
| 02760-64041 | 3a | 12 WK | P/R | yes | | Y93H | | N142S (>99%) |
| 03060-64249 | 3a | 12 WK RBV | NAIVE-ALL | yes | | S38P (8.6%) Y93H | | N142T (3.1%) E237G (2.3%) |
| 04421-64013 | 3a | 24 WK | NAIVE-ALL | yes | P58A | Y93H | | E237G (1.5%) |
| 05665-64208 | 3a | 12 WK RBV | NAIVE-ALL | yes | | M28V (1.7%) Y93H | | |
| 08118-64117 | 3a | 12 WK | NAIVE-ALL | yes | | Y93H | | |
| 09891-64195 | 3a | 24 WK | P/R | yes | | M28T (2.2%) Y93H | | |

Bolded subjects are in the recommended regimen being indicated.

In ASTRAL 4, there were 13 total GT3 virologic failures in all the arms; 6 in the SOF/VEL 12 week arm, 2 in the SOF/VEL 12 week + RBV arm and 5 in the SOF/VEL 24 week arm (Table 52). Two of the GT3 relapsers had baseline NS5A RAPs (P58A and Y93H). Twelve of the 13 relapsers had emergent NS5A resistance-associated substitutions and all 13 had Y93H at failure (Fig. 6 and Table 53; FDA analysis). Other emergent substitutions included M28T/V, S38P/Y and H58T. The 2 GT3 virologic failures in the 12 week SOF/VEL + RBV arm had S38P + Y93H and M28V + Y93H emerge at failure.

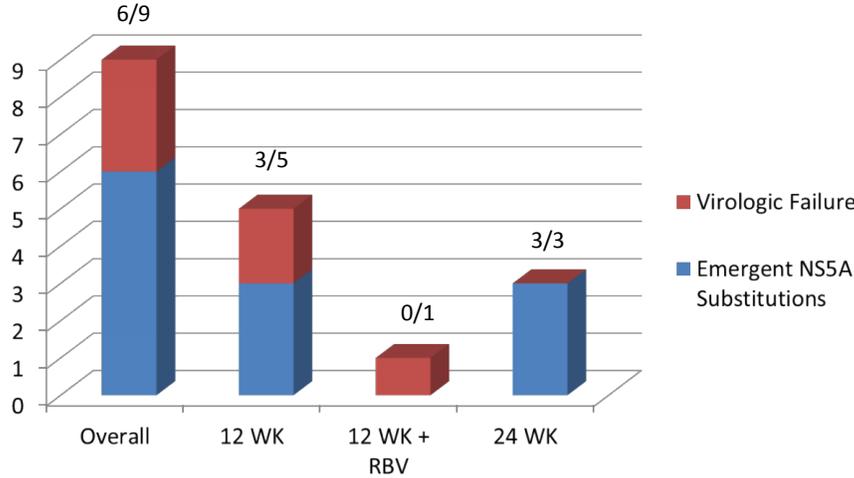
DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

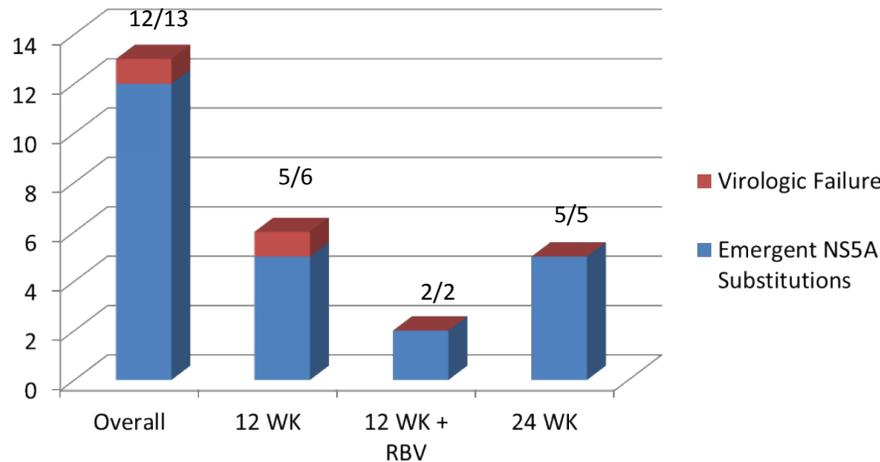
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Figure 5. Emergent NS5A Resistance Substitutions in GT1 Decompensated Failures: ASTRAL 4



Emergent NS5A resistance substitutions: Y93H/N = 6/6; Q30H/R = 2/6; L31M= 2/6; H58D = 1/6)

Figure 6. Emergent NS5A Resistance Substitutions in GT3 Decompensated Failures: ASTRAL 4



Emergent NS5A resistance substitutions: Y93H (n=12), M28T/V (n=2), S38P/Y (n=2) and H58T (n=1)

Table 53. Summary of NS5A Resistance in ASTRAL 4 GT1 and GT3 Virologic Failures

| | Number of Virologic Failures | Number with Baseline RAPs | Emergent NS5A Resistance-Associated Substitutions |
|--------------------|------------------------------|---------------------------|---|
| GT1 | 9 | 4 | 6 (67%) |
| SOF/VEL 12 WK | 5 (56%) | 2 | 3 (60%) |
| SOF/VEL 12 WK +RBV | 1 (11%) | | 0 |
| SOF/VEL 24 WK | 3 (33%) | 2 | 3 (100%) |
| 1a | 6 (67%) | 1 | 2 |
| 1b | 3 (33%) | 3 | 3 |

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

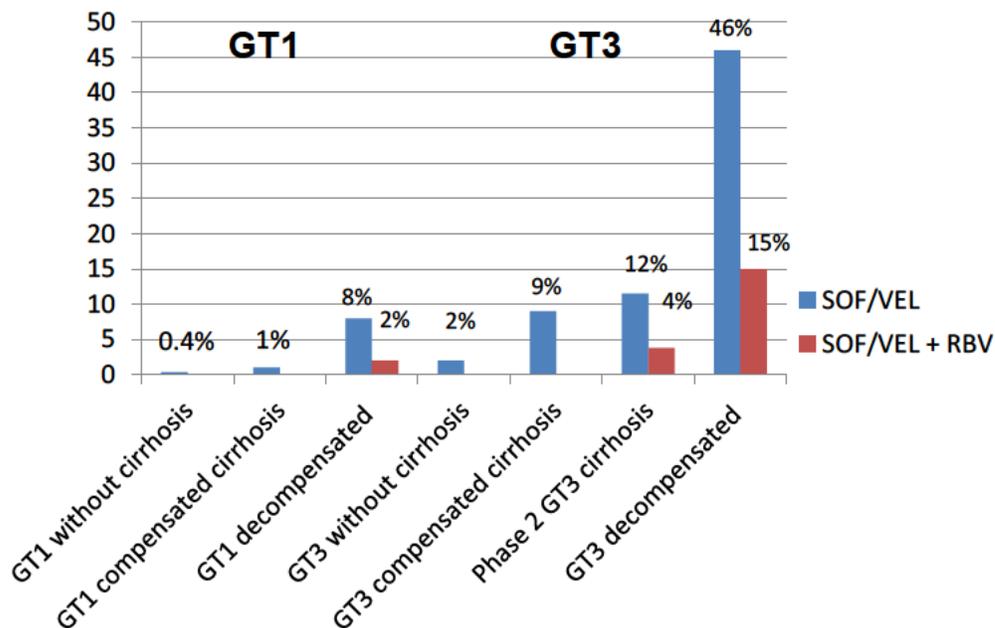
| | | | |
|--------------------|---------|---|----------|
| Q30H or R | | | 2 (33%) |
| L31M | | | 2 (33%) |
| H58D | | | 1 (17%) |
| Y93H or N | | | 6 (100%) |
| GT3 | 13 | 2 | 12 (92%) |
| SOF/VEL 12 WK | 6 (46%) | | 5 (83%) |
| SOF/VEL 12 WK +RBV | 2 (15%) | | 2 (100%) |
| SOF/VEL 24 WK | 5 (38%) | | 5 (100%) |
| Y93H | | | 12 |

12 WEEK SOF/VEL + RBV OPTION FOR GT3 SUBJECTS WITH COMPENSATED CIRRHOSIS

Relapse rates for GT3 compensated cirrhotics in ASTRAL 3 are 9% compared to 1% for GT1 compensated cirrhotics (Fig. 7). Even though the overall relapse rates are low with SVR12 rates >90%, the relapse rates for certain subpopulations of GT3 cirrhotics (e.g. subjects with NS5A RAPs) are higher. In ASTRAL 3, relapse rates are 33% (3/9) for GT3 subjects with compensated cirrhosis and baseline NS5A RAPs. Additionally, 2 of 2 GT3 subjects with Y93H relapsed.

Data from the ASTRAL4 study of GT3 decompensated cirrhotics showed the addition of RBV to 12 week SOF/VEL reduced relapse rates to 15% (2/13) compared to 46% (6/13) without RBV (Fig. 7). There was insufficient data to determine the impact of HCV NS5A RAPs in GT3 subjects in this study. However, for GT1 subjects with NS5A RAPs, relapse rates were 0% (0/17) for subjects receiving RBV+ SOV/VEL 12 weeks compared to 17% (2/12) in the SOV/VEL 12 week no RBV arm.

Figure 7. Summary of Relapse Results in GT1 and GT3 Subjects with and without Cirrhosis



DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

At the time of the EOP2 meeting, based on the available efficacy data in GT3 subjects, we recommended the sponsor evaluate a longer treatment duration (>12 weeks) to optimize SVR rates in the “harder to treat” patient population such as subjects with cirrhosis. The sponsor did not study a >12 week regimen or a 12-week + RBV regimen in GT3 subjects with compensated cirrhosis in the registrational phase 3 studies. Thus, unfortunately, we do not have phase 3 data of the SOF/VEL 12 week regimen with RBV in GT3 compensated cirrhotics. However, we do have phase 2 data from Study 109.

Phase 2 Study 109 Results

Study 109 was a phase 2 study of 2 doses (25 mg and 100mg) of VEL in combination with SOF 400 mg with or without RBV. This study had 12 arms examining GT1 and GT3 subjects with and without cirrhosis. Arms 7 and 8 are the pertinent arms examining 12 week SOF + 100 mg VEL with and without RBV in GT3 subjects with compensated cirrhosis. The SVR12 rate was 88% (23/26) for the SOF/VEL arm compared to 96% (25/26) for the SOF/VEL + RBV arm (Table 54; FDA analysis). The four subjects (#61030, #61073, #61105, #61045) who relapsed on the proposed dose and duration regimen of SOF 400 mg+VEL 100 mg (one received RBV) were all GT3a. Only one of these subjects had baseline NS5A resistance polymorphisms A30K and L31M, but all the relapse subjects had emergent Y93H at the post-treatment timepoint. None of the 4 relapsers have pre or post-treatment NS5B nucleoside analog inhibitor resistance substitutions. The data from this study provides support for improved SVR12 rates when adding RBV to 12 week SOF/VEL for GT3 subjects with compensated cirrhosis.

Table 54. GT3 Subjects with Compensated Cirrhosis

| | SOF/VEL 100 mg | SOF/VEL 100 mg + RBV |
|-----------------|-----------------------|-----------------------------|
| SVR12 | 88.5% (23/26) | 96.2% (25/26) |
| Relapse | 11.5% (3/26) | 3.8% (1/26) |
| BL RAPs | 1 (A30K, L31M) | 0 |
| Y93H at failure | 2/3 | 1/1 |

Because of the concern for the consequences of virologic failure with development of Y93H in all failures and loss of subsequent treatment options, we pushed for a consideration for adding RBV to 12 week SOF/VEL in the GT3 compensated cirrhotic population. Specifically we were concerned for those with baseline NS5A RAPs, but did not have enough data to support screening all patients for NS5A RAPs before treatment with SOF/VEL. Our virology proposal to the review team was to add a footnote to Table 1 in Section 2, Dosage and Administration stating “SOF/VEL + RBV for 12 weeks can be considered for GT3 patients with compensated cirrhosis [see Clinical 14 and Microbiology 12.4].

The benefits of our consideration included: 1) relapse rates could be reduced for GT3 patients with compensated cirrhosis who could take RBV. Based on a bridging assessment by Karen Qi, Ph.D., statistical reviewer, relapse rates of 9% for GT3 cirrhotics could be reduced to 2-3% with the addition of RBV, 2) relapse rates are 33% for cirrhotic subjects with baseline NS5A RAPs, so adding RBV would be a better option for these subjects and it would not be necessary to screen for RAPs and 3) adding RBV could reduce failure with the Y93H resistance substitution would would have

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 **SDN:** 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

consequences for future treatment options. The cons of this approach are that 1) there are no phase 3 data for GT3 cirrhotics with RAPs in the 12 Week SOF/VEL + RBV arm, 2) there would be potentially unnecessary RBV use in some patients, and 3) would have to determine GT/subtype at screening even though SOF/VEL is a pangenotypic regimen.

The justifications for adding the consideration footnote include:

- Phase 2 data showing the increase in SVR12 rate with RBV (89% (23/26) to 96% (25/26).
- Cirrhosis is a continuum from compensated from decompensated so results in decompensated can inform treatment for compensated.
- Results from ASTRAL 4 in decompensated patients show better SVR rates with RBV in both GT3 subjects (46% without compared to 15% with RBV) and GT1 subjects (8% without and 2% with RBV).

In Section 14 Clinical Studies, Table 14 shows the SVR12 rates with compensated cirrhosis and treatment-experienced patients. Study 109 phase 2 results showing SVR12 rates improved from 88% (23/26) to 96% (25/26) with the addition of RBV to SOF/VEL for 12 weeks can be added after Table 14. Also in Section 14, the results of ASTRAL 4 are included with the statement "Treatment with SOF/VEL plus RBV results in higher SVR12 rates that treatment with SOF/VEL for 12 weeks or 24 weeks."

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

5. CONCLUSION

This NDA for a fixed-dose combination of velpatasvir (VEL) and sofosbuvir (SOF) is approvable from a virology perspective for the treatment of adult patients with GT1, 2, 3, 4, 5, and 6 HCV infection. The recommended treatment regimen for patients without cirrhosis and patient with compensated cirrhosis (Child-Pugh A) is 12 weeks of SOF/VEL. This recommendation is based on the high SVR12 rates in ASTRAL 1, 2, and 3. There were only 2 GT1 virologic failures (one with GT1a and one with GT1c/h) who relapsed in ASTRAL 1 and there were no virologic failures in ASTRAL 2. For GT2, GT4, GT5 and GT6 subjects, SVR12 rates were 100% with or without the presence of baseline NS5A RAPs. The GT1a relapser had emergent Y93N at failure with an 805-fold reduced susceptibility to VEL. The GT1c/h relapser had cirrhosis with baseline NS5A RAPs Q30R, L31M and H58P (above 15% threshold) and had emergent L24M/T, L31I/V and Y93H with a 763-fold reduced susceptibility to VEL.

For GT3 subjects, relapse rates were higher for subjects with cirrhosis in both treatment arms; 9% (7/80) for the SOF/VEL arm and 29% (23/78) for the SOF+RBV arm. GT3 subjects with compensated cirrhosis treated with 12 weeks SOF/VEL had a relapse rate of 9% compared to 2% for GT3 subjects without cirrhosis. In addition, for cirrhotic subjects treated with SOF/VEL for 12 weeks, relapse rates were higher for subjects with NS5A RAPs (33%; 3/9) than subjects without RAPs (6%; 4/71). There were 11 GT3 virologic failures in the SOF/VEL 12 week arm compared to 38 relapsers in the SOF+RBV 24 week arm. Four of the the relapsers in the SOF/VEL arm had baseline NS5A RAPs, 3 had Y93H and 1 had A30K. Eight of the 11 relapsers had emergent NS5A resistance-associated substitutions; all 8 had emergent Y93H (1 from a mixture at baseline), 1 had emergent P58L at 2% and 1 had emergent A30V at 12%. In total, 10 of the 11 failures had Y93H at failure. If the one subject who is suspected of being reinfected is removed, then all 10 relapsers had Y93H at failure.

This virology review supports adding a footnote to consider adding RBV to 12 week SOF/VEL for GT3 subjects with compensated cirrhosis, because relapse rates were higher overall in this population and the consequences of failure with resistance to all NS5A inhibitors and potentially SOF for the cirrhotic population are significant. Importantly, relapse rates were much higher (33%) in GT3 compensated cirrhotic subjects who had baseline NS5A resistance-associated polymorphisms (RAPs). Furthermore, all the GT3 virologic failures with compensated cirrhosis had the Y93H NS5A resistance substitution at failure, which confers high level resistance to all current NS5A inhibitors and may compromise future treatment options. Thus, it is important to optimize chances of virologic success for this advanced patient population. The data support adding RBV to 12 weeks SOF/VEL to optimize SVR12 rates in GT3 patients with compensated cirrhosis.

The recommended treatment regimen for patients with decompensated cirrhosis (Child-Pugh B and C) is 12 weeks of SOF/VEL + RBV. The basis for the indication in decompensated cirrhosis came from the data in the ASTRAL 4 study. For GT1 subjects in this study, the overall relapse rates were lower for the 12-week SOF/VEL + RBV arm (2%; 1/66) compared to 8% (5/65) and 4% (3/68) for the SOF/VEL 12-week and 24-week treatment arms, respectively. For subjects with NS5A RAPs, relapse rates were 0% (0/17) for the 12 week SOF/VEL + RBV containing arm compared to 17% and 11%

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

for the 12-week SOF/VEL and 24-week SOF/VEL regimens, respectively. For subjects without NS5A RAPs, relapse rates were 2% (1/49) for the 12 week SOF/VEL + RBV containing arm compared to 6% (3/52) and 2% (1/48) for the 12-week SOF/VEL and 24-week SOF/VEL regimens, respectively.

For GT3 subjects, overall relapse rates were also lower for the 12 week SOF/VEL+RBV (15% (2/13) compared to 46% (6/13) and 45% (5/11) for the SOF/VEL 12-week and 24-week treatment arms, respectively. There are insufficient data to determine the impact of HCV NS5A RAPs in GT3 decompensated cirrhotics.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

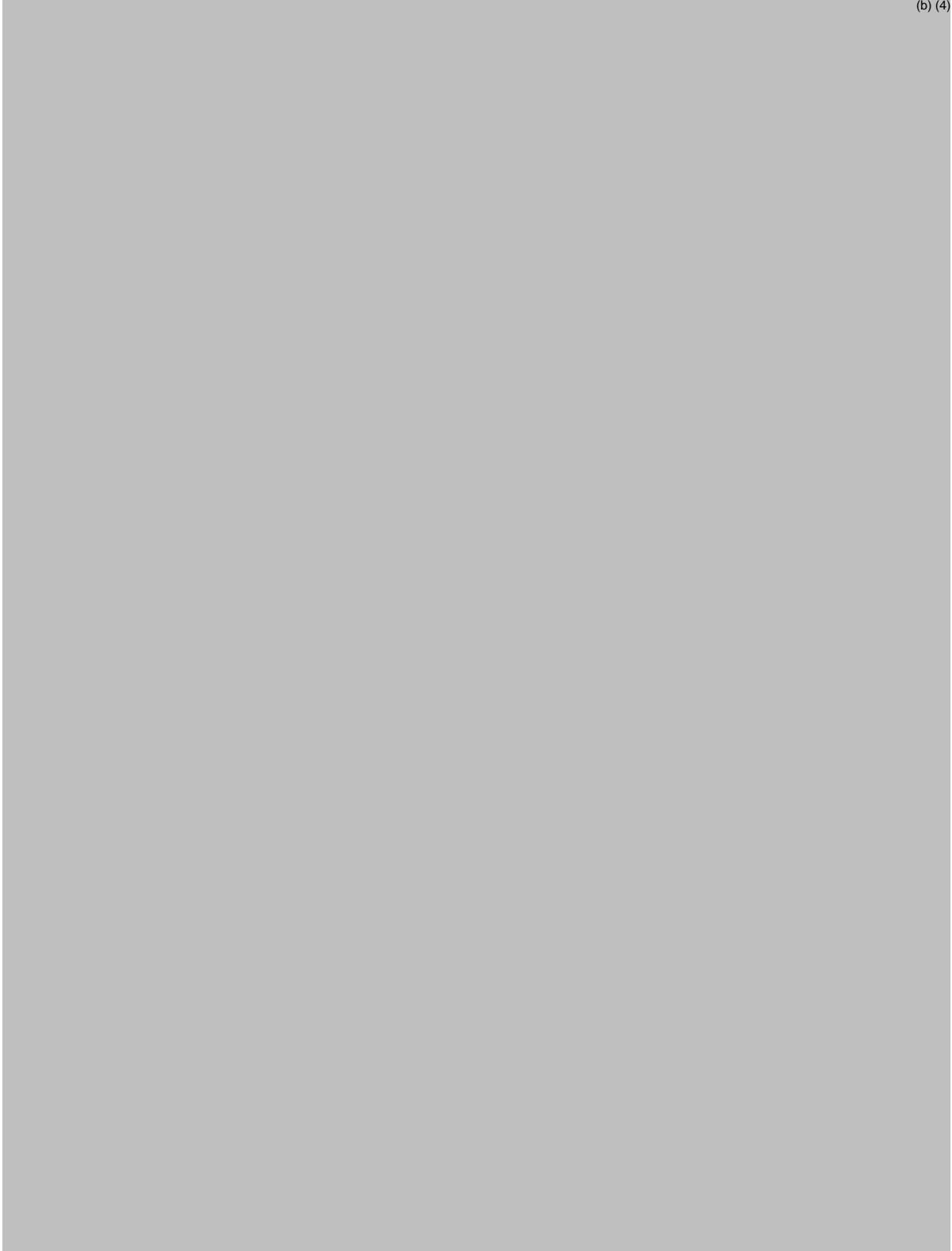
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DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

6. PACKAGE INSERT

6.1 SPONSOR-PROPOSED PACKAGE INSERT



(b) (4)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

6.2 FDA-NEGOTIATED PACKAGE INSERT

12.4 Microbiology

Mechanism of Action

Sofosbuvir is an inhibitor of the HCV NS5B RNA-dependent RNA polymerase, which is required for viral replication. Sofosbuvir is a nucleotide prodrug that undergoes intracellular metabolism to form the pharmacologically active uridine analog triphosphate (GS-461203), which can be incorporated into HCV RNA by the NS5B polymerase and acts as a chain terminator. In a biochemical assay, GS-461203 inhibited the polymerase activity of the recombinant NS5B from HCV genotype 1b, 2a, 3a and 4a with an IC_{50} value ranging from 0.36 to 3.3 micromolar. GS-461203 is neither an inhibitor of human DNA and RNA polymerases nor an inhibitor of mitochondrial RNA polymerase.

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

Antiviral Activity

The EC_{50} values of sofosbuvir and velpatasvir against full-length or chimeric replicons encoding NS5B and NS5A sequences from the laboratory strains are presented in Table 6. The EC_{50} values of sofosbuvir and velpatasvir against clinical isolates are presented in Table 7.

Table 6 Activity of Sofosbuvir and Velpatasvir Against Full Length or Chimeric Laboratory Replicons

| Replicon Genotype | Sofosbuvir EC_{50} , nM ^a | Velpatasvir EC_{50} , nM ^a |
|-------------------|--|---|
| 1a | 40 | 0.014 |
| 1b | 110 | 0.016 |
| 2a | 50 | 0.005-0.016 ^c |
| 2b | 15 ^b | 0.002-0.006 ^c |
| 3a | 50 | 0.004 |
| 4a | 40 | 0.009 |
| 4d | 33.4 | 0.004 |
| 5a | (b) (4) | 0.021-0.054 ^d |
| 6a | (b) (4) | 0.006-0.009 |
| 6e | (b) (4) | 0.130 ^d |

- Mean value from multiple experiments of same laboratory replicon.
- Stable chimeric 1b replicons carrying NS5B genes from genotype 2b, 5a or 6a were used for testing.
- Data from various strains of full length NS5A replicons or chimeric NS5A replicons carrying full-length NS5A genes that contain L31 or M31 polymorphisms.
- Data from a chimeric NS5A replicon carrying NS5A amino acids 9-184.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

Table 7 Activity of Sofosbuvir and Velpatasvir Against Transient Replicons Containing NS5A or NS5B from Clinical Isolates

| Replicon Genotype | Replicons containing NS5B from clinical isolates | | Replicons containing NS5A from clinical isolates | |
|-------------------|--|---|--|--|
| | Number of clinical isolates | Median sofosbuvir EC ₅₀ , nM (range) | Number of clinical isolates | Median velpatasvir EC ₅₀ , nM (range) |
| 1a | 67 | 62 (29-128) | 23 | 0.019 (0.011-0.078) |
| 1b | 29 | 102 (45-170) | 34 | 0.012 (0.005-0.500) |
| 2a | (b) (4) | (b) (4) | 8 | 0.011 (0.006-0.364) |
| 2b | (b) (4) | (b) (4) | 16 | 0.002 (0.0003-0.007) |
| 3a | 106 | 81 (24-181) | 38 | 0.005 (0.002-1.871) |
| 4a | NA | NA | 5 | 0.002 (0.001-0.004) |
| 4d | NA | NA | 10 | 0.007 (0.004-0.011) |
| 4r | NA | NA | 7 | 0.003 (0.002-0.006) |
| 5a | NA | NA | 42 | 0.005 (0.001-0.019) |
| 6a | NA | NA | 26 | 0.007 (0.0005-0.113) |
| 6e | NA | NA | 15 | 0.024 (0.005-0.433) |

NA=Not Available

Velpatasvir was not antagonistic in reducing HCV RNA levels in replicon cells when combined with sofosbuvir or IFN- α , RBV, a HCV NS3/4A protease inhibitor, (b) (4) or HCV NS5B non-nucleoside inhibitors (b) (4)

Resistance

In Cell Culture

HCV replicons with reduced susceptibility to sofosbuvir have been selected in cell culture for multiple genotypes including 1b, 2a, 2b, 3a, 4a, 5a, and 6a. Reduced susceptibility to sofosbuvir was associated with the NS5B substitution S282T in all replicon genotypes examined. An M289L substitution developed along with the S282T substitution in genotype 2a, 5 and 6 replicons. Site-directed mutagenesis of the S282T substitution in replicons of genotypes 1 to 6 conferred 2- to 18-fold reduced susceptibility to sofosbuvir.

HCV genotype 1a, 1b, 2a, 3a, 4a, 5a, and 6a replicon variants with reduced susceptibility to velpatasvir were selected in cell culture. Variants developed amino acid substitutions at NS5A resistance-associated positions 24, 28, 30, 31, 32, 58, 92, and 93. Phenotypic analysis of site-directed mutant replicons of the selected NS5A substitutions showed that single and double combinations of L31V and Y93H/N in genotype 1a, the combination of L31V +Y93H in genotype 1b, Y93H/S in genotype 3a, and L31V and

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

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P32A/L/Q/R in genotype 6 conferred >100-fold reduction in velpatasvir susceptibility. In the GT2a replicon, the single mutant F28S and Y93H showed 91-fold and 46-fold, respectively, in reduced susceptibility to velpatasvir. The single mutant Y93H conferred 3-fold reduced susceptibility to velpatasvir in genotype 4a replicons. Combinations of these NS5A substitutions often showed greater reductions in susceptibility to velpatasvir than single substitutions alone.

In Clinical Trials

Studies in Subjects without Cirrhosis and Subjects with Compensated Cirrhosis

In a pooled analysis of subjects without cirrhosis or with compensated cirrhosis who received [TRADENAME] for 12 weeks in Phase 3 trials (ASTRAL-1, ASTRAL-2 and ASTRAL-3), 12 subjects (2 with genotype 1 (1a, 1c/h) and 10 with genotype 3a) qualified for resistance analysis due to virologic failure. No subjects with genotype 2, 4, 5, or 6 HCV infection experienced virologic failure.

Of the 2 genotype 1 virologic failure subjects, one subject had virus with emergent NS5A resistance substitution Y93N and the other had virus with emergent NS5A resistance substitutions K24M/T, L31I/V and Y93H at virologic failure. The latter subject had virus at baseline harboring NS5A resistance polymorphisms (Q30R, L31M, H58P). No NS5B nucleoside analog inhibitor resistance substitutions were observed at failure in the two subjects.

Of the 10 genotype 3a virologic failure subjects, Y93H was observed in all 10 subjects at failure (7 had Y93H emerge post-treatment and 3 subjects had Y93H at baseline and post-treatment). [REDACTED] (b) (4)

Studies in Subjects with Decompensated Cirrhosis

In the ASTRAL-4 trial in subjects with decompensated cirrhosis who received [TRADENAME]+RBV for 12 weeks, 3 subjects (1 with genotype 1a and 2 with genotype 3a) qualified for resistance analysis due to virologic failure. No subjects with genotype 2 or 4 HCV infection in the [TRADENAME]+RBV 12 Weeks group experienced virologic failure.

The genotype 1 virologic failure subject had no NS5A or NS5B resistance substitutions at failure.

The 2 genotype 3a virologic failure subjects had NS5A resistance substitutions S38P and/or Y93H emerge at failure. One of these subjects also developed low levels (<5%) of NS5B nucleoside analogue inhibitor resistance substitutions N142T and E237G at failure.

In the ASTRAL-4 trial, 2 GT3a subjects treated with [TRADENAME] for 12 or 24 weeks without ribavirin had emergent NS5B S282T at low levels (<5%) along with L159F.

Persistence of Resistance-Associated Substitutions

No data are available on the persistence of sofosbuvir or velpatasvir resistance-associated substitutions. NS5A resistance-associated substitutions for other NS5A inhibitors have been found to persist for >1 year in [REDACTED] (b) (4) patients. The long-term clinical

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 SDN: 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

impact of the emergence or persistence of virus containing sofosbuvir or velpatasvir resistance-associated substitutions is unknown.

Effect of Baseline HCV Polymorphisms on Treatment Response

Analyses were conducted to explore the association between relapse rates and pre-existing baseline NS5A RAPs (any change from reference at NS5A amino acid positions 24, 28, 30, 31, 58, 92, or 93) identified by population or deep sequencing analysis at a sensitivity threshold of 15% or higher for subjects without cirrhosis or with compensated cirrhosis in ASTRAL-1 and ASTRAL-3 and subjects with decompensated cirrhosis in ASTRAL-4.

Studies in Subjects without Cirrhosis and Subjects with Compensated Cirrhosis

Among the subjects who received treatment with [TRADENAME] for 12 weeks, 18% ([REDACTED] (b) (4)) 63% (73/115), 9% (3 (b) (4)) and 83% (35/42) of subjects with genotype 1a, 1b, 2, 3, 4, 5 and 6 HCV, respectively, had baseline virus with NS5A resistance-associated polymorphisms (RAPs).

Genotype 1: Among the [REDACTED] (b) (4) genotype 1 subjects who had baseline NS5A RAPs, one subject (1%) with Q30R, L31M and H58P polymorphisms at baseline and compensated cirrhosis relapsed.

Genotype 3: Among the 56 genotype 3 subjects who had baseline NS5A RAPs, 4 subjects (7%) relapsed (3 with baseline Y93H and 1 with baseline A30K). Overall, [REDACTED] (b) (4) % (3/15) of genotype 3 subjects with the Y93H polymorphism at baseline relapsed.

For genotype 3 subjects with compensated cirrhosis, relapse rates were 33% (3/9) for subjects with baseline NS5A RAPs compared to 6% (4/71) for subjects without baseline NS5A RAPs.

Genotypes 2, 4, 5 and 6: The presence of baseline NS5A RAPs did not affect relapse rates for subjects with genotypes 2, 4, 5 and 6, because all achieved SVR12.

The NS5B nucleoside analog inhibitor resistance substitution S282T was not detected in the baseline NS5B sequence of any subject in Phase 3 trials. SVR12 was achieved in all 77 subjects who had baseline NS5B nucleoside analog inhibitor resistance polymorphisms including N142T, L159F, E/N237G, C/M289L/I, L320F/I/V, V321A/I, and S282G+V321I.

Studies in Subjects with Decompensated Cirrhosis

In ASTRAL 4, the prevalence of NS5A RAPs at baseline was 24% (48/198), 60% (6/10), 11% (4/37), and 63% (5/8) in GT1, GT2, GT3, and GT4 HCV subjects, respectively. No subjects with genotypes 2, 4 and 6 relapsed. There were no subjects with genotype 5 HCV infection in this trial.

For genotype 1 subjects, the overall relapse rates were numerically lower for the 12-week [TRADENAME] plus RBV arm (2%; 1/66) compared to [TRADENAME] 12-week and 24-week treatment arms. For subjects with NS5A RAPs, relapse rates were 0% (0/17) compared to 2% (1/49) for subjects without NS5A RAPs in the 12 week [TRADENAME] plus RBV containing arm.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530) VIROLOGY REVIEW

NDA: 208341 **SDN:** 000

DATE REVIEWED: 03/17/2015

Virology Reviewer: Lisa K. Naeger, Ph.D.

For genotype 3 subjects, overall relapse rates were numerically lower for the 12 week [TRADENAME] plus RBV (15% (2/13) compared to [TRADENAME] 12-week and 24-week treatment arms. There are insufficient data to determine the impact of HCV NS5A RAPs in genotype 3 subjects decompensated cirrhotics.

Three subjects in the [TRADENAME] plus RBV 12 week group had baseline NS5B nucleoside analog inhibitor polymorphisms (N142T and L159F) and all three subjects achieved SVR12.

Cross Resistance

Both sofosbuvir and velpatasvir were fully active against substitutions associated with resistance to other classes of direct-acting antivirals with different mechanisms of action, such as NS5B non-nucleoside inhibitors and NS3 protease inhibitors. The efficacy of [TRADENAME] has not been established in patients who have previously failed treatment with other regimens that include an NS5A inhibitor.

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/s/

LISA K NAEGER
03/29/2016

JULIAN J O REAR
03/29/2016

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

NDA#: 208341

Serial #: 0002

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

Sponsor's Name and Address:

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

Initial Submission Dates:

Correspondence Date: October 28, 2015
CDER Receipt Date: October 28, 2015
Assigned Date: October 29, 2015
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Amendments:

| SDN | Date Submitted | Date Received | Date Assigned |
|-----|----------------|---------------|---------------|
| 002 | 10/20/2015 | 10/20/2015 | 10/27/2015 |
| 017 | 02/24/2016 | 2/24/2016 | 3/2/2016 |

Related/Supporting Documents: IND115670, IND106739, NDA204671

| Product Names | Sofosbuvir (GS-7977) | Velpatasvir (GS-5816) |
|-------------------|--|---|
| Structures | (b) (4) | |
| 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 | (b) (4) |
| Molecular formula | C ₂₂ H ₂₉ FN ₃ O ₉ P | C ₄₉ H ₅₄ N ₈ O ₈ |
| Molecular weight | 529.46 | 883.00 |

Drug category: Antiviral

Indication: SOF/VEL is a fixed-dose combination of sofosbuvir, a hepatitis C virus (HCV) nucleotide analog inhibitor of NS5B polymerase, and velpatasvir, an HCV NS5A inhibitor, and is indicated for the treatment of HCV infection.

Dosage Form/Route of administration: Oral

Dispensed: Rx

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

Abbreviations: BL, baseline; DAA, direct acting antiviral; EC₅₀, effective concentration at 50%; FC, fold-change; FDA, Food and Drug Administration; FDC, fixed-dose combination; GT, genotype; HCV, hepatitis C virus; IC₅₀, inhibitory concentration at 50%; IFN, recombinant human interferon; LDV, ledipasvir; LFVD, Low Frequency Variant Detector; NGS, next generation sequencing; NRTIs, nucleoside reverse transcriptase inhibitors; PBL, peripheral blood lymphocytes; PVD, Probabilistic Variant Detector; PDVF, protocol defined virologic failure; PI, NS3/4A protease inhibitor; P/R, pegylated interferon/ribavirin; QbVD, Quality-based Variant Detector; RAP, resistance-associated polymorphism; RAS, resistance-associated substitution; RBV, ribavirin; SDM, site-directed mutants; SOF, sofosbuvir; SUBS, substitutions; SVR, sustained virologic response; SVR12, sustained virologic response at 12 week after end of treatment; VEL, velpatasvir; WT, wild-type.

Table of Contents

| | |
|--|-----------|
| EXECUTIVE SUMMARY | 3 |
| BACKGROUND AND SUMMARY | 4 |
| Rationale for Requesting and Analyzing NGS Data | 6 |
| NGS Data Analysis Pipeline | 7 |
| NGS Analysis Parameters and Overview of Data Analysis | 7 |
| NGS Analysis Pipeline Output | 8 |
| NGS Data Comparison | 9 |
| CLINICAL STUDIES | 9 |
| Resistance Analysis Population | 11 |
| ASTRAL-1: GS-US-342-1138 | 11 |
| ASTRAL-1 (GS-US-342-1138) Resistance Conclusions from the Sponsor..... | 13 |
| ASTRAL-1 (GS-US-342-1138) DAVP Analysis | 13 |
| ASTRAL-1 (GS-US-342-1138) DAVP conclusions: | 14 |
| ASTRAL 2: GS-US-342-1139 | 14 |
| ASTRAL-2 (GS-US-342-1139) Resistance Conclusions from the Sponsor..... | 15 |
| ASTRAL-2 (GS-US-342-1139) DAVP Analysis | 15 |
| ASTRAL-2 (GS-US-342-1139) DAVP conclusions: | 16 |
| ASTRAL 3: GS-US-342-1140 | 16 |
| ASTRAL-3 (GS-US-342-1140) Resistance Conclusions from the Sponsor..... | 19 |
| ASTRAL-3 (GS-US-342-1140) DAVP Analysis | 19 |
| ASTRAL-3 (GS-US-342-1140) DAVP Conclusions: | 19 |
| Astral 4: GS-US-342-1137 | 20 |
| ASTRAL-4 (GS-US-342-1137) Resistance Conclusions from the Sponsor..... | 23 |
| ASTRAL-4 (GS-US-342-1137) DAVP Analysis | 23 |
| ASTRAL-4 (GS-US-342-1137) DAVP Conclusions: | 25 |
| COMBINED RESISTANCE ANALYSIS..... | 26 |
| CONCLUSIONS..... | 28 |
| POST MARKETING RECOMMENDATIONS | 29 |
| ADMINISTRATIVE..... | 29 |
| Reviewer’s Signature(s) | 29 |
| Concurrence(s) | 29 |
| APPENDICES | 30 |
| METHODS..... | 30 |

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

EXECUTIVE SUMMARY

This review focused on the independent assessment of next generation sequencing (NGS) data provided in support of New Drug Application (NDA) 208341 for the fixed-dose combination (FDC) of sofosbuvir (SOF) and velpatasvir (VEL; SOF/VEL). The NDA for the SOF/VEL FDC is seeking an indication, with and without ribavirin (RBV), for the treatment of adult patients with chronic Hepatitis C virus (HCV) infection. SOF was previously approved for use in combination with other agents for the treatment of HCV infection (NDA 205834; Harvoni®; approved October 10, 2014). VEL is an investigational agent for which the sponsor is seeking approval for its use in combination with SOF in this NDA. Overall, assessment of the NGS data by the Division of Antiviral Products (DAVP) indicated that the data and analyses provided by the sponsor, Gilead Sciences (GSI), were acceptable and this NDA is approvable with respect to clinical virology.

Sofosbuvir is a nucleotide analog inhibitor of the HCV NS5B RNA-dependent RNA polymerase, which is required for HCV genome replication. The SOF prodrug is hydrolyzed by cellular esterases to a uridine analog monophosphate that is subsequently phosphorylated by cellular kinases to generate a uridine analog triphosphate inhibitor. The uridine analog is incorporated into the nascent HCV RNA by the NS5B polymerase and acts as a chain terminator. Velpatasvir is an inhibitor of the HCV NS5A protein, which is required for HCV replication. Resistance selection experiments in cell culture and cross-resistance studies indicate that VEL targets NS5A as its mode of action. The sponsor has shown that SOF and VEL have antiviral activity against HCV genotypes (GT) 1, 2, 3, 4, 5, and 6. The EC₅₀ values for SOF range from 15 to 264 nM against laboratory replicons and the EC₅₀ values for VEL range from 0.004 to 0.130 nM. Against clinical isolates, median EC₅₀ values range from 29 – 102 nM and 0.002 – 0.024 nM for SOF and VEL, respectively.

Four pivotal phase 3 clinical trials were conducted to determine the efficacy of the FDC of SOF/VEL. In ASTRAL-1 (GS-US-342-1138), SOF/VEL was compared with placebo for 12 weeks in treatment-naïve and treatment-experienced subjects with chronic genotype 1, 2, 4, 5, or 6 HCV infection. In ASTRAL-2 (GS-US-342-1139), 12 weeks of SOF/VEL was compared with 12 weeks of SOF+RBV in treatment-naïve and treatment-experienced subjects with chronic genotype 2 HCV infection. In ASTRAL-3 (GS-US-342-1140), 12 weeks of SOF/VEL was compared with 24 weeks of SOF+RBV in treatment-naïve and treatment-experienced subjects with chronic genotype 3 HCV infection. For the SOF/VEL FDC for 12 weeks in ASTRAL-1 (GS-US-342-1138), ASTRAL-2 (GS-US-342-1139), and ASTRAL-3 (GS-US-342-1140), the SVR12 rates were >95% across all HCV genotypes (GT1 (n=328), 98.5%; GT2 (n=238), 99.6%; GT3 (n=277), 95.3%; GT4 (n=116), 100%; GT5 (n=35), 97.1%, and GT6 (n=41), 100%). In ASTRAL-4 (GS-US-342-1137), a comparison was made between SOF/VEL±RBV for 12 weeks and SOF/VEL for 24 weeks in treatment-naïve and treatment-experienced subjects with chronic genotypes 1, 2, 3, 4, 5, or 6 HCV infection with Child-Pugh-Turcotte class cirrhosis. The overall SVR12 rate for subjects in the SOF/VEL+RBV 12 Week group was 94.3% (n=87), the SVR12 rate for subjects in the SOF/VEL 12 Week group was 83.3% (n=90), and the SVR12 rate for subjects in the SOF/VEL 24 Week group was 85.6% (n=90). For GT3 infected subjects in this study, the highest response rate was in the SOF/VEL+RBV 12 Week group (84.6% (n=13) compared to SVR12 rates of 50% (n=14) in the SOF/VEL 12 Week group and 50% (n=12) in the SOF/VEL 24 Week group.

Several amino acid positions have been associated with resistance to SOF and VEL. For SOF, substitutions at NS5B positions 159, 282, 320, and 321 are considered resistance-associated. Substitutions at other positions, some specific to a particular HCV genotype, have been identified and these are included in the SOF label. For VEL, the following NS5A positions were determined to be resistance-associated: 24, 28, 30, 31, 32, 58, 92, and 93. The goal of the independent assessment of NGS data was to confirm the results reported by the sponsor, to determine which known resistance-associated substitutions were present in the virus of subjects who failed treatment, and to determine if additional substitutions occurring in two or more subjects could be associated with treatment failure.

Overall, the NGS analyses results reported by the sponsor were in agreement with the results generated by DAVP, with a few exceptions. In general, most disagreements occurred at low frequency

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

where differences in program parameters likely contributed to these discrepancies. However, there were a few disagreements that occurred at high frequency at positions known to be resistance-associated and so, substitutions that occurred at known positions were counted if they were detected by any of the variant detectors used (including one by the sponsor and three by DAVP). For VEL resistance, the predominant resistance-associated substitutions that were detected across all four phase 3 trials were Y93H/N (n=28: 19 in GT3a, 5 in GT1a, 3 in GT1b, and 1 in GT1c), Q30R/H (GT1a and b) or A30A30V/K (GT3) (n=5: 3 in GT1a or b and 2 in GT3a), P58L/T/A (GT3a) or H58P/R/D (GT1a) (n=5, 3 in GT3a and 2 in GT1a) H85Y (GT3a) or S85N (GT1a) (n=4, 2 each for GT3a and GT1a) and M28V/T (n=3, 2 in GT3a and 1 in GT1a). For SOF resistance, the predominant resistance-associated substitutions that were detected across all four phase 3 trials were L159F (n=9, 7 in GT3a and 1 each in GT1a and 1b), S282N/T/G (n=6, 4 in GT3a and 1 each in GT1a and 1b), and L320F/I (n=4, all 4 in GT3a). Importantly, 6 L159F, 3 S282N, and 1 L320F resistance-associated substitutions were detected in subjects enrolled in the SOF+RBV 24 Week group of ASTRAL-3, indicating that longer exposure to SOF may produce more resistance-associated substitutions in those whole fail treatment.

Of note, using the SUBS10 criteria, resistance-associated substitutions L314F/I were detected in the NS5B polymerase protein of HCV viruses from four subjects infected with HCV GT3a who relapsed after treatment ended. Three of the four subjects were in the SOF/RBV 24 Week Group of ASTRAL-3 and were exposed to longer durations of SOF, which may have selected for this newly detected resistance-associated substitution. One treatment-naïve subject from the FDC 12 Week group of ASTRAL-3 also developed this substitution. Of note, this substitution was not detected in any subject at baseline but was detected at a frequency of greater than 30% at the final post-treatment timepoint. In addition, position L314 is highly conserved among HCV GT3 isolates with 100% conservation reported by the sponsor at that position. Low frequency analysis at this position identified one treatment-naïve subject (02127-64256) infected with HCV GT3a who relapsed in the SOF/VEL 12 Week group of ASTRAL-4 (GS-US-342-1137) whose virus had L314P at 3.5% at the post-treatment timepoint. Clinical virology will add this resistance-associated substitution to the label and make a Post Marketing Request to have the sponsor determine the phenotype of L314F and L314I in an HCV GT3a replicon.

BACKGROUND AND SUMMARY

Sofosbuvir is a uridine nucleotide analog inhibitor of the HCV NS5B RNA-dependent RNA polymerase, which is required for HCV genome replication. The SOF prodrug is hydrolyzed by cellular esterases to a uridine analog monophosphate that is subsequently phosphorylated to generate a uridine analog triphosphate inhibitor. The uridine analog is incorporated into the nascent HCV RNA by the NS5B polymerase and acts as a chain terminator.

Velpatasvir (VEL) is an inhibitor of the HCV NS5A protein, which is an HCV protein that is required for HCV replication, although its exact function in the RNA genomic replication process is not fully understood. Resistance selection experiments in cell culture and cross-resistance studies indicate velpatasvir targets NS5A as its mode of action. The sponsor has shown that SOF and VEL have antiviral activity against HCV GTs 1, 2, 3, 4, 5, and 6. The EC₅₀ values for SOF range from 15 to 264 nM against laboratory replicons. EC₅₀ values for VEL range from 0.004 to 0.130 nM against laboratory replicons. Against clinical isolates, median EC₅₀ values range from 29 – 102 nM and 0.002 – 0.024 nM for SOF and VEL, respectively.

Several amino acid positions have been associated with resistance to SOF and VEL. For SOF, substitutions at NS5B positions 159, 282, 320, and 321 are considered resistance-associated. Substitutions at other positions, some specific to a particular HCV genotype, have also been identified and these are included in the label for SOF. For VEL, the following NS5A positions were determined to be resistance-associated: 24, 28, 30, 31, 32, 58, 92, and 93.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

Next generation sequencing of the HCV NS5A and NS5B (and in some cases NS3/4A) genes was performed for all subjects at baseline and at the timepoint closest to failure for all subjects who relapsed or otherwise met the criteria for treatment failure in four pivotal phase 3 clinical studies. These studies included ASTRAL-1 (GS-US-342-1138), ASTRAL-2 (GS-US-342-1139), ASTRAL-3 (GS-US-342-1140), and ASTRAL-4 (GS-US-342-1137) (Table 1). The sponsor provided the NGS data as part of their resistance analysis for the four clinical trials (Table 1).

Table 1. Phase 3 SOF/VEL studies analyzed for resistance by NGS (Table 1, page 10, Summary of Clinical Efficacy).

| Study Number | Study Design | Treatment Regimen ^a | Subject Population | | | Location of Study Summary and CSR |
|---|---|---|--|--|--|---|
| | | | HCV Genotype (N) ^b | Prior HCV Treatment | Cirrhosis Status | |
| SOF/VEL Phase 3 Efficacy Studies | | | | | | |
| GS-US-342-1138 (ASTRAL-1) | Phase 3, randomized, double-blind, placebo-controlled multicenter study | SOF/VEL or placebo for 12 weeks | 1, 2, 4, 5, or 6 (Genotype 1: 393; Genotype 2: 125; Genotype 4: 138; Genotype 5: 35; Genotype 6: 49) | Treatment-naïve and treatment-experienced subjects | Up to 20% of subjects may have had cirrhosis | Section 2.3.1; m5.3.5.1, GS-US-342-1138 Interim CSR |
| GS-US-342-1139 (ASTRAL-2) | Phase 3, randomized, active-controlled, open-label multicenter study | SOF/VEL or SOF+RBV for 12 weeks | 2 (266) | Treatment-naïve and treatment-experienced subjects | Up to 20% of subjects may have had cirrhosis | Section 2.3.2; m5.3.5.1, GS-US-342-1139 Interim CSR |
| GS-US-342-1140 (ASTRAL-3) | Phase 3, randomized, active-controlled, open-label multicenter study | SOF/VEL for 12 weeks or SOF+RBV for 24 weeks | 3 (552) | Treatment-naïve and treatment-experienced subjects | Up to 20% of subjects may have had cirrhosis | Section 2.3.3; m5.3.5.1, GS-US-342-1140 Interim CSR |
| SOF/VEL Phase 3 Special Population Study: HCV-Infected Subjects with Decompensated Cirrhosis | | | | | | |
| GS-US-342-1137 (ASTRAL-4) | Phase 3, randomized, open-label multicenter study | SOF/VEL for 12 weeks with or without RBV, or SOF/VEL for 24 weeks | 1, 2, 3, 4, or 6 (Genotype 1: 207; Genotype 2: 12; Genotype 3: 39; Genotype 4: 8; Genotype 6: 1) | Treatment-naïve and treatment-experienced subjects | All subjects had decompensated cirrhosis (CPT class B) | Section 2.4.1; m5.3.5.1, GS-US-342-1137 Interim CSR |

BID = twice daily; CPT = Child-Pugh-Turcotte; Peg-IFN = pegylated interferon; QD = once daily

a The dose for SOF/VEL FDC was 400/100 mg once daily, the dose for RBV was 1000 or 1200 mg/day divided BID (ie, for subjects who weighed < 75 kg, the dose of RBV was 1000 mg/day divided BID; and for subjects who weighed ≥ 75 kg, the RBV dose was 1200 mg/day divided BID), and the dose for Peg-IFN was 180 µg weekly.

b N = Subjects who received at least 1 dose of study drug, unless otherwise specified.

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 (not all cases), NS5A, and NS5B) for each subject at baseline and near the time of failure for all treatment failure samples that were successfully sequenced using Illumina technology; 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 three 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.
4. Previous reviews of NGS data have identified cases of amplification artifacts and cross-contamination of large numbers of samples evaluated in parallel indicating that sample preparation errors are also a concern.

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 this information 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 and which only identifies minority populations present as a high percentage of the 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 the appropriate HCV reference sequence. Three 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).

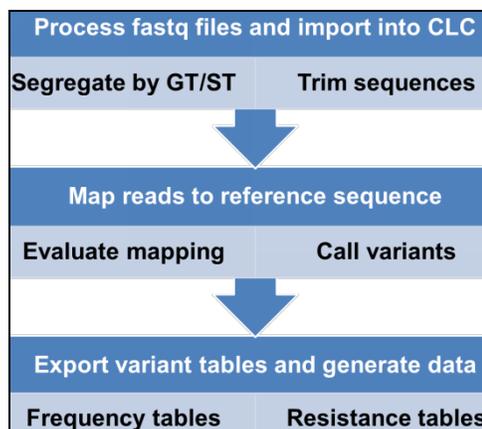


Figure 1. Overview of the NGS analysis Pipeline using CLC Genomics Workbench (DAVP Analysis).

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 sofosbuvir 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; AF011753), GT1b (Con1; AJ238799), HCV GT1c (HCG9; D14853), HCV GT2a (JFH1; AB047639), HCV GT2b (MD2b1; AF238486), and GT3a (S52; GU814263) 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, three algorithms were used to call variants based on independent criteria, and variant tables were generated for each sequence run and variant detection method. Of note, previously, only two variant detection methods were used; however, in CLC Genomics Workbench, version 7.5, an additional low frequency variant detection caller was added and so variants from this algorithm were also compared in this analysis. The variant tables included the following column headers: Reference Position, Type, Length Reference, Allele Linkage, Zygoty, Count Coverage, Frequency, Forward/reverse balance, Average quality, Overlapping annotations, Coding region change, and Amino acid change. The three 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 three detection methods were:
 - a. **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.
 - b. **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.
 - c. **Low Frequency Variant Detection (LFVD)** – according to the CLC Genomics Workbench Manual: *“a statistical test is performed at each site to determine if the nucleotides observed in the reads at that site could be due simply to sequencing errors, or if they are significantly better explained by there being one (or more) alleles than the reference present in the sample at some unknown frequency. If the latter is the case, a variant corresponding to the significant allele will be called, with estimated frequency”.*
 - d. **Frequency tables** – Tables were generated by exporting the variant tables for all three variant detection methods (LFVD, PVD75, and QBVD) for each mapping and then reformatting the data to reflect variation at the amino acid level with these pertinent changes:
 - i. The variant tables were combined by genotype/subtype and study
 - ii. The variant tables were filtered to remove synonymous substitutions
 - iii. The variant tables were reformatted to be directly comparable to the frequency tables submitted by the sponsor
5. **Generating resistance analysis tables.** Excel macros were 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:

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

- a. **SUBS10 criteria** – Identified all substitutions that were not detected at baseline (<0.01 frequency) but were detected at a frequency of 10% (0.10) or greater at later timepoints or detected at baseline at a frequency of 10% (0.10) and not detected at later timepoints.
- b. In addition, known resistance-associated substitutions that have been identified in NS5A and NS5B were analyzed at the 1% frequency, given that amino acid replacements at these sites may represent viral populations that are of low fitness and therefore may rapidly be overgrown once treatment stops. For NS5A, low frequency analysis was conducted for positions 24, 28, 30, 31, 32, 38, 58, 92, and 93. For NS5B, low frequency analysis was conducted for positions 159, 282, 316, 320, 321, and 355. NS5B position 314 was included in the low frequency analysis when it was determined to be a potentially resistance-associated site.

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 four algorithms (the sponsor's algorithm (GIL) and LFVD, 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 four pivotal phase 3 clinical trials and three phase 2 trials that were provided to support the NDA for the NS5B uridine nucleotide analog inhibitor sofosbuvir and the NS5A inhibitor velpatasvir (SOF/VEL) FDC tablet. This portion of the virology NDA review focused exclusively on the independent analysis of NGS data from treatment failures from the phase 3 clinical trials. The primary review of the 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 208341 SDN 000).

The NGS data submitted for these 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 3,854 NGS files for the four clinical trials for 1,825 subjects, with study GS-US-342-1138 (ASTRAL 1) containing the most subjects sequenced (n=740) and the most NGS files (n=1510) (Table 2).

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

Table 2. Next Generation Sequencing data submitted for NDA 208341 (DAVP Analysis).

| Trial Name | Protocol Number | Total Subjects | Treatment Failures | Total NGS Files | NGS Files Analyzed |
|--------------------------------------|-----------------|----------------|---|-----------------|--------------------|
| ASTRAL 1 | GS-US-342-1138 | 740 | 2 | 1510 | 6 |
| ASTRAL 2 | GS-US-342-1139 | 266 | 6 | 546 | 14 |
| ASTRAL 3 | GS-US-342-1140 | 552 | 11 ^a /39 ^b | 1222 | 105 |
| ASTRAL 4 | GS-US-342-1137 | 267 | 11 ^c /3 ^d /8 ^e | 576 | 44 |
| ^a SOF/VEL 12 Week Arm | | 1825 | 80 | 3854 | 169 |
| ^b SOF+RBV 24 Week Arm | | | | | |
| ^c SOF/VEL 12 Week Arm | | | | | |
| ^d SOF/VEL+RBV 12 Week Arm | | | | | |
| ^e SOF/VEL+RBV 24 Week Arm | | | | | |

The sponsor submitted a Virology Study Report that described the individual and integrated resistance analyses results for the four phase 3 clinical studies that evaluated the SOF/VEL FDC in treatment-naïve and treatment-experienced subjects chronically infected with HCV genotype 1 through 6. Table 3 presents an overview of the clinical studies included in this virology report.

Table 3. Clinical studies included in the Virology Study Report (Table 2-1, page 15, Integrated Virology Report).

| Study | Study Design | Treatment Regimen ^a | Subject Population | | |
|---|--|--|--|--|--|
| | | | HCV Genotypes (N) ^b | Prior HCV Treatment | Cirrhosis Status |
| SOF/VEL Phase 3 Efficacy Studies | | | | | |
| GS-US-342-1138 (ASTRAL-1) | Phase 3, Multicenter, Randomized, Double Blind, Placebo Controlled Study | SOF/VEL FDC for 12 weeks or SOF/VEL Placebo for 12 weeks | 1, 2, 4, 5, or 6 (GT 1: n = 393; GT 2: n = 125; GT 4: n = 138; GT 5: n = 35; GT 6: n = 49) | Treatment-naïve and treatment experienced subjects | Up to 20% of subjects may have had cirrhosis |
| GS-US-342-1139 (ASTRAL-2) | Phase 3, Multicenter, Randomized, Open-Label Study | SOF/VEL FDC for 12 weeks or SOF+RBV for 12 weeks | 2 (GT 2: 266) | Treatment-naïve and treatment experienced subjects | Up to 20% of subjects may have had cirrhosis |
| GS-US-342-1140 (ASTRAL-3) | Phase 3, Multicenter, Randomized, Open-Label Study | SOF/VEL FDC for 12 weeks or SOF+RBV for 24 weeks | 3 (GT 3: n = 552) | Treatment-naïve and treatment experienced subjects | Up to 20% of subjects may have had cirrhosis |
| SOF/VEL Phase 3 Special Population Study: HCV-Infected Subjects with Decompensated Cirrhosis | | | | | |
| GS-US-342-1137 (ASTRAL-4) | Phase 3, Multicenter, Randomized, Open-Label Study | SOF/VEL FDC for 12 weeks or SOF/VEL FDC + RBV for 12 weeks or SOF/VEL FDC for 24 weeks | 1, 2, 3, 4, or 6 (GT 1: n = 207; GT 2: n = 12; GT 3: n = 39; GT 4: n = 8; GT 6: n = 1) | Treatment-naïve and treatment experienced subjects | All subjects had decompensated cirrhosis (CPT class B) |

SOF/VEL = sofosbuvir/velpatasvir; CPT = Child-Pugh-Turcotte

a The dose for SOF/VEL FDC was 400/100 mg once daily, the dose for RBV was 1000 or 1200 mg/day divided twice daily (ie, for subjects who weighed < 75 kg, the dose of RBV was 1000 mg/day divided twice daily; and for subjects who weighed ≥ 75 kg, the RBV dose was 1200 mg/day divided twice daily), and the dose for Peg-IFN was 180 µg weekly.

b N = Subjects who received at least 1 dose of Study drug, unless otherwise specified.

Source: SOF/VEL FDC m2.7.3, Section 1.2

Resistance Analysis Population

The Virology Analysis Plan for all phase 2 and phase 3 studies evaluating SOF/VEL-containing regimens was previously submitted by the sponsor (PC-342-2001) and a mock NGS data set for this submission was reviewed (IND 118605 SDN 081).

The sponsor explained that deep nucleotide sequence analysis of HCV NS5A and NS5B genes was performed at baseline for all subjects enrolled in the studies listed in Table 3, and that the NS3/4A gene was also subjected to deep sequencing at baseline for subjects with previous NS3 protease inhibitor (PI) treatment experience. The NGS data for the NS3/4A gene was not evaluated.

The sponsor reported the following criteria for resistance testing: Resistance Analysis will be performed for any subject in the Resistance Analysis Population with HCV RNA >1000 IU/mL, and the Resistance Analysis Population would include any subject in the Safety Analysis Set who received at least 1 dose of a SOF/VEL-containing regimen and had a virologic outcome based on the following criteria:

1. SVR12
 - a. SVR12: HCV RNA <lower limit of quantitation (LLOQ) 12 weeks after the completion of treatment
2. Virologic Failure (VF)
 - a. Relapse
 - i. Virologic Relapse: HCV \geq LLOQ during the post-treatment period after having achieved <LLOQ at the end of treatment, confirmed with 2 consecutive values or last available post-treatment measurement
 - b. On-Treatment VF
 - i. Virologic Breakthrough: $>1 \log_{10}$ IU/mL increase in HCV RNA over nadir or HCV RNA above the lower limit of quantitation (LLOQ) after having previously had HCV RNA <LLOQ during the on treatment period, confirmed with 2 consecutive values (second confirmation value could be post-treatment)
 - ii. Virologic Rebound: $>1 \log_{10}$ IU/mL increase in HCV RNA from nadir while on treatment, confirmed with 2 consecutive values (second confirmation value could be post-treatment), or last available on-treatment measurement with no subsequent follow-up value
 - iii. Non-Responsive: HCV RNA \geq LLOQ through 8 weeks of treatment

As part of the resistance analysis, the sponsor included deep sequencing of the HCV NS5A and NS5B genes at post-treatment time points for subjects who experienced virologic failure. In addition, phenotypic analysis was attempted for the majority of subjects with virologic failure and was successful for a subset of subjects.

ASTRAL-1: GS-US-342-1138

The sponsor reported that a total of 740 treatment-naïve and treatment-experienced subjects were enrolled in ASTRAL-1 and received study drug in a double-blind manner in one of the following treatment groups: 1) SOF/VEL FDC (400/100 mg) once daily for 12 weeks (N = 624) and 2) Placebo once daily for 12 weeks (N = 116). The sponsor reported that of the 6 of 624 subjects (1.0%) who did not achieve SVR12 in the SOF/VEL 12 Week group, only two subjects had virologic failure due to relapse:

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

- Subject 00529-63184, who was infected with HCV GT1a, was treatment-naive without cirrhosis and was determined to have relapsed at the post-treatment Week 4 visit.
- Subject 00529-63312, who was determined to be infected by HCV GT1b by LiPA/TRUGENE, and then later determined to be infected with HCV GT1c/1h by sequence-based BLAST (described below), was treatment-experienced (TE) with cirrhosis and was determined to have relapsed at the post-treatment Week 4 visit.

The sponsor reported that of the 624 subjects in the SOF/VEL 12 Week group, four subjects did not achieve SVR12 with a virologic outcome listed as “other” as defined in the Statistical Analysis Plan (SAP), and these subjects were not included in the Resistance Analysis Population. The Resistance Analysis Population included 620 subjects.

ASTRAL-1 (GS-US-342-1138) Virologic Failure Analyses

HCV Sequence and Phenotypic Analyses in Subjects with Virologic Failure

The sponsor reported that a total of two subjects in the SOF/VEL 12 Week arm experienced virologic failure and both subjects were determined to have relapsed post-treatment. Table 4 presents the results from the baseline and post-treatment nucleotide sequence analysis of the NS5A and NS5B genes and the sponsor’s analysis for these two subjects. The sponsor reported that both subjects had baseline NS5A resistance-associated amino acid polymorphisms Q30R or Q30R/L + L31M and developed additional NS5A resistance-associated amino acid substitutions Y93N or Y93H at relapse. The NS5A resistance-associated substitutions at post-treatment time points were Y93N in the subject infected with HCV GT1a and Q30R+ L31M/I/V+Y93H in the subject infected with HCV GTa1c/1h. The sponsor also reported that there were no NS5B resistance-associated polymorphisms or substitutions observed in these two subjects at baseline or at relapse. These are the details that the sponsor reported for these two subjects:

1. Subject 00529-63184 (treatment-naive, non-cirrhotic, HCV GT1a)
 - a. This subject had Q30R (2.6%) at baseline. Q30R as a full site-directed mutant conferred a 2.2-fold change in EC₅₀ value to VEL in a HCV GT1a replicon. At relapse, Q30R was no longer detectable; however, the Y93N resistance-associated substitution that conferred a 805-fold change from wild type reference in EC₅₀ value to VEL emerged in >99% of the viral population at the time of relapse (post-treatment Week 4) and was maintained through post-treatment Week 12 (Table 4). No NS5B resistance-associated polymorphisms (RAPs) or resistance-associated substitutions were observed at baseline or relapse in this subject. No phenotypic change (<1.3-fold) in susceptibility to SOF was observed for this subject at baseline or relapse time points.
2. Subject 05294-63312 (treatment-experienced, cirrhotic, HCV GT1c/1h)
 - a. This subject was determined to have HCV GT1b infection by LiPA/TRUGENE assay at screening; however, sequencing-based BLAST analyses of the NS3/4a, NS5A, and NS5B sequences indicated that this subject was infected with HCV GT1c/1h. The baseline virus in this subject had Q30L (1.1%), Q30R (98.7%), and L31M (>99%) NS5A resistance-associated polymorphisms with 1.4-fold shift from wild-type HCV GT1a reference in EC₅₀ value to VEL. At relapse, this subject had HCV NS5A Q30R (>99%), L31M (>99%) and emergent Y93H that conferred 763-fold change in EC₅₀ value to VEL (from the HCV GT1a reference). At post-treatment Week 12, L31V/I minor variants were also detected in the viral population of this subject, indicating evolution of the L31M NS5A variant (Table 4). No NS5B RAPs or resistance-associated substitutions were observed at baseline or relapse in this subject. No phenotypic analysis of NS5B was done as only amino acids 1-325 of NS5B were amplified for baseline and relapse samples for this subject.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

Table 4. ASTRAL-1 (GS-US-342-1138) subjects with virologic failure following treatment with SOF/VEL for 12 weeks (1% cutoff) (Table 3-15, page 41, Integrated Virology Report).

| Subject | GT | NS5A | | | | NS5B | |
|-------------|-------|---|------------------------------|---|------------------------------|---------------------------|---|
| | | Baseline | | Posttreatment | | Baseline | Posttreatment |
| | | Variants (%) ^a | VEL FC from Ref ^b | Variants (%) ^a | VEL FC from Ref ^b | Variants (%) ^a | Posttreatment Variants (%) ^a |
| 00529-63184 | 1a | Q30R (2.6%) | 0.81 | FU-4: Y93N (>99%) | 805 | None | FU-4: None |
| | | | | FU-12: Y93N (>99%) | ND | | FU-12: None |
| 05294-63312 | 1c/1h | Q30L (1.1%) Q30R (98.7%) L31M (>99%) H58P (>99%) | 1.4 | FU-4: Q30R (>99%) L31M (>99%) H58P (>99%) Y93H (99.0%) | 763 | None | FU-4: None |
| | | | | FU-12: K24M (1.6%) K24T (1.3%) Q30R (>99%) L31I (2.8%) L31M (88.4%) L31V (8.6%) H58P (>99%) Y93H (72.3%) | ND | | FU-12: None |

FC = fold-change; Ref = wild-type reference control; BL = baseline; GT = genotype; FU-x = follow-up visit at x weeks after discontinuing treatment; ND = not determined

a For NS5A and NS5B NI variants, RAPs are indicated in plain and RAVs are indicated in **bold**.

b Phenotypic fold change of EC₅₀ compared with wild-type reference control or Baseline.

Source: Appendix; PC-342-2003 Phase 3 iVSR [Virology Listings 5, 6, and 7](#)

ASTRAL-1 (GS-US-342-1138) Resistance Conclusions from the Sponsor

The sponsor concluded that deep sequence analyses indicated that subjects in the SOF/VEL 12 Week arm had a diverse population of HCV with more than 30 subtypes across genotypes 1, 2, 4, 5, 6, and 7.

Overall, approximately 41.7% (257 of 616) and 8.7% (52 of 599) of subjects in the SOF/VEL 12 Week arm had baseline NS5A resistance-associated polymorphisms and NS5B resistance-associated polymorphisms, respectively. Baseline NS5A or NS5B resistance-associated polymorphisms had no impact on SVR12, with high SVR12 rates (99.6% to 100%) observed across all subtypes/genotypes regardless of the presence of NS5A resistance-associated polymorphisms or NS5B resistance-associated polymorphisms. Two subjects had virologic failure and both had baseline NS5A resistance-associated polymorphisms, but with no reduced susceptibility to VEL at baseline (as determined by cell culture based phenotypic assays). At the virologic failure time points, both subjects developed NS5A resistance-associated substitutions (Y93N and Y93H) that conferred high-level resistance to VEL (>700 fold-change in EC₅₀ value). No NS5B resistance-associated polymorphisms were detected at baseline or post-treatment in either subject with virologic failure.

ASTRAL-1 (GS-US-342-1138) DAVP Analysis

For the most part, the DAVP analysis of data from ASTRAL-1 (GS-US-342-1138) was consistent with the results reported by the sponsor (Table 5). For subject 00529-63184, who was infected with HCV GT1a, NS5A resistance-associated polymorphisms Q30R and L31Q were detected at low frequency at baseline and Y93N emerged at high frequency at later timepoints (Table 5). No other substitutions appeared to be associated with resistance for this subject.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

For subject 05294-63312, who was infected with HCV GT1c/1h, NS5A resistance-associated polymorphisms Q30R, Q30L, and L31M were detected at low frequency at baseline and Y93H emerged at high frequency at later timepoints (Table 5). Of note, the sponsor reported that H58P was detected at baseline and at Follow-up Weeks 4 and 12 at greater than 99% frequency at all of these timepoints (Table 4); however, this substitution was not detected by the three variant detection algorithms used by DAVP (Table 5). For NS5B, the three DAVP variant detectors detected a D62N polymorphism at baseline at a frequency >99% and this substitution was maintained at that frequency at Follow-up Weeks 4 and 12. NS5B substitution D62N was identified in the SOF liver study as a substitution of interest, and it is possible that this polymorphism at baseline contributed to the failure of SOF in this regimen for this subject infected with HCV GT1c/1h.

Table 5. Comparison of NGS results for ASTRAL-1 (GS-US-342-1138) treatment failures (DAVP analysis). Only substitutions detected by multiple DAVP variant callers are shown unless the substitution was detected at a site of interest. BL, polymorphisms detected at high frequency at baseline (>10%); SUBS10, substitutions meeting the treatment-emergent criteria explained in the text; LF SUB, low frequency substitution (<10%). Variant detectors shown in the AGREE column were defined as: G, Gilead; L, Low Frequency Variant Detector; P, Probabilistic Variant Detector; Q, Quality-based Variant Detector.

| STUDY | ARM | HCV GT | GENE | BL | SUBS10 | LF SUB | No. SUBJ | AGREE | |
|----------|--------|--------|-------|--------|--------|-----------|----------|-------|------|
| ASTRAL-1 | FDC12W | 1a | NS5A | | Y93N | | 1 | GLPQ | |
| | | | | | | Q30R | 1 | GQ | |
| | | | | | | L31Q | 1 | Q | |
| | | | | | | E32Y | 1 | G | |
| | | | | | | T213A | 1 | GLQ | |
| | | | | | | A377T | 1 | GLQ | |
| | | | NS5B | | F101L | | 1 | GPQ | |
| | | | | E202G | | 1 | GLPQ | | |
| | | | | D444N* | | 1 | LP | | |
| | | | 1c/1h | NS5A | | Y93H | | 1 | GLPQ |
| | | | | | | K24T/K24M | 1 | GLQ | |
| | | | | | | Q30L/R | 1 | GLPQ | |
| | | | | | | L31V/M/I | 1 | GLPQ | |
| | | | | | | L153V | 1 | LPQ | |
| | | | | | | E171D | 1 | LQ | |
| | | V442T | | | 1 | G | | | |
| | NS5B | | D62N | | 1 | LPQ | | | |
| | | | N231A | 1 | LPQ | | | | |
| | | | V253I | 1 | LPQ | | | | |

* Includes L443N444delinsLD

ASTRAL-1 (GS-US-342-1138) DAVP conclusions: In general, there was agreement between the variants detected by the sponsor and those detected by DAVP. Of the two subjects who had virologic failure, both had baseline NS5A substitutions, but both subjects developed NS5A resistance-associated substitutions Y93N and Y93H that likely lead to failure of VEL. For subject 05294-63312, who was infected with HCV GT1c/1h, a D62N polymorphism in NS5B was detected at >99% frequency and it is possible that this polymorphism at baseline contributed to the failure of SOF in this regimen for this subject.

ASTRAL 2: GS-US-342-1139

The sponsor reported that a total of 266 treatment-naïve and treatment-experienced subjects were randomized into two cohorts in this clinical trial: 1) 134 subjects in the SOF/VEL 12 Week arm and 2) 132 subjects in the SOF+RBV 12 Week arm. In the SOF/VEL 12 Week arm, 1 of 134 subjects (0.7%) (The one subject was categorized as “other”) did not achieve SVR12. No subject had virologic failure

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

due to relapse. In the SOF+RBV 12 Week group, 8 of 132 subjects (6.1%) did not achieve SVR12. Six subjects had virologic failure due to relapse. Two additional subjects (categorized as “other”) did not achieve SVR12.

According to the sponsor, the Resistance Analysis Population was defined as all subjects in the SOF/VEL 12 Week and SOF+RBV 12 Week arms in the Safety Analysis Set with a virologic outcome. Of the 266 subjects, 3 subjects with virologic outcome of “other” as defined in the SAP were not included in the Resistance Analysis Population. The Resistance Analysis Population included 263 subjects, 133 in the SOF/VEL 12 Week group and 130 in the SOF+RBV 12 Week group.

Resistance analysis was performed for the six subjects who failed treatment with SOF+RBV for 12 Weeks due to relapse, and five of these subjects were infected with HCV GT2b and one with HCV GT2a. The L159F resistance-associated substitution emerged on-treatment and was detected at low frequency in two of these subjects (one infected with HCV GT2a and one with HCV GT2b) at post-treatment follow-up (Table 6).

Table 6. ASTRAL-2 (GS-US-342-1139) subjects with virologic failure following treatment with SOF+RBV for 12 weeks (1% cutoff) (Table 4-10, page 50, Integrated Virology Report).

| Subject | HCV GT | TN or TE | Cirrhotic | NS5B | |
|-------------|--------|----------|-----------|---------------------------|---------------------------|
| | | | | Baseline | Posttreatment |
| | | | | Variants (%) ^a | Variants (%) ^a |
| 05518-65054 | 2b | TN | No | None | None |
| 02760-65165 | 2b | TN | Yes | None | None |
| 01543-65238 | 2b | TE | No | None | L159F (2.0%) |
| 04371-65253 | 2a | TN | No | None | L159F (1.1%) |
| 01543-65167 | 2b | TE | No | None | None |
| 02760-65132 | 2b | TE | No | None | None |

GT = genotype; RAP = resistance-associated polymorphism; RAV = resistance-associated variant; TE = treatment experience; TN = treatment naïve

a For NS5A and NS5B NI variants, RAPs are indicated in plain and RAVs are indicated in **bold**.

ASTRAL-2 (GS-US-342-1139) Resistance Conclusions from the Sponsor

Deep sequence analyses indicated that HCV GT2a and 2b were the predominant HCV subtypes in subjects who were randomized and treated in this study. Approximately 60.2% (80 of 133) and 9.8% (13 of 133) of subjects in the SOF/VEL 12 Week group had baseline NS5A resistance-associated polymorphisms and NS5B resistance-associated polymorphisms, respectively. Despite the presence of baseline NS5A resistance-associated polymorphisms and NS5B resistance-associated polymorphisms, no subjects in the SOF/VEL 12 Week group experienced virologic failure. Among the 6 subjects who relapsed in the SOF+RBV 12 Week group, low levels of the NS5B resistance-associated substitution L159F emerged in 2 of 6 subjects.

ASTRAL-2 (GS-US-342-1139) DAVP Analysis

In general, there was agreement between the variants detected by the sponsor and those detected by DAVP. NS5B L159F was detected in two subjects (one infected with HCV GT2a and one with HCV GT2b) at post-treatment follow-up Week 4 (Table 6). However, three additional NS5B polymorphisms of interest were detected by DAVP, including a A252V polymorphism in the virus of two subjects infected with HCV GT2b; an S62A polymorphism at baseline in the virus of one subject infected with HCV GT2b; and R355I was detected at ~1% in the virus of the subject infected with HCV GT2b whose virus had L159F emerge at low frequency (Table 7). Of note, NS5B amino acid positions 62 and 355 were previously identified by DAVP as potential resistance-associated sites.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

Table 7. Comparison of NGS results for ASTRAL-2 (GS-US-342-1139) treatment failures (DAVP analysis). Only substitutions detected by multiple DAVP variant callers are shown unless the substitution was detected at a site of interest. BL, variants detected at high frequency at baseline (>10%); SUBS10, substitutions meeting the treatment-emergent criteria explained in the text; LF SUB, low frequency substitution (<10%). Variant detectors shown in the AGREE column were defined as: G, Gilead; L, Low Frequency Variant Detector; P, Probabilistic Variant Detector; Q, Quality-based Variant Detector.

| STUDY | ARM | HCV GT | GENE | BL | SUBS10 | LF SUB | No. SUBJ | AGREE |
|---------|-------|--------|------|----|--------|--------|----------|-------|
| ASTRAL2 | SR12W | 2a | NS5B | | | L159F | 1 | GLQ |
| | | 2B | NS5B | | | L159F | 1 | G |
| | | | | | | R355I | 1 | GLQ |
| | | | | | A252V | | 2 | GLPQ |
| | | | | | S62A | | 1 | GL |

ASTRAL-2 (GS-US-342-1139) DAVP conclusions: In general, there was agreement between the polymorphisms/substitutions detected by the sponsor and those detected by DAVP. L159F was detected in two subjects (one infected with HCV GT2a and one with HCV GT2b) at post-treatment follow-up Week 4 and likely contributed to the failure of SOF in this regimen. In addition, three additional NS5B polymorphisms of interest were detected by DAVP, including an A252V polymorphism in the virus of two subjects infected with HCV GT2b; an S62A polymorphism at baseline in the virus of one subject infected with HCV GT2b; and R355I detected at ~1% in the virus of the subject infected with HCV GT2b whose virus had L159F emerge at low frequency. These polymorphisms/substitutions may have contributed to the failure of SOF in this regimen.

ASTRAL 3: GS-US-342-1140

The sponsor reported that ASTRAL 3 is an ongoing Phase 3, randomized, open-label multicenter study designed to assess the antiviral efficacy, safety, and tolerability of 12 weeks of SOF/VEL treatment compared with 24 weeks of SOF+RBV treatment in treatment-naïve and treatment-experienced subjects with chronic HCV GT3 infection. The sponsor reported that a total of 552 subjects were randomized in a 1:1 ratio into one of the following treatment groups:

- 1. SOF/VEL 12 Week group (Group 1):** SOF/VEL FDC (400/100 mg) Tablet once daily for 12 weeks (N = 277)
- 2. SOF+RBV 24 Week group (Group 2):** SOF (400 mg) Tablet once daily + RBV (1000 or 1200 mg/day divided twice daily) Tablets for 24 weeks (N = 275)

The sponsor reported that in the SOF/VEL 12 Week group, 13 of 277 subjects (4.7%) did not achieve SVR12. Of these, none of the subjects experienced on-treatment virologic failure, but 11 subjects had virologic failure due to relapse and 2 additional subjects (categorized as “other”) did not achieve SVR12. In the SOF+RBV 24 Week group, 54 of 275 subjects (19.6%) did not achieve SVR12. Of these, one subject was categorized as an on-treatment virologic failure (nonresponsive), 38 subjects had virologic failure due to relapse, and 15 additional subjects (categorized as “other”) did not achieve SVR12.

The sponsor stated that the Resistance Analysis Population was defined as all subjects in the SOF/VEL 12 Week and SOF+RBV 24 Week groups in the Safety Analysis Set with a virologic outcome. Of the 552 subjects, 17 subjects (SOF/VEL 12 Week group, n = 2; SOF+RBV 24 Week group, n=15) with virologic outcome of “other” as defined in the SAP were not included in the Resistance Analysis Population. The Resistance Analysis Population included 275 subjects in the SOF/VEL 12 Week group and 260 subjects SOF+RBV 24 Week group.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

In the SOF/VEL 12 Week group, 11 of 275 (4.0%) subjects experienced virologic failure, including 10 subjects who relapsed and 1 subject who was reportedly re-infected with a different genotype of HCV.

Table 8 presents the baseline and post-treatment nucleotide sequence analysis of the NS5A and NS5B genes for these 11 subjects. One subject (Subject 01069-62225) had genotype 3a HCV infection at baseline and had genotype 1a HCV infection at virologic failure as determined by NS5B sequencing, which the sponsor concluded that this subject likely experienced reinfection and not a relapse of baseline virus. According to the sponsor, this subject did not have NS5A or NS5B baseline resistance-associated polymorphisms or post-treatment resistance-associated substitutions.

According to the sponsor's analysis, five of the 10 remaining subjects with virologic relapse had baseline NS5A resistance-associated polymorphisms (A30K, n = 1; Y93H, n = 4) that were maintained (A30K, n = 1; Y93H, n = 2) or enriched (Y93H, n = 2) at relapse. The subject with A30K at baseline had the NS5A resistance-associated substitution Y93H emerge at the time of relapse. In addition, the NS5A resistance-associated substitution Y93H emerged in the other 5 subjects without baseline NS5A resistance-associated polymorphisms who relapsed. Y93H was observed in all 10 subjects at relapse. The sponsor noted that Y93H, as site-directed mutant in a phenotypic assay, confers 724-fold reduction in susceptibility to VEL in HCV GT3a. The sponsor reported that no NS5B resistance-associated polymorphisms or resistance-associated substitutions were observed in these 10 subjects at baseline or at the time of relapse.

Table 8. ASTRAL-3 (GS-US-342-1140) subjects with virologic failure following treatment with SOF/VEL for 12 weeks (1% cutoff) (Table 5-8, page 57, Integrated Virology Report).

| Subject | GT | TN or TE | Cirrhotic | NS5A | | | | NS5B | |
|-------------|----|--------------|-----------|---------------------------|------------------------------|--|------------------------------|---------------------------|---------------------------|
| | | | | Baseline | | Posttreatment | | Baseline | Posttreatment |
| | | | | Variants (%) ^a | VEL FC from Ref ^b | Variants (%) ^a | VEL FC from Ref ^b | Variants (%) ^a | Variants (%) ^a |
| 04472-62202 | 3a | TE (PEG+RBV) | Yes | A30K (>99%) | 30 | A30K (>99%) P58L (1.6%) Y93H (97.2%) | 35154 | None | None |
| 03314-62107 | 3a | TE (PEG+RBV) | No | Y93H (2.8%) | 1 | Y93H (>99%) | 385 | N142S (80.5%) | None |
| 00529-62147 | 3a | TN | Yes | Y93H (>99%) | 347 | Y93H (>99%) | 302 | N142S (1.5%) | None |
| 01589-62011 | 3a | TN | No | Y93H (>99%) | 1073 | Y93H (>99%) | 1221 | None | None |
| 00472-62512 | 3a | TN | Yes | Y93H (15.2%) | ND | A30V (11.5%) Y93H (>99%) | ND | None | None |
| 00529-62069 | 3a | TE (PEG+RBV) | Yes | None | 0.23 | Y93H (>99%) | 74 | None | None |
| 01065-62502 | 3a | TE (PEG+RBV) | No | None | 0.61 | Y93H (>99%) | 698 | None | None |
| 02080-62118 | 3a | TE (PEG+RBV) | Yes | None | 1.32 | Y93H (>99%) | 1138 | None | None |
| 05873-62186 | 3a | TE (PEG+RBV) | Yes | None | 0.63 | Y93H (>99%) | 170 | None | None |
| 05730-62185 | 3a | TN | Yes | None | 0.31 | Y93H (>99%) | 202 | None | None |
| 01069-62225 | 3a | TE (PEG+RBV) | No | None (GT3a) | ND | None (GT1a) | ND | None (GT3a) | None (GT1a) |

EC₅₀ = concentration of a compound inhibiting virus replication by 50%; FC = fold-change; Ref = wild-type reference control; BL = baseline; GT = genotype; RAP = resistance-associated polymorphism; RAV = resistance-associated variant; TE = treatment experienced; TN = treatment naive; ND = not determined

a For NS5A and NS5B NI variants, RAPs are indicated in plain and RAVs are indicated in bold.

b Phenotypic fold change of EC₅₀ compared with wild-type reference control or Baseline

Source: Appendix; PC-342-2003 Phase 3 iVSR Virology Listing 5, 6, and 7.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

In the SOF+RBV 24 Week group, a total of 39 subjects in the study experienced virologic failure with Subject 01086-62044 experiencing on-treatment failure and while 38 additional subjects experienced post-treatment relapse.

Table 9 presents the sponsor's results from the baseline and post-treatment nucleotide sequence analysis of the NS5B gene for these 39 subjects. According to the sponsor, seven of the 39 subjects (17.9%) had NS5B resistance-associated substitutions (N142T, L159F or V321A) emerge post-treatment. Additionally, 2 subjects had an N142T polymorphism at baseline which was no longer detectable at post-treatment timepoints, from which the sponsor concluded that natural drift occurred in low level variants at this position. In addition, the sponsor noted that the NS5B resistance-associated substitution S282T was not detected in any subject in this group. The sponsor also reported that in accordance with their expectations, no significant changes in the NS5A gene or emergent NS5A resistance-associated substitutions were observed in the subjects who relapsed after SOF+RBV 24 week treatment.

Note: The sponsor reported that the NS5B polymorphism N142T was a position of interest and they performed analyses to determine if this position was associated with resistance. By DAVP analysis, NS5B N142T was not associated with resistance in the four studies analyzed in this NDA.

Table 9. ASTRAL-3 (GS-US-342-1140) subjects with virologic failure following treatment with SOF+RBV for 24 weeks (1% cutoff) (Table 5-9, page 59, Integrated Virology Report).

| Subject | HCV GT | TN or TE | Cirrhotic | Baseline NS5B Variants (%) | Posttreatment NS5B Variants (%) |
|----------------------------------|--------|-----------------|-----------|---|---|
| 01086-62044 (on-treatment VF) | 3a | TE (PEG+RBV) | No | None | None |
| 03314-62080 | 3a | TE (PEG+RBV) | No | N142S (>99%) L159F (2.8%) | N142S (>99%) L159F (10.2%) |
| 01119-62444 | 3a | TE (PEG+RBV) | No | N142T (>99%) | N142T (>99%) |
| 00521-62068 | 3a | TE (PEG) | No | N142T (1.8%) | L159F (1.2%) |
| 00494-62550 | 3a | TE (PEG) | No | N142T (4.7%) | V321A (5.2%) |
| 08667-62228 | 3a | TE (PEG+RBV) | Yes | None | L159F (>99%) |
| 02803-62242 | 3a | TE (PEG+RBV) | No | None | L159F (3.0%) |
| 01249-62021 | 3a | TN | No | None | L159F (5.8%) |
| 08118-62145 | 3a | TE (PEG+RBV) | Yes | None | V321A (3.9%) |
| 01415-62419 | 3a | TE (PEG+RBV) | No | None | N142T (4.8%) L159F (2.7%) V321A (1.2%) |
| 01415-62410 | 3a | TN | No | N142S (74.6%) | N142S (81.6%) |
| Other Relapse subjects (N = 28) | 3a | Mixed | Mixed | None | None |

EC₅₀ = concentration of a compound inhibiting virus replication by 50%; FC = fold-change; Ref = wild-type reference control; BL = baseline; GT = genotype; RAP = resistance-associated polymorphism; RAV = resistance-associated variant; VF = virologic failure

For NS5B NI variants, RAPs are indicated in plain and RAVs are indicated in bold.

Source: Appendix; PC-342-2003 Phase 3 iVSR [Virology Listing 6](#)

ASTRAL-3 (GS-US-342-1140) Resistance Conclusions from the Sponsor

The sponsor reported that baseline NS5A and NS5B resistance-associated polymorphisms were present in 15.7% (43 of 274) and 4.4% (12 of 272) of the subjects in the SOF/VEL group. The sponsor concluded that despite the presence of baseline NS5A resistance-associated polymorphisms in subjects with HCV GT3 infection, 38 of 43 subjects (88%) achieved SVR12. Moreover, the sponsor noted that subjects with NS5B resistance-associated polymorphisms in the SOF/VEL 12 Week group achieved SVR12. The sponsor reported that a total of 10 subjects in SOF/VEL group relapsed and 1 subject was re-infected. The sponsor concluded that 10 subjects had the NS5A resistance-associated substitutions Y93H detected at relapse time points and indicated that no SOF/VEL-treated subjects had NS5B resistance-associated substitutions that emerged at relapse. However, the sponsor reported that NS5B resistance-associated substitutions (N142T, L159F, or V321A) emerged in 7 of 39 subjects in the SOF+RBV 24 week group who relapsed.

ASTRAL-3 (GS-US-342-1140) DAVP Analysis

The independent analysis of the NGS data for both groups in ASTRAL-3 indicated that analyses performed by the sponsor and by DAVP were largely in agreement; however there were a few notable exceptions. In Table 8 showing the analysis results of treatment failures from the SOF/VEL for 12 weeks group, DAVP's analysis was in complete agreement with the results provided by the sponsor except for two subjects: DAVP was unable to confirm the results for subject 00529-62069, who was reported by the sponsor to have an HCV GT3a virus with the resistance-associated substitution Y93H present at >99% at the time of relapse and subject 01589-62011, who was reported by the sponsor to have >99% frequency of resistance-associated polymorphism Y93H at baseline and at the time of relapse, as these polymorphisms/substitutions were not detected by DAVP with any of the three variant detectors used. It is not clear why a high frequency substitution reported by one analysis pipeline was unable to be detected by another. In the SOF+RBV for 24 weeks group (Table 9), the sponsor provided specific data for 11 subjects, for which DAVP analyses confirmed and was in complete agreement. However, among the 28 subjects listed in that table as other relapsers with no baseline or treatment-emergent resistance-associated substitutions in NS5B, DAVP identified several substitutions that may have played a role in relapse. S282N was detected in three subjects at post-treatment follow-up week 4 (01815-62181 at 22%; 01815-62351 at 1.4%; and 01815-62101 at 7%) and L320F was detected at a frequency of 1% in subject 01815-62174 at baseline.

In addition, several treatment-emergent substitutions were identified in more than one subject, and these are potentially resistance-associated substitutions (Table 10). Of particular interest is NS5B substitution L314F, which was detected (using the SUBS10 criteria) in 4 subjects (3 in the SOF+RBV for 24 weeks group and 1 in the SOF/VEL for 12 weeks group). L314 is highly conserved among HCV isolates and occurs on the same beta sheet as the catalytic triad in the active site of the polymerase and substitutions at this position could alter the functionality of this essential viral enzyme (Table 10).

ASTRAL-3 (GS-US-342-1140) DAVP Conclusions: The independent analysis of the NGS data for both groups in ASTRAL-3 indicated that analyses performed by the sponsor and by DAVP were largely in agreement; however DAVP identified several inconsistencies between analysis methods, and the DAVP analysis identified additional substitutions that may be important for resistance to the SOF/VEL FDC. Of particular interest is NS5B substitution L314F, which was detected (using the SUBS10 criteria) in 4 subjects (3 in the SOF+RBV for 24 weeks group and 1 in the SOF/VEL for 12 weeks group) where it was not present at baseline but was present at greater than 35% at the time of failure. L314 is highly conserved (100%) among HCV isolates and occurs on the same beta sheet as the catalytic triad in the active site of the polymerase and substitutions at this position could alter the functionality of this viral enzyme.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

sponsor reported that a total of 267 subjects were randomized (1:1:1) to one of the following three treatment groups:

1. **SOF/VEL 12 Week group (Group 1):** SOF/VEL FDC (400/100 mg) once daily for 12 weeks (N = 90)
2. **SOF/VEL+RBV 12 Week group (Group 2):** SOF/VEL FDC once daily + RBV (1000 or 1200 mg/day divided twice daily) Tablets for 12 weeks (N = 87)
3. **SOF/VEL 24 Week group (Group 3):** SOF/VEL FDC once daily for 24 weeks (N = 90)

The sponsor reported that the virologic failure rates by treatment group in this population with decompensated cirrhosis were as follows:

1. **SOF/VEL 12 Week group:** 12.2% of subjects (11 of 90) did not achieve SVR12
2. **SOF/VEL+RBV 12 Week group:** 3.4% of subjects (3 of 87) did not achieve SVR12
3. **SOF/VEL 24 Week group:** 8.9% of subjects (8 of 90) did not achieve SVR12
4. The sponsor reported that eleven additional subjects were categorized as “other” and therefore did not achieve SVR12.

The Resistance Analysis Population was defined as all subjects in the SOF/VEL 12 Week, SOF/VEL+RBV 12 Week, and SOF/VEL 24 Week groups in the Safety Analysis Set with a virologic outcome. The sponsor reported that of the 267 subjects, 11 subjects (SOF/VEL 12 Week group, n=4; SOF/VEL+RBV 12 Week group, n=2; SOF/VEL 24 Week group, n=5) with virologic outcome of “other” as defined in the SAP were not included in the Resistance Analysis Population. Therefore, the Resistance Analysis Population included 256 total subjects, with 86 in the SOF/VEL 12 Week group, 85 in the SOF/VEL+RBV 12 Week group, and 85 in the SOF/VEL 24 Week group.

The sponsor noted that across all treatment groups, 8.6% of subjects (22 of 256) experienced virologic failure in the Resistance Analysis Population (20 relapses and 2 on-treatment virologic failures). Table 10 presents baseline and post-treatment NS5A and NS5B resistance-associated polymorphisms or resistance-associated substitutions (1% cut off) for these subjects. Variants at NS5A position 93, predominantly Y93H, were observed in 19 of 22 subjects (86.4%) at the time of virologic failure. Treatment-emergent NS5B resistance-associated substitutions at the time of virologic failure were less common, occurring in 5 of 22 subjects (22.7%).

In the SOF/VEL 12 Week group, the sponsor reported that 11 subjects experienced virologic failure (5 with HCV GT1 infections and 6 with HCV GT3 infections). Of the 5 subjects with HCV GT1 infection who experienced virologic failure, the virus of 3 (60%) had emergent NS5A resistance-associated substitutions (L31V and Y93N) at virologic failure (Table 11).

The sponsor also reported that the three subjects with HCV GT1 infection and treatment-emergent NS5A resistance-associated substitutions showed phenotypic resistance to VEL with the absence of wild type virus at relapse. One subject with HCV GT1b infection had virus with low levels of NS5B resistance-associated substitutions L159F and S282T emerge at virologic failure; this subject also had L31I/M+Y93H at baseline and failure. While this subject had >100 fold resistance to VEL at virologic failure, the sponsor reported that no phenotypic resistance to SOF was observed likely due to >80% of viral population being NS5B wild type virus at relapse (Table 11).

Of the 6 subjects with HCV GT3 who relapsed, 2 had Y93H NS5A resistance-associated polymorphisms at baseline that were maintained at relapse and 4 had Y93H emerge at relapse. All genotype 3 subject isolates with Y93H as full variants showed >100 fold resistance to VEL.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

One subject with HCV GT3a infection and Y93H at baseline and virologic failure also had the L320I NS5B resistance-associated substitution emerge at virologic failure. The sponsor noted that no phenotypic SOF resistance was detected in any subject at baseline or post-treatment (Table 11).

Table 11. ASTRAL-4 (GS-US-342-1137) subjects with virologic failure following treatment with SOF/VEL regimens (1% cutoff) (Table 6-11, page 75, Integrated Virology Report).

| Treatment Group | Subject | HCV GT | NS5A | | | | NS5B | | | |
|--|--------------------------|-------------|---|-----------------|---|-----------------|--------------|-----------------|--------------------------------|-----------------|
| | | | Baseline | | Posttreatment | | Baseline | | Post Treatment | |
| | | | Variants (%) | VEL FC from Ref | Variants (%) | VEL FC from Ref | Variants (%) | SOF FC from Ref | Variants (%) | SOF FC from Ref |
| SOF/VEL 12 Weeks (Group 1) | 00380-64032 | 1a | M28V (6.1%), H58P (2.4%), H58R (2.7%) | 1.08 | None | 1.22 | None | 0.74 | None | 0.75 |
| | 07275-64065 | 1a | K24Q (3.9%) | 1.02 | Y93N (> 99%) | 789 | None | 0.79 | None | 0.69 |
| | 01651-64225 | 1a | None | 1.19 | None | 1.27 | None | 1.13 | None | 0.87 |
| | 08224-64040 | 1b | R30Q (58.6%), L31I (8.3%), L31M (2.6%), Y93H (60.0%) | 1.47 | R30Q (> 99%), L31M (80.8%), L31V (10.1%), Y93H (> 99%) | 122 | None | 0.93 | L150F (13.9%), S282T (3.6%) | 0.82 |
| | 02127-64167 | 1b | A92T (1.1%), A92V (1.8%), Y93H (80.2%) | 1.98 | L31M (49.9%), L31V (48.8%), Y93H (> 99%) | 180 | None | 0.96 | None | 0.86 |
| | 02760-64041 | 3a | None | 1.06 | Y93H (> 99%) | 108 | N142S (>99%) | 1.29 | N142S (>99%) | 1.15 |
| | 01248-64072 | 3a | None | 1.58 | Y93H (> 99%) | 800 | None | 1.02 | None | 0.92 |
| | 00331-64095 | 3a | Y93H (>99%) | 881 | S38Y (1.1%), P58T (1.3%), Y93H (> 99%) | 1029 | None | 1.21 | S96Y (1.0%), L320I (1.1%) | 1.23 |
| | 08118-64117 | 3a | None | 0.89 | Y93H (> 99%) | 1185 | None | 1.06 | None | 1.00 |
| | 02127-64256 | 3a | None | 0.50 | Y93H (> 99%) | 203 | None | 1.11 | None | 1.10 |
| 00585-64263 | 3a | Y93H (4.9%) | 2.59 | Y93H (> 99%) | 828 | None | 0.94 | None | 1.09 | |
| SOF/VEL+ RBV 12 Weeks (Group 2) | 01039-64172 | 1a | None | 1.05 | None | 0.93 | None | 0.92 | None | 0.88 |
| | 05665-64208 | 3a | None | NA | M28V (1.7%), Y93H (> 99%) | NA | None | NA | None | NA |
| | 03060-64249 ^a | 3a | Y93H (2.9%) | 1.07 | S38P (8.6%), Y93H (> 99%) | 535 | None | 1.14 | N142T (3.1%), E237G (2.3%) | 0.98 |
| SOF/VEL 24 Weeks (Group 3) | 08734-64091 | 1a | H58P (3.1%) | 1.22 | Q30R (94.5%), H58D (94.5%), Y93N (4.2%) | 64 | None | 0.95 | None | 1.07 |
| | 02127-64221 | 1a | Q30H (64.0%), Y93H (57.4%), Y93N (1.2%) | 695 | Q30H (> 99%), Y93H (> 99%) | 934 | None | 0.93 | L150F (96.3%), S282T (3.0%) | 1.38 |
| | 05069-64193 | 1b | L31M (> 99%) | NA | L31M (97.9%), L31V (1.7%), Y93H (> 99%) | NA | None | NA | None | NA |
| | 04421-64013 | 3a | P58A (>99%) | 1.25 | P58A (>99%) Y93H (97.8%) | 2434 | None | 1.26 | E237G (1.5%) | 1.13 |
| | 00619-64062 | 3a | None | NA | Y93H (98.8%) | NA | None | NA | None | NA |
| | 01657-64105 | 3a | None | 0.86 | Y93H (98.9%) | 458 | None | 1.43 | None | 1.69 |
| | 05801-64195 | 3a | None | NA | M28T (2.2%), Y93H (> 99%) | NA | NA | 0.95 | None | 1.06 |
| 00407-64262 | 3a | None | NA | Y93H (> 99%) | NA | None | NA | None | NA | |

EC₅₀ = concentration of a compound inhibiting virus replication by 50%; FC = fold-change; Ref = wild-type reference control;

BL = baseline; GT = genotype; RAP = resistance-associated polymorphism; RAV = resistance-associated

variant; TE = treatment experienced; TN = treatment naive; ND = not determined

a For NS5A and NS5B NI variants. RAPs are indicated in plain and RAVs are indicated in bold.

b Phenotypic fold change of EC₅₀ compared with wild-type reference control or Baseline

c Subject was consistent with nonadherence to study drug.

Source: Appendix: PC-342-2003 Phase 3 iVSR Virology Listings 12, 13, 14, 15

The sponsor reported that in the SOF/VEL+RBV 12 Week group, 3 subjects (1 with HCV GT1a and 2 with HCV GT3) experienced virologic failure. One subject with HCV GT1a infection had no NS5A or NS5B resistance-associated substitutions at virologic failure, and no observable phenotypic resistance to VEL or SOF. One subject with HCV GT3 had Y93H emerge in NS5A at virologic failure. Another subject (Subject 03060-64249) with HCV GT3 infection had the Y93H NS5A resistance-associated polymorphism at baseline and relapse; and also developed low levels of N142T+E237G NS5B resistance-associated substitutions at virologic failure (Table 11).

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

The sponsor noted that detectable phenotypic VEL resistance was observed at failure, consistent with the enrichment of Y93H to a full variant in this subject. No phenotypic resistance to SOF was observed at baseline or virologic failure. The sponsor noted that this subject had undetectable drug levels, consistent with study drug non-adherence. The sponsor reported that in the SOF/VEL+RBV 24 Week group, 8 subjects (2 with HCV GT1a, 1 with HCV GT1b, and 5 with HCV GT3a) experienced virologic failure. All genotype 1 subjects were observed to have multiple NS5A resistance-associated substitutions at failure (including Y93 variants) including emergent NS5A resistance-associated substitutions Q30R+H58D+Y93N in one subject, L31V+Y93H in another subject, and enrichment of Q30H+Y93H to full double mutant in the third subject. The third subject had Q30H+Y93H NS5A resistance-associated polymorphisms at baseline and virologic failure (of note, the EC₅₀ value fold change to VEL was >100) and developed L159F in combination with low levels of S282T at relapse (the EC₅₀ value fold change to SOF was 1.38). All 5 subjects with genotype 3a HCV infection had Y93H NS5A resistance-associated substitutions emerge at virologic failure; one of these subjects also developed low levels of E237G NS5B resistance-associated substitution at virologic failure.

ASTRAL-4 (GS-US-342-1137) Resistance Conclusions from the Sponsor

The sponsor noted that overall subjects in the SOF/VEL 12 Week group or SOF/VEL 24 Week treatment groups had lower SVR12 rates than subjects in the SOF/VEL+RBV 12 Week group. The sponsor's analysis across treatment groups indicated that 28.2% (72 of 255) and 3.2% (8 of 251) of subjects had baseline NS5A resistance-associated polymorphisms and NS5B resistance-associated polymorphisms respectively, in this population of subjects with advanced liver disease. The sponsor stated that the presence of baseline NS5A resistance-associated polymorphisms did not impact treatment outcome in subjects with HCV GT1 infection in the SOF/VEL+RBV 12 Week group. They reported that the small number of subjects with HCV GT 2, 3, 4, and 6 infections with baseline NS5A resistance-associated polymorphisms limited the interpretation of the effect of NS5A resistance-associated polymorphisms on treatment outcome. The sponsor noted that no effect of baseline NS5B resistance-associated polymorphisms was observed.

The sponsor observed that the majority of subjects had NS5A resistance-associated substitutions present at virologic failure; however, they reported that NS5B resistance-associated substitutions were less common and typically observed at low levels. The most common NS5A resistance-associated substitution reported by the sponsor was Y93H as the predominant virus in the population (at high frequency >90%). Of the 9 subjects infected with HCV GT1 who experienced virologic failure, 5 had treatment-emergent NS5A resistance-associated substitutions in the infecting virus population, one maintained a NS5A resistance-associated polymorphism in the viral population from baseline, and 3 subjects failed treatment with no NS5A resistance-associated substitutions detected.

The virus from two HCV GT1-infected subjects had L159F and S282T NS5B resistance-associated substitutions present at virologic failure and both of these subjects had multiple NS5A resistance-associated polymorphisms at baseline that reduced susceptibility to VEL when tested as site-directed mutants. The sponsor reported that all 13 subjects with HCV GT3 infection who experienced virologic failure had the Y93H NS5A resistance-associated substitution at virologic failure and 10 of 13 were treatment-emergent. The virus from three subjects infected with HCV GT3 had low levels of NS5B resistance-associated substitutions emerge at virologic failure.

ASTRAL-4 (GS-US-342-1137) DAVP Analysis

The independent analysis of the NGS data for the three groups in ASTRAL-4 indicated that analyses performed by the sponsor and by DAVP were largely in agreement; however there were a few notable exceptions that will be described in detail below. The substitutions detected by DAVP and the sponsor were compared in Table 12, which shows substitutions at previously identified positions of interest and at new positions if novel substitutions occurred in the virus of more than one subject (Table 12). In

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

general, there were no novel NS5A or NS5B resistance-associated substitutions identified in the analysis of the NGS data for ASTRAL-4.

Table 12. Comparison of NGS results for ASTRAL-4 (GS-US-342-1137) treatment failures (DAVP analysis). Only substitutions detected by multiple DAVP variant callers are shown unless the substitution was detected at a site of interest. BL, variants detected at high frequency at baseline (>10%); SUBS10, substitutions meeting the treatment-emergent criteria explained in the text; LF SUB, low frequency substitution (<10%). Variant detectors shown in the AGREE column were defined as: G, Gilead; L, Low Frequency Variant Detector; P, Probabilistic Variant Detector; Q, Quality-based Variant Detector.

| ARM | HCV GT | GENE | BL | SUBS10 | LF SUB | No. SUBJ | AGREE |
|---------|--------|-------|-------------|----------|--------|----------|-------------|
| FDC12W | 1a | NS5A | | Y93N | | 1 | GLPQ |
| | | | | | K24Q | 1 | GLQ |
| | | | | | M28V | 1 | GLQ |
| | | | | | S38Y | 1 | LQ |
| | | | | G49A | | 1 | LPQ |
| | | | | | H58P/R | 1 | GLQ |
| | | | NS5B | | C316R | 1 | GLQ |
| | 1b | NS5A | Y93H | | | 2 | GLPQ |
| | | | Q30R | | | 1 | LPQ |
| | | | L31M/I/V | L31M/V | | 2 | G |
| | | NS5B | | L159F | | 1 | GLQ |
| | 3a | NS5A | | | S282T | 1 | GQ |
| | | | | | K355Q | 1 | LPQ |
| | | | Y93H | Y93H (4) | | 5 | GLPQ |
| | | | | | E92* | 2 | G |
| | | | | | S38Y | 1 | GQ |
| | | | | | P58T | 1 | GLQ |
| | | | | V/T183A | | 2 | GLQ |
| | | | | T/V349A | | 2 | G |
| | | | | S/L383P | | 2 | G |
| | | | G444N | | 2 | G | |
| | NS5B | L320F | | L320I | 2,1 | LPQ, G | |
| | | | G549S/S549N | | 2 | G | |
| FDCR12W | 1a | NS5A | | | | 0 | |
| | | NS5B | | | | 0 | |
| | 3a | NS5A | | Y93H | | 2 | GLPQ |
| | | | | | M28V | 1 | GQ |
| | NS5B | | | S38P | 1 | G | |
| | | | | | 0 | | |
| FDC24W | 1a | NS5A | Y93H/N | Y93H | Y93N | 3 | GLPQ, G, GQ |
| | | | Q30H | Q30R | | 2 | GLPQ |
| | | | | H58D | | 1 | GLPQ |
| | | NS5B | | L159F | 1 | G | |
| | | | | S282T | 1 | GLQ | |
| | 1b | NS5A | | N62S | | 1 | G |
| | | | | Y93H | | 1 | GLPQ |
| | | | | | L31V | 1 | G |
| | 3a | NS5B | | K355Q | | 1 | LPQ |
| | | | | | | 1 | LPQ |
| | | NS5A | | Y93H | | 4 | GLPQ |
| | | | | | M28T | 1 | GQ |
| | NS5B | P58A | | | 1 | LPQ | |
| | | | | L159F | 1 | LQ | |
| | | | | S282G | 1 | LQ | |
| | | | S442P/P442S | | 2 | G | |

In the SOF/VEL 12 Week group among the three subjects infected with HCV GT1a, DAVP and the sponsor's analysis agreed with the following exceptions:

1. DAVP detected a treatment-emergent C316R substitution at 1.3% in subject 01651-64225 at post-treatment follow-up Week 4. C316 was previously determined to be a site of interest for SOF.
2. The virus of subject 07275-64025 had S38Y detected at 1% at FUW12 by the sponsor but not by DAVP.
3. Given the low frequency of these substitutions, these differences are not considered significant.

In the SOF/VEL 12 Week group among the two subjects infected with HCV GT1b, L31M/V was reported as present at baseline or treatment-emergent in two subjects (08224-64040 and 02127-64167), but was not detected by the three variant detection callers employed by DAVP. The analyses at the other positions were in complete agreement.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

In the SOF/VEL 12 Week group among the six subjects infected with HCV GT3a, DAVP and the sponsor's analysis were in complete agreement for four of these subjects with the following exceptions:

1. The sponsor reported that Y93H was present at baseline and at the time of relapse at a frequency of >99% for the virus of subject 00331-64095, and this substitution was not detected by DAVP. In NS5B, DAVP detected L320F at >98% at baseline and at the time of relapse in this same virus, while the sponsor reported L320I at 1% post-treatment.
2. In the virus of subject 01249-64072, DAVP detected L320F at baseline and at relapse at a frequency >98%, but this substitution was not detected by the sponsor.
3. Low frequency analysis at position L314 identified one treatment-naïve subject (02127-64256) infected with HCV GT3a who relapsed in the SOF/VEL 12 Week group whose virus had L314P at 3.5% at the post-treatment timepoint.

In the SOF/VEL+RBV 12 Week group among the three subjects infected with HCV GT1b, DAVP variant detectors did not detect L31M/V in two subjects whose viruses were reported to have this substitution by the sponsor. In addition, in the virus of subject 03060-64249 the sponsor detected treatment-emergent S38P at 8.6% at the post-treatment timepoint and this was not detected by DAVP.

In the SOF/VEL 24 Week group among the two subjects infected with HCV GT1a, there were the following disagreements for both subjects:

1. In the virus from subject 08734-64091: H58P was detected by the sponsor at 3.1% at baseline but not detected by DAVP
2. In the virus from subject 02127-64221: The sponsor detected L159F at 96.3% at the time of failure and this substitution was not detected by DAVP.

In the SOF/VEL 24 Week group in the subject infected with HCV GT1b who failed treatment, the sponsor detected L31M/V at greater than 97% frequency at baseline and relapse in subject 05969-64193 and this substitution was not detected by DAVP. In the SOF/VEL 24 Week group among the five treatment failures infected with HCV GT3a, there was complete agreement between 3 subjects, with low frequency variations where DAVP detected L159F at 1.1% frequency and S282G at 1.2% frequency and these were not reported by the sponsor. In addition, the sponsor reported that an Y93H substitution emerged at a frequency of greater than 93% in the virus of subject 00407-64262, but this substitution was not detected by DAVP.

NOTE: In general, the disagreements that occurred at high frequency that were not detected by the three variant detectors used by DAVP, but were reported by the sponsor, occurred in or adjacent to regions in the short read alignments that contained multiple low frequency inserts. Looking at the alignments for each of the disagreements verified that the substitutions for the disagreements were present.

ASTRAL-4 (GS-US-342-1137) DAVP Conclusions: The independent analysis of the NGS data for all three groups in ASTRAL-4 indicated that analyses performed by the sponsor and by DAVP were largely in agreement; however DAVP identified several inconsistencies between analysis methods. In general, most disagreements occurred at low frequency where differences in program parameters likely contributed to these discrepancies. However, there were a few disagreements that occurred at high frequency at positions known to be resistance-associated and so, substitutions that occurred at known positions were counted if they were detected by any of the variant detectors used (including one by the sponsor and three by DAVP). Of note, low frequency analysis at L314 identified one treatment-naïve subject (02127-64256) infected with HCV GT3a who relapsed in the SOF/VEL 12 Week group of ASTRAL-4 (GS-US-342-1137) whose virus had L314P at 3.5% at the post-treatment timepoint.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

COMBINED RESISTANCE ANALYSIS

DAVP's combined analysis of NS5A resistance-associated substitutions that were detected across all four phase 3 clinical trials in the virus of subjects treated with the SOF/VEL FDC are shown in Table 13.

Table 13. NS5A resistance-associated substitutions detected across all phase 3 studies (DAVP Analysis). Red, sites of known resistance-associated substitutions. Shades distinguish different positions.

| Trial | ARM | GT | SUB | No. | AAPOS | Total | Status | Rationale |
|---------|---------|----|----------|-----|-------|-------|-----------|---|
| ASTRAL1 | FDC12W | 1a | Y93H | 1 | 93 | 28 | Known RAS | |
| ASTRAL1 | FDC12W | 1c | Y93H | 1 | | | | |
| ASTRAL3 | FDC12W | 3a | Y93H | 8 | | | | |
| ASTRAL4 | FDC12W | 1a | Y93N | 1 | | | | |
| ASTRAL4 | FDC12W | 1b | Y93H | 2 | | | | |
| ASTRAL4 | FDC12W | 3a | Y93H | 5 | | | | |
| ASTRAL4 | FDC24W | 1a | Y93H/N | 3 | | | | |
| ASTRAL4 | FDC24W | 1b | Y93H | 1 | | | | |
| ASTRAL4 | FDC24W | 3a | Y93H | 4 | | | | |
| ASTRAL4 | FDCR12W | 3a | Y93H | 2 | | | | |
| ASTRAL3 | FDC12W | 3a | P383S | 3 | 383 | 6 | unlikely | Polymorphic |
| ASTRAL4 | FDC12W | 3a | S/L383P | 2 | | | | |
| ASTRAL4 | FDCR12W | 3a | P383Q | 1 | | | | |
| ASTRAL3 | FDC12W | 3a | G411E | 5 | 411 | 6 | unlikely | Polymorphic |
| ASTRAL4 | FDCR12W | 3a | G411E | 1 | | | | |
| ASTRAL3 | FDC12W | 3a | A30V/K | 2 | 30 | 5 | Known RAS | |
| ASTRAL4 | FDC12W | 1b | Q30R | 1 | | | | |
| ASTRAL4 | FDC24W | 1a | Q30R/H | 2 | | | | |
| ASTRAL3 | FDC12W | 3a | P58L | 1 | 58 | 5 | Known RAS | |
| ASTRAL4 | FDC12W | 1a | H58P/R | 1 | | | | |
| ASTRAL4 | FDC12W | 3a | P58T | 1 | | | | |
| ASTRAL4 | FDC24W | 1a | H58D | 1 | | | | |
| ASTRAL4 | FDC24W | 3a | P58A | 1 | | | | |
| ASTRAL3 | FDC12W | 3a | A183V | 2 | 183 | 5 | unlikely | Polymorphic |
| ASTRAL4 | FDC12W | 1b | P183L | 1 | | | | |
| ASTRAL4 | FDC12W | 3a | V/T183A | 2 | | | | |
| ASTRAL2 | SR12W | 2b | D331E | 1 | 331 | 5 | unlikely | Polymorphic |
| ASTRAL3 | FDC12W | 3a | A331E | 2 | | | | |
| ASTRAL4 | FDC12W | 1a | S331K | 1 | | | | |
| ASTRAL4 | FDC24W | 1a | E331K | 1 | | | | |
| ASTRAL3 | FDC12W | 3a | T382S | 3 | | | | |
| ASTRAL4 | FDC12W | 3a | P382T | 1 | | | | |
| ASTRAL4 | FDCR12W | 3a | P382T | 1 | | | | |
| ASTRAL3 | FDC12W | 3a | N444S | 2 | 444 | 5 | unlikely | Polymorphic |
| ASTRAL4 | FDC12W | 1a | N444D | 1 | | | | |
| ASTRAL4 | FDC12W | 3a | G444N | 2 | | | | |
| ASTRAL3 | FDC12W | 3a | H85Y | 2 | | | | |
| ASTRAL4 | FDC24W | 1a | S85N | 2 | | | | |
| ASTRAL3 | FDC12W | 3a | S197A | 3 | 197 | 4 | unlikely | Polymorphic |
| ASTRAL4 | FDC12W | 1a | T197A | 1 | | | | |
| ASTRAL4 | FDC12W | 3a | D389N | 1 | 389 | 4 | unlikely | Polymorphic |
| ASTRAL4 | FDC24W | 1a | A389T | 1 | | | | |
| ASTRAL3 | FDC12W | 3a | T401A | 1 | 401 | 4 | unlikely | Polymorphic |
| ASTRAL4 | FDC12W | 1a | S401P | 1 | | | | |
| ASTRAL4 | FDC12W | 1b | S401F | 1 | | | | |
| ASTRAL4 | FDCR12W | 3a | T401A | 1 | | | | |
| ASTRAL1 | FDC12W | 1c | V442T | 1 | 442 | 4 | unlikely | Polymorphic |
| ASTRAL4 | FDC12W | 1a | A442T | 1 | | | | |
| ASTRAL4 | FDC24W | 1a | A442T | 1 | | | | |
| ASTRAL4 | FDCR12W | 3a | G442S | 1 | | | | |
| ASTRAL3 | FDC12W | 3a | D7N/W/A | 2 | 7 | 3 | unlikely | Polymorphic |
| ASTRAL4 | FDCR12W | 3a | D7T | 1 | | | | |
| ASTRAL4 | FDC12W | 1a | M28V | 1 | 28 | 3 | Known RAS | |
| ASTRAL4 | FDCR12W | 3a | M28V | 1 | | | | |
| ASTRAL4 | FDC24W | 3a | M28T | 1 | | | | |
| ASTRAL4 | FDC12W | 1b | L31M/I/V | 2 | 31 | 3 | Known RAS | |
| ASTRAL4 | FDC24W | 1b | L31V | 1 | | | | |
| ASTRAL4 | FDC12W | 1a | S38Y | 1 | 38 | 3 | Known RAS | |
| ASTRAL4 | FDC12W | 3a | S38Y | 1 | | | | |
| ASTRAL4 | FDCR12W | 3a | S38P | 1 | | | | |
| ASTRAL4 | FDC12W | 1b | A92T/V | 1 | 92 | 3 | Known RAS | |
| ASTRAL4 | FDC12W | 3a | E92* | 2 | | | | |
| ASTRAL1 | FDC12W | 1c | E171D | 1 | 171 | 3 | unlikely | Polymorphic |
| ASTRAL3 | FDC12W | 3a | E171D | 1 | | | | |
| ASTRAL4 | FDC12W | 1b | D171E | 1 | | | | |
| ASTRAL3 | FDC12W | 3a | P248L | 1 | 248 | 3 | unlikely | Polymorphic |
| ASTRAL4 | FDC12W | 1a | D248N | 1 | | | | |
| ASTRAL4 | FDC12W | 3a | L248P | 1 | | | | |
| ASTRAL4 | FDC12W | 1b | D333G | 1 | 333 | 3 | unlikely | Conserved at >95% in all three GT, but position is unlikely |
| ASTRAL4 | FDC24W | 1a | G333D | 1 | | | | |
| ASTRAL4 | FDCR12W | 3a | G333D | 1 | | | | |
| ASTRAL2 | SR12W | 2b | L359P | 1 | 359 | 3 | unlikely | Polymorphic |
| ASTRAL3 | FDC12W | 3a | R359K | 2 | | | | |
| ASTRAL3 | FDC12W | 3a | S385Q | 2 | 385 | 3 | unlikely | Polymorphic |
| ASTRAL4 | FDC12W | 3a | P385S | 1 | | | | |
| ASTRAL2 | SR12W | 2b | A398V | 2 | 398 | 3 | unlikely | Polymorphic |
| ASTRAL3 | FDC12W | 3a | Q398H | 1 | | | | |

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

For VEL resistance, the predominant resistance-associated substitutions that were detected across all four phase 3 trials were Y93H/N (n=28: 19 in GT3a, 5 in GT1a, 3 in GT1b, and 1 in GT1c), Q30R/H (GT1a and b) or A30A30V/K (GT3) (n=5: 3 in GT1a or b and 2 in GT3a), P58L/T/A (GT3a) or H58P/R/D (GT1a) (n=5, 3 in GT3a and 2 in GT1a) H85Y (GT3a) or S85N (GT1a) (n=4, 2 each for GT3a and GT1a) and M28V/T (n=3, 2 in GT3a and 1 in GT1a). Additional substitutions that were detected in 3 or more subjects occurred at positions that were polymorphic (Table 13).

DAVP's combined analysis of NS5B resistance-associated substitutions that were detected across all four phase 3 clinical trials in the virus of subjects treated with the SOF/VEL FDC are shown in Table 14.

Table 14. (DAVP analysis). NS5B resistance-associated substitutions detected across all phase 3 studies (DAVP Analysis). Red, sites of known resistance-associated substitutions. Shades distinguish different positions.

| TRIAL | ARM | GT | SUB | NO. | AAPOS | TOTAL | STATUS | RATIONALE |
|---------|---------|----|-------------|-----|-------|-------|-----------|--|
| ASTRAL3 | SR24W | 3a | L159F | 6 | 159 | 9 | Known RAS | |
| ASTRAL4 | FDC12W | 1b | L159F | 1 | | | | |
| ASTRAL4 | FDC24W | 1a | L159F | 1 | | | | |
| ASTRAL4 | FDCR12W | 3a | L159F | 1 | | | | |
| ASTRAL3 | SR24W | 3a | S282N | 3 | 282 | 6 | Known RAS | |
| ASTRAL4 | FDC12W | 1b | S282T | 1 | | | | |
| ASTRAL4 | FDC24W | 1a | S282T | 1 | | | | |
| ASTRAL4 | FDCR12W | 3a | S282G | 1 | | | | |
| ASTRAL2 | SR12W | 2b | R77K | 1 | 77 | 5 | unl kely | Polymorphic position |
| ASTRAL3 | SR24W | 3a | R77K | 3 | | | | |
| ASTRAL4 | FDCR12W | 3a | R77G | 1 | | | | |
| ASTRAL3 | SR24W | 3a | K377R/K377A | 3 | 377 | 5 | unl kely | Conserved (95.99%) but detected in subjects who achieved SVR12 |
| ASTRAL4 | FDC12W | 3a | K377E | 1 | | | | |
| ASTRAL4 | FDCR12W | 3a | K377E | 1 | | | | |
| ASTRAL3 | FDC12W | 3a | S569T | 1 | 569 | 5 | unl kely | Polymorphic position |
| ASTRAL3 | SR24W | 3a | T569S/A/I | 4 | | | | |
| ASTRAL3 | SR24W | 3a | A90T/G | 3 | 90 | 4 | unl kely | Polymorphic position |
| ASTRAL4 | FDCR12W | 3a | T90A | 1 | | | | |
| ASTRAL3 | FDC12W | 3a | K100R | 2 | 100 | 4 | unl kely | Polymorphic position |
| ASTRAL4 | FDC24W | 3a | K100R | 1 | | | | |
| ASTRAL4 | FDCR12W | 3a | K100R | 1 | | | | |
| ASTRAL3 | FDC12W | 3a | K304R | 1 | 304 | 4 | unl kely | Polymorphic position |
| ASTRAL4 | FDC12W | 3a | K304M | 1 | | | | |
| ASTRAL4 | FDC24W | 1a | R304Q | 1 | | | | |
| ASTRAL4 | FDCR12W | 3a | R304K | 1 | | | | |
| ASTRAL3 | FDC12W | 3a | L314F | 1 | 314 | 4 | RAS | Highly conserved (100%), not detected at BL in subjects who achieved SVR12 |
| ASTRAL3 | SR24W | 3a | L314F | 3 | | | | |
| ASTRAL3 | SR24W | 3a | L320F | 1 | 320 | 4 | Known RAS | |
| ASTRAL4 | FDC12W | 3a | L320F/I | 3 | | | | |
| ASTRAL2 | SR12W | 2b | Q334R | 1 | 334 | 4 | unl kely | Polymorphic position |
| ASTRAL3 | SR24W | 3a | A334V/I/T | 3 | | | | |
| ASTRAL3 | FDC12W | 3a | R50K | 2 | | | | |
| ASTRAL4 | FDCR12W | 3a | R50K | 1 | 50 | 3 | unl kely | Polymorphic position |
| ASTRAL2 | SR12W | 2b | T189P | 1 | | | | |
| ASTRAL4 | FDC24W | 1a | N189S | 1 | 189 | 3 | unl kely | Polymorphic position |
| ASTRAL4 | FDCR12W | 3a | S189P | 1 | | | | |
| ASTRAL2 | SR12W | 2b | A252V | 2 | | | | |
| ASTRAL3 | SR24W | 3a | V252A | 1 | 252 | 3 | unl kely | Conserved (98.88%), detected in multiple subjects who achieved SVR12 |
| ASTRAL3 | SR24W | 3a | D330E/N | 3 | | | | |
| ASTRAL3 | SR24W | 3a | A353V/T | 2 | 353 | 3 | unl kely | Conserved (96.95%), detected in multiple subjects who achieved SVR12 |
| ASTRAL4 | FDCR12W | 3a | A353T | 1 | | | | |
| ASTRAL3 | FDC12W | 3a | Q355R | 1 | 355 | 3 | unl kely | Conserved (3a: 98.88%; 1b: 81.83%), detected at BL in multiple subjects who achieved SVR12 |
| ASTRAL4 | FDC12W | 1b | K355Q | 1 | | | | |
| ASTRAL4 | FDC24W | 1b | K355Q | 1 | | | | |
| ASTRAL2 | SR12W | 2b | N442D | 1 | 442 | 3 | unl kely | Polymorphic position |
| ASTRAL4 | FDC24W | 3a | S442P/P442S | 2 | | | | |
| ASTRAL3 | FDC12W | 3a | A506S | 1 | 506 | 3 | unl kely | Conserved (3a: 99.93%; 1a: 71.97%), detected at BL in multiple subject who achieved SVR12 |
| ASTRAL3 | SR24W | 3a | A506T | 1 | | | | |
| ASTRAL4 | FDC12W | 1a | N506S | 1 | | | | |

For SOF resistance, the predominant resistance-associated substitutions that were detected across all four phase 3 trials were L159F (n=9, 7 in GT3a and 1 each in GT1a and 1b), S282N/T/G (n=6, 4 in GT3a and 1 each in GT1a and 1b), and L320F/I (n=4, all 4 in GT3a) (Table 14). Importantly, 6 L159F, 3 S282N, and 1 L320F resistance-associated substitutions were detected in subjects enrolled in the

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

SOF+RBV 24 Week group of ASTRAL-3, indicating that longer exposure to SOF may produce more resistance-associated substitutions in those who fail treatment.

Of note, resistance-associated substitutions L314F/I were detected in the NS5B polymerase protein of HCV viruses from four subjects infected with HCV GT3a who relapsed after treatment ended. Three of the four subjects were in the SOF/RBV 24 Week Group of ASTRAL-3 and were exposed to longer durations of SOF, which may have selected for this newly detected resistance-associated substitution. One treatment-naïve subject from the FDC 12 Week group of ASTRAL-3 also developed this substitution. Of note, this substitution was not detected in any subject at baseline but was detected at a frequency of greater than 30% at the final post-treatment timepoint. In addition, position L314 is highly conserved among HCV GT3 isolates with 100% conservation reported by the sponsor at that position. Low frequency analysis at this position identified one treatment-naïve subject (02127-64256) infected with HCV GT3a who relapsed in the SOF/VEL 12 Week group of ASTRAL-4 (GS-US-342-1137) whose virus had L314P at 3.5% at the post-treatment timepoint. Clinical virology will add this resistance-associated substitution to the label and make a Post Marketing Request to have the sponsor determine the phenotype of L314F and L314I in an HCV GT3a replicon.

CONCLUSIONS

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

Overall, the SOF/VEL 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 addition, the NGS analyses results reported by the sponsor were in agreement with the results generated by DAVP, with a few exceptions. In general, most disagreements occurred at low frequency where differences in program parameters likely contributed to these discrepancies. However, there were a few disagreements that occurred at high frequency at positions known to be resistance-associated and so substitutions that occurred at known positions were counted if they were detected by any of the variant detectors used (including one by the sponsor and three by DAVP). For VEL resistance, the predominant resistance-associated substitutions that were detected across all four phase 3 trials were Y93H/N (n=28: 19 in GT3a, 5 in GT1a, 3 in GT1b, and 1 in GT1c), Q30R/H (GT1a and b) or A30A30V/K (GT3) (n=5: 3 in GT1a or b and 2 in GT3a), P58L/T/A (GT3a) or H58P/R/D (GT1a) (n=5, 3 in GT3a and 2 in GT1a) H85Y (GT3a) or S85N (GT1a) (n=4, 2 each for GT3a and GT1a) and M28V/T (n=3, 2 in GT3a and 1 in GT1a). For SOF resistance, the predominant resistance-associated substitutions that were detected across all four phase 3 trials were L159F (n=9, 7 in GT3a and 1 each in GT1a and 1b), S282N/T/G (n=6, 4 in GT3a and 1 each in GT1a and 1b), and L320F/I (n=4, all 4 in GT3a). Importantly, 6 L159F, 3 S282N, and 1 L320F resistance-associated substitutions were detected in subjects enrolled in the SOF+RBV 24 Week group of ASTRAL-3, indicating that longer exposure to SOF may produce more resistance-associated substitutions in those whole fail treatment.

Of note, resistance-associated substitutions L314F/I were detected in the NS5B polymerase protein of HCV viruses from four subjects infected with HCV GT3a who relapsed after treatment ended. Three of the four subjects were in the SOF+RBV 24 Week group of ASTRAL-3 and were exposed to longer durations of SOF, which may have selected for this newly detected resistance-associated substitution. One treatment-naïve subject from the FDC 12 Week group of ASTRAL-3 also developed this

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208341 SDN 002 DATE REVIEWED: 03/21/2016

substitution. Of note, this substitution was not detected in any subject at baseline but was detected at a frequency of greater than 30% at the final post-treatment timepoint. In addition, position L314 is highly conserved among HCV GT3 isolates with 100% conservation reported by the sponsor at that position. Low frequency analysis at this position identified one treatment-naïve subject (02127-64256) infected with HCV GT3a who relapsed in the SOF/VEL 12 Week group of ASTRAL-4 (GS-US-342-1137) whose virus had L314P at 3.5% at the post-treatment timepoint. Clinical virology will add this resistance-associated substitution to the label and make a Post Marketing Request to have the sponsor determine the phenotype of L314F and L314I in an HCV GT3a replicon.

Taken together the results of this independent analysis confirm that SOF/VEL was highly effective against all HCV GTs, and has a high resistance to barrier. However, additional substitutions should be evaluated to determine if these impact the efficacy of SOF/VEL FDC. These include: NS5B_L314F/I/P in HCV GT3a.

For complete labeling details, please see the review of NDA 208341 SDN 000 by Senior Clinical Virology Reviewer Lisa Naeger, Ph.D.

POST MARKETING RECOMMENDATIONS

1. Phenotypic assessment of NS5B_L314F, NS5B_L314I, and NS5B_L314P in the HCV GT3a replicon

ADMINISTRATIVE

Reviewer's Signature(s)

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

Concurrence(s)

HFD-530/Clin Micro TL/J O'Rear

Date: _____

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

APPENDICES

METHODS (Copied from the NDA)

Resistance Analysis Methodologies

A full description of the resistance analysis methodologies is summarized in the virology analysis plan (PC-342-2001). A brief description is summarized below.

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

Deep sequencing of full-length HCV NS3/4A, NS5A, and NS5B coding regions was performed by (b) (4) (b) (4) or the (b) (4) (b) (4) using reverse transcriptase polymerase chain reaction (RT-PCR) and then deep sequencing using the Illumina MiSeq deep sequencing platform. The NS3/4A, NS5A and NS5B sequences were utilized to confirm the results of HCV genotyping and subtyping by the INNO-LiPA assay (Innogenetics) performed at screening. All deep sequencing analyses of NS3/4A, NS5A, and NS5B regions were conducted with 1% and/or 15% cutoffs.

Table A1. Overview of Gilead's deep sequencing analysis pipeline (Table 3, page 11, Virology Analysis Plan).

| Step | Description |
|------|-------------|
| | (b) (4) |

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/s/

ERIC F DONALDSON
03/24/2016

JULIAN J O REAR
03/24/2016