CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

206619Orig1s000

MICROBIOLOGY / VIROLOGY REVIEW(S)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
CLINICAL VIROLOGY REVIEW (ADDENDUM)
NDA: 206619 **SDN**: 000 (Original NDA) **REVIEW COMPLETED:** 11/17/2014
Clinical Virology Reviewer: Patrick R. Harrington, Ph.D.

NDA#: 206619 **SDN:** 000 (Original NDA)
Reviewer's Name(s): Patrick R. Harrington, Ph.D.

Sponsor: AbbVie, Inc.
1 N. Waukegan Road, Dp. PA77/Bldg. AP30
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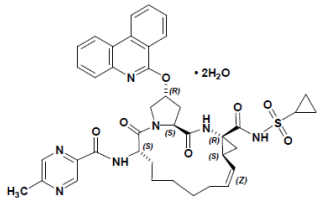
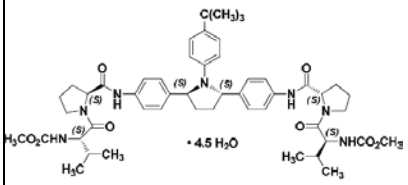
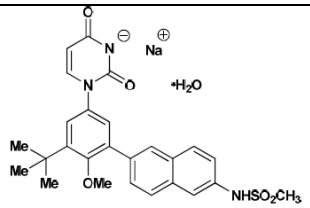
Troy ZumBrunnen, PharmD
Director, Regulatory Affairs

Initial Complete Submission Dates:
Correspondence Date: 4/21/2014
CDER Receipt Date: 4/21/2014
Assigned Date: 4/22/2014
PDUFA Date: 12/21/2014

Proprietary Name	**Viekira Pak™ (combination product: paritaprevir/ritonavir, ombitasvir and dasabuvir)**		
Individual Drug Names [class]	paritaprevir (PTV, ABT-450) [NS3/4A protease inhibitor]	ombitasvir (OMB, ABT-267) [NS5A inhibitor]	dasabuvir (DBV, ABT-333) [non-nucleoside NS5B-palm polymerase inhibitor]
Individual IND #s	103526	108434	101636
Chemical Names	(2R,6S,12Z,13aS,14aR,16aS)-N-(Cyclopropylsulfonyl)-6-{[(5-methylpyrazin-2-yl)carbonyl] amino}-5,16-dioxo-2-(phenanthridin-6-yloxy)-1,2,3,6,7,8,9,10,11,13a,14,15,16,16atetradecahydrocyclopropa[e]pyrrolo[1,2-a][1,4] diazacyclopentadecine-14a(5H)-carboxamide dihydrate	Dimethyl ([(2S,5S)-1-(4-tert-butylphenyl)pyrrolidine-2,5-diyl]bis{benzene-4,1-diylcarbamoyl(2S)pyrrolidine-2,1-diyl[(2S)-3-methyl-1-oxobutane-1,2-diyl]}) biscarbamate hydrate	Sodium 3-(3-tert-butyl-4-methoxy-5-{6-[(methylsulfonyl)amino] naphthalen-2-yl}phenyl)-2,6-dioxo-3,6-dihydro-2H-pyrimidin-1-ide hydrate (1:1:1)
Structures	 **paritaprevir**	 **ombitasvir**	 **dasabuvir**
Molecular Formulas	$C_{40}H_{43}N_7O_7S \cdot 2H_2O$	$C_{50}H_{67}N_7O_8 \cdot 4.5H_2O$	$C_{26}H_{26}N_3O_5S{\bullet}Na{\bullet}H_2O$ (salt); $C_{26}H_{27}N_3O_5S$ (acid)
Molecular Weights	765.88 (anhydrate), 801.91 (hydrate)	894.11 (anhydrate); 975.20 (hydrate)	533.57 (salt); 493.57 (acid)

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Amendments/submissions covered in this addendum (SDN/eCTD): 036/035 (M14-004 report), 039/038 (revised labeling), 041/040 (response to requests for additional data/information from M14-004), 045/044 (updated labeling), 053/051 (agreed PMRs/PMCs), 054/052 (updated labeling)

Related/Supporting Documents:

- Previously reviewed NDA 206619 SDNs/eCTDs: 001/000, 002/001 (Presubmission), 003/002 (Presubmission), 004/003 (Completed NDA), 007/006 (Response to 5/7/2014 request), 008/007 (Response to 5/12/2014 request), 010/009 (Preliminary data from liver transplant trial M12-999), 018/017 (Updated SVR data), 021/020 (Response to 7/17/2014 request), 022/021 (Sponsor's mid-cycle meeting minutes), 024/023 (Updated report and datasets from liver transplant trial M12-999), 027/026 (Updated draft labeling), 030/029 (120-day safety update), 032/031 (Response to initial labeling edits), 033/032 (Response to 8/27/2014 request), 034/033 (Response to 9/11/2014 request)

Dosage Form and Route of Administration: 12.5 mg/75 mg/50 mg (ombitasvir/paritaprevir/ritonavir) fixed-dose combination tablet, 250 mg dasabuvir tablet; Oral
Dispensed: Rx **x** OTC__

Proposed Indication(s): Treatment of chronic HCV GT1 infection in adults, including those with compensated cirrhosis, who are either treatment-naïve or previously treated with pegylated interferon (pegIFN) and ribavirin

Abbreviations: 3TC, lamivudine; ARV, antiretroviral; ATV, atazanavir; DAA, direct acting antiviral agent; DBV, dasabuvir; EOT, end of treatment; FDC, fixed-dose combination; FTC, emtricitabine; GT, genotype; HCV, hepatitis C virus; HIV(-1), human immunodeficiency virus (type 1); LLOQ, lower limit of quantification; NRTI, nucleos(t)ide reverse transcriptase inhibitor; OMB, ombitasvir; Peg-IFNα, pegylated interferon alfa; P/R, pegylated interferon alfa plus ribavirin; PTV, paritaprevir; PTW, post-treatment week; rtv or /r, ritonavir; RAL, raltegravir; RBV, ribavirin; RT-PCR, reverse transcription polymerase chain reaction; SOF, sofosbuvir; SVR, sustained virologic response; TD, target detected; TDF, tenofovir disoproxil fumarate; TND, target not detected; vBT, virologic breakthrough; VF, virologic failure;

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CLINICAL VIROLOGY REVIEW ADDENDUM

1. INTRODUCTION

The purpose of this review addendum is to document the following:

- Clinical Virology review of clinical trial M14-004 (HCV/HIV-1 coinfected subjects) interim analysis
- Additional exploratory analysis of clinical trial M11-099 (subjects with cirrhosis)
- Final agreed labeling for Section 12.4 Microbiology
- Final agreed PMRs/PMCs related to Clinical Virology

2. REVIEW OF M14-004 INTERIM ANALYSIS

2.1 Summary of Trial Design and Study Population

Clinical trial M14-004 is a Phase 2/3, randomized, open-label study evaluating the safety and efficacy of the 3-DAA + RBV regimen dosed for 12 or 24 weeks in subjects with chronic HCV genotype 1 infection and human immunodeficiency virus type 1 (HIV-1) coinfection. The study consists of a Phase 2 pilot cohort (Part 1a and Part 1b) and a Phase 3 cohort (Part 2).

This report covers the Part 1a pilot phase. Eligible subjects included those who were either naïve to anti-HCV therapy or Peg-IFNα/RBV treatment-experienced. Subjects with compensated cirrhosis were also eligible.

With respect to HIV-related eligibility criteria, eligible subjects in Part 1a were to have unquantifiable plasma HIV-1 RNA (HIV-1 RNA <40 copies/mL) based on the Abbott RealTime™ HIV-1 Assay and a $CD4^+$ T-cell count ≥200 cells/mm^3 or $CD4^+$ T-cell % ≥14% while on a stable antiretroviral (ARV) treatment regimen. Because of drug interaction concerns with the anti-HCV regimen (including DAAs and ritonavir), the only eligible HIV-1 ARV regimens in the original protocol included ritonavir-boosted atazanavir (ATV, HIV-1 protease inhibitor) or raltegravir (RAL, integrase strand transfer inhibitor) in combination with the following nucleos(t)ide reverse transcriptase inhibitors (NRTIs): tenofovir disoproxil fumarate (TDF) plus emtricitabine (FTC) or TDF plus lamivudine (3TC). The protocol has since been amended to add a second pilot cohort (Part 1b) of subjects on a darunavir-based (HIV-1 protease inhibitor) ARV regimen. Eligible ARV regimens in Part 2 will depend on the results from Part 1b, and the overall design of Part 2 with respect to anti-HCV treatment regimen(s), treatment duration and study population is still under discussion.

Because the HIV-1 protease inhibitor ritonavir is included as a PK enhancer in Viekira Pak™, any HCV/HIV-1 coinfected patients treated with Viekira Pak™ ± RBV should be on a suppressive ARV regimen to prevent exposure to functional ritonavir HIV-1 monotherapy and the selection of HIV-1 populations with protease inhibitor resistance-associated substitutions.

2.2 Anti-HCV Efficacy and Resistance

A total of 63 subjects were treated in Part 1a, with 31 subjects randomized to Arm A (12-week duration) and 32 subjects randomized to Arm B (24-week duration). Randomization was stratified by prior HCV treatment history and the presence or absence of cirrhosis. Anti-HCV treatment-naïve subjects were further stratified by IL28B rs12979860 genotype, and P/R treatment-experienced

subjects were further stratified by prior P/R treatment response (null response, partial response, relapse). The primary efficacy endpoint was SVR12, defined as HCV RNA <LLOQ 12 weeks following treatment. Resistance analyses were conducted for subjects who experienced virologic failure but the data were not submitted for independent review.

Efficacy results are summarized in Table 1. SVR12 was achieved in 29/31 (94%) subjects in the 12-week treatment arm and 29/32 (91%) subjects in the 24-week treatment arm, with no clear difference in SVR12 rates among key subgroups according to treatment arm.

Table 1. SVR12 rates (intent-to-treat) for M14-004 Part 1a. vBT, virologic breakthrough

	SVR12	
Group	**12-Week Arm**	**24-Week Arm**
All Subjects	**29/31 (94%)**	**29/32 (91%)**
Genotype 1a	25/27 (93%)	26/29 (90%)
Genotype 1b	4/4 (100%)	3/3 (100%)
Genotype 1a, cirrhotic	5/6 (83%) *{1 relapse}*	4/5 (80%) *{1 vBT}*
Genotype 1a, non-cirrhotic	20/21 (95%) *{1 Pre-DC/LTFU}*	22/24 (92%) *{2 relapse, poss. re-infection}*

Table 2 summarizes the 5 subjects who did not achieve SVR12 in Part 1a. Four of these subjects experienced virologic failure (3 relapse, 1 breakthrough). All 4 virologic failure subjects were infected with HCV genotype 1a. One subject (101902) withdrew consent during treatment with HCV RNA <LLOQ Target Not Detected at the last visit (Treatment Week 10). Additional analyses, described in greater detail below, indicated that two of the subjects who experienced virologic relapse (Subjects 101907 and 101908, both in 24-week arm) possibly were not true relapsers to anti-HCV therapy, but rather were re-infected with another HCV strain during the post-treatment follow-up period. To this reviewer's knowledge this would be highly unusual for an HCV DAA clinical trial, particularly so early following treatment.

Table 2. Subjects who did not achieve SVR12 in M14-004 Part 1a. vBT, virologic breakthrough; VF, virologic failure

USUBJID	Arm	Type of Failure	HCV GT	Tx-History	Cirrhosis?	IL28B GT
M14004-15601-101902	12-Week	Non-VF	1A	Naïve	N	TT
M14004-33471-105901	12-Week	Relapse	1A	P/R Null	Y	TT
M14004-15601-101907	24-Week	Relapse/Re-inf.	1A	Naïve	N	CT
M14004-15601-101908	24-Week	Relapse/Re-inf.	1A	Naïve	N	CC
M14004-9818-119906	24-Week	vBT	1A	P/R Null	Y	TT

Table 3 summarizes the HCV RNA results from Subjects 101907 and 101908, which clearly indicated the confirmed detection of high levels of HCV RNA during the follow-up period. There are multiple lines of evidence supporting possible re-infection rather than post-treatment relapse for these subjects. First, it is somewhat unexpected that these two subjects experienced virologic relapse as both were non-cirrhotic, treatment-naïve, and received a 24-week duration of therapy. Among *cirrhotic* treatment-naïve subjects in Phase 3 clinical trial M13-099, virologic relapse occurred in only 1/56 (2%) subjects who received 24 weeks of 3-DAA + RBV treatment, and relapse would likely be even less common for noncirrhotic subjects receiving 24 weeks of treatment. The other two subjects who experienced virologic failure in M14-004 Part 1a were both prior P/R null responders with

cirrhosis, which is a patient population that tended to have a higher rate of virologic failure in Phase 3 clinical trial M13-099.

Table 3. HCV RNA data for Subjects 101907 and 101908, who were possibly re-infected with HCV during the follow-up period.

USUBJID	Visit Day	Visit	HCV RNA (IU/mL)
M14004-15601-101907	-28	DAY 1/BASELINE	1,860,000
	1	DAY 1/BASELINE	565,000
	6	WEEK 1	2,560
	17	WEEK 2	<25 IU/ML HCV RNA DETECTED
	29	WEEK 4	HCV RNA NOT DETECTED
	44	WEEK 6	HCV RNA NOT DETECTED
	57	WEEK 8	HCV RNA NOT DETECTED
	70	WEEK 10	HCV RNA NOT DETECTED
	84	WEEK 12	HCV RNA NOT DETECTED
	114	WEEK 16	HCV RNA NOT DETECTED
	142	WEEK 20	HCV RNA NOT DETECTED
	169	WEEK 24	HCV RNA NOT DETECTED
	183	PTW2	HCV RNA NOT DETECTED
	198	PTW4	HCV RNA NOT DETECTED
	225	PTW8	14,200,000
	238	PTW8	16,300,000
	252	PTW12	12,500,000
M14004-15601-101908	-32	DAY 1/BASELINE	4,190,000
	1	DAY 1/BASELINE	11,200,000
	9	WEEK 1	94
	16	WEEK 2	HCV RNA NOT DETECTED
	28	WEEK 4	HCV RNA NOT DETECTED
	43	WEEK 6	HCV RNA NOT DETECTED
	57	WEEK 8	HCV RNA NOT DETECTED
	71	WEEK 10	HCV RNA NOT DETECTED
	85	WEEK 12	HCV RNA NOT DETECTED
	113	WEEK 16	HCV RNA NOT DETECTED
	142	WEEK 20	HCV RNA NOT DETECTED
	170	WEEK 24	HCV RNA NOT DETECTED
	185	PTW2	HCV RNA NOT DETECTED
	198	PTW4	HCV RNA NOT DETECTED
	225	PTW8	HCV RNA NOT DETECTED
	254	PTW12	38,200,000
	261	PTW12	21,000,000

Nucleotide sequencing and phylogenetic analysis of HCV DAA target genes from Baseline and Post-Treatment isolates are consistent with possible HCV genotype 1a reinfection during the follow-up period for Subjects 101907 and 101908. As shown in Table 4 (Report pg. 63), across all three DAA target genes the nucleotide sequence identity between Baseline and Post-Treatment isolates ranged from 90-94%, which is lower than the 98-99% sequence identity observed between Baseline and Post-Treatment isolates from virologic failure Subjects 105901 and 119906. This is further illustrated in phylogenetic analyses of each drug target gene; data for NS5B as a representative example are shown in Figure 1 (modified from Report pg. 6048). Nucleotide sequences from Post-treatment isolates from Subjects 101907 and 101908 did not cluster with sequences from the respective Pre-treatment isolates, but rather appeared to align as if they were different isolates from different subjects.

Table 4. Nucleotide sequence identity between Baseline and Virologic failure HCV isolates from subjects who experienced virologic failure in M14-004 Part 1a.

	Nucleotide Identity (%)		
Subject	**NS3**	**NS5A**	**NS5B**
105901	99	98	99
119906	99	99	99
101907	92	91	94
101908	91	90	93

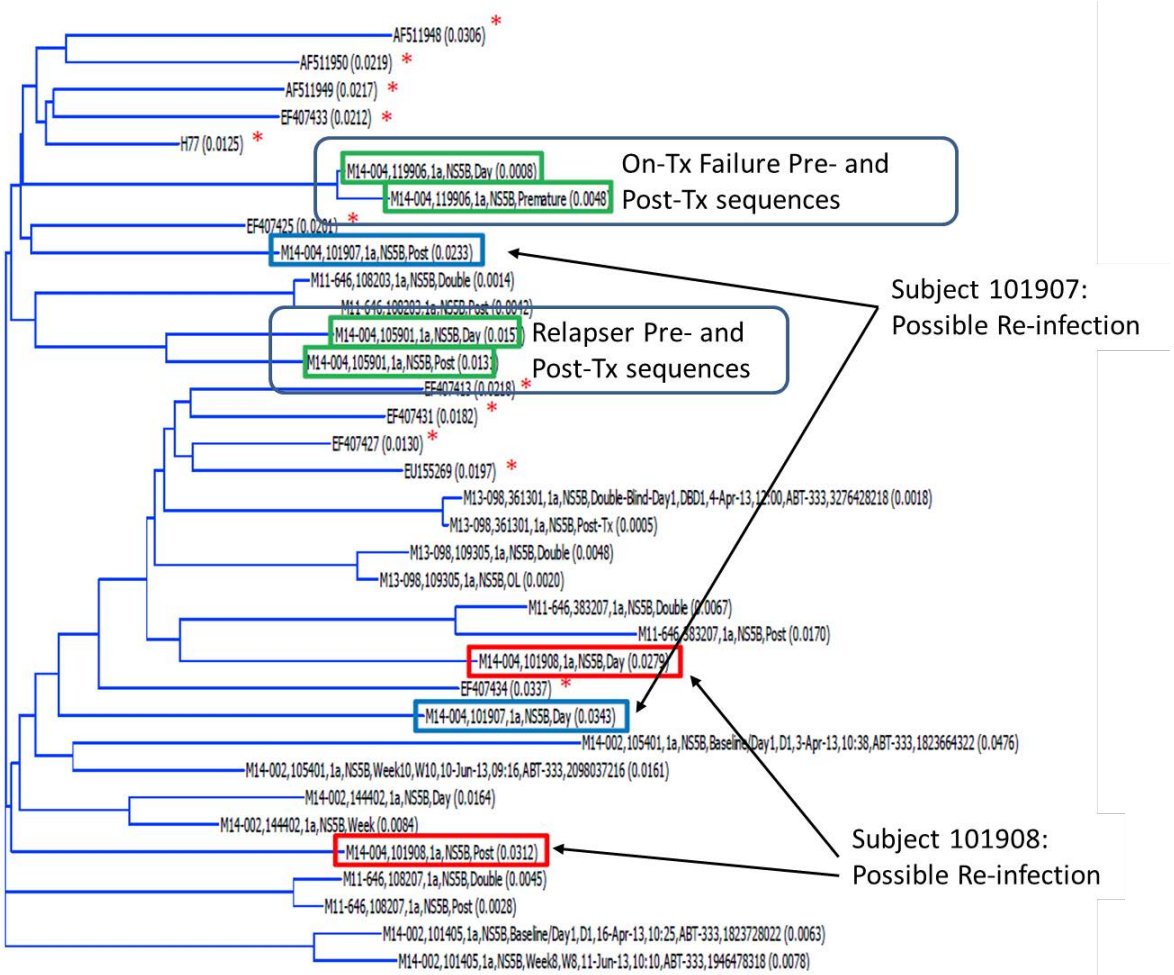

Figure 1. Phylogenetic analysis of NS5B sequences for Baseline and Virologic failure HCV isolates from subjects who experienced virologic failure in M14-004 Part 1a. Sequences from other HCV genotype 1a isolates and reference strains are included for comparison.

Finally, analyses of specific HCV amino acid positions associated with resistance to the HCV DAAs are also consistent with reinfection, based on lack of clear treatment-emergent resistance, rather than post-treatment virologic relapse for Subjects 101907 and 101908. Among HCV genotype 1a infected subjects in Phase 2b/3 trials who received the 3-DAA +/- RBV regimen for 12 or 24 weeks and

experienced virologic failure, 93%, 86% and 53% of subjects had treatment-emergent resistance-associated substitutions in ≥1 drug target, ≥2 drug targets, and all 3 drug targets, respectively (see the 'parent' Clinical Virology review of NDA 206619). Although M14-004 Subjects 105901 and 119906 had treatment-emergent resistance-associated substitutions in all 3 drug targets, no sponsor-defined resistance-associated substitutions emerged in any drug target for Subjects 101907 and 101908 (Table 5; Report pg. 62). Note that NS3 Q80K was found to possibly impact treatment efficacy when detected as a natural polymorphism, but no subjects in the Phase 2b/3 trials of 3-DAA +/- RBV regimens had treatment-emergent Q80K and therefore it is not considered a common treatment-emergent substitution with this regimen. Also note that in genotype 1a NS5A H58 is considered "wild-type". These results seem to rule out the possibility that pre-existing coinfection with multiple different HCV strains explains the phylogenetic analysis results illustrated in Figure 1.

Table 4. Treatment-emergent HCV resistance-associated substitutions for subjects who experienced virologic failure in M14-004 Part 1a.

Subject	Part 1a Arm	Genotype	Reason for Nonresponse	Timepoint Sequenced	NS3	NS5A	NS5B
105901	A	1a	Relapse at PTW4	Baseline	Q80K	None[b]	None[b]
				PTW4[a]	Q80K, D168V, P334S	M28T	S556G
119906	B	1a	Rebound at TW16	Baseline	Q80K	None[b]	None[b]
				PTW2[a]	V55I, Q80K, I132I/V, R155K	Q30R	S556G
101907	B	1a	Relapse at PTW8	Baseline	none[b]	H58P	None[b]
				PTW8[a]	Q80K	P58H	None[b]
101908	B	1a	Relapse at PTW12	Baseline	none[b]	None[b]	None[b]
				PTW12[a]	Q80K	None[b]	None[b]

a. The sample closest in time after virologic failure with an HCV RNA level ≥ 1,000 IU/mL where a product could be amplified.

b. None = variants at resistance-associated amino acid positions were not detected.

According to additional descriptive information provided by the sponsor, Subject 101907 denied any injection drug use, but reported having unprotected anal intercourse throughout the treatment and post-treatment period with a single partner who also had chronic HCV infection. Similarly, Subject 101908 denied any injection drug use but reported having unprotected anal intercourse with multiple sexual partners throughout the post-treatment period, although the subject was unaware of the HCV or HIV serostatus of recent sexual partners. HCV is believed to be transmissible among men who have sex with men (e.g., CDC 2011; MMWR 22; 60(28): 945-50).

Another possible explanation for these observations for Subjects 101907 and 101908 is that Baseline and/or Post-Treatment isolates were mistakenly analyzed from the wrong subjects. We have recommended that the sponsor conduct nucleotide sequence analyses of additional pre-treatment and post-treatment isolates from these subjects to rule out this possibility and confirm reinfection (data to be submitted at a later date). Nevertheless, efficacy of the 3-DAA + RBV regimen has been

demonstrated in subjects with HCV/HIV-1 coinfection, and the precise cause of "relapse" for Subjects 101907 and 101908 has no impact on this conclusion.

2.3 Summary of HIV-1 Outcomes

Plasma HIV-1 RNA levels and $CD4^+$ T cell levels were monitored throughout the study. A total of 28 (44%) subjects were on ATV-based ARV therapy and 35 (56%) were on RAL-based ARV therapy during the start of anti-HCV therapy. One subject (102906) switched from ATV to RAL during treatment while HIV-1 RNA levels were consistently <40 copies/mL. No subjects changed background NRTIs during the anti-HCV treatment period. According to the sponsor, no subjects in either treatment arm were required to switch ARV regimens due to loss of HIV-1 RNA suppression.

HIV-1 RNA Levels

A pooled analysis of HIV-1 RNA levels was conducted to identify subjects with quantifiable (≥40 copies/mL) HIV-1 RNA levels either at the last available on anti-HCV treatment visit or at the last available post-treatment follow-up visit (Table 5). One subject who did not have available follow-up data (101902) had HIV-1 RNA <40 copies/mL at all available visits.

Table 5. HIV-1 RNA levels for the last available anti-HCV on-treatment or follow-up visits.

	Last On-Tx Visit	Last Follow-Up Visit
HIV-1 RNA <40 copies/mL	61/63 (97%)	60/63 (95%)
HIV-1 RNA ≥40 copies/mL	2/63 (3%)	2/63 (3%)
Missing/Lost to Follow-Up	none	1/63 (2%)

A total of 4 subjects had HIV-1 RNA ≥40 copies/mL at the end of anti-HCV treatment or at the end of follow-up. None of the 4 subjects switched their ARV regimen during the study, and the 2 subjects with HIV-1 RNA ≥40 copies/mL (all values <200 copies/mL) at the end of anti-HCV treatment re-suppressed their HIV-1 RNA to <40 copies/mL during follow-up. HIV-1 RNA levels for all available visits for the 2 subjects with HIV-1 RNA ≥40 copies/mL at the end of follow-up are shown in Table 6.

Table 6. Subjects with HIV-1 ≥40 copies/mL at the end of follow-up.

USUBJID	Day	Visit	HIV-1 RNA (copies/mL)	ATV/RAL	NRTIs
M14004-15601-101903	-31	DAY 1/BASELINE	NO HIV-1 RNA DETECTED	RAL	TDF/FTC
M14004-15601-101903	1	DAY 1/BASELINE	NO HIV-1 RNA DETECTED	RAL	TDF/FTC
M14004-15601-101903	16	WEEK 2	NO HIV-1 RNA DETECTED	RAL	TDF/FTC
M14004-15601-101903	31	WEEK 4	NO HIV-1 RNA DETECTED	RAL	TDF/FTC
M14004-15601-101903	57	WEEK 8	NO HIV-1 RNA DETECTED	RAL	TDF/FTC
M14004-15601-101903	85	WEEK 12	NO HIV-1 RNA DETECTED	RAL	TDF/FTC
M14004-15601-101903	115	WEEK 16	NO HIV-1 RNA DETECTED	RAL	TDF/FTC
M14004-15601-101903	141	WEEK 20	NO HIV-1 RNA DETECTED	RAL	TDF/FTC
M14004-15601-101903	171	WEEK 24	NO HIV-1 RNA DETECTED	RAL	TDF/FTC
M14004-15601-101903	197	PTW4	NO HIV-1 RNA DETECTED	RAL	TDF/FTC
M14004-15601-101903	253	PTW12	7,016	RAL	TDF/FTC
M14004-15601-101903	268	PTW12	1,164	RAL	TDF/FTC
M14004-15601-101903	283	PTW12	218	RAL	TDF/FTC
M14004-6246-102904	-36	DAY 1/BASELINE	NO HIV-1 RNA DETECTED	RAL	TDF/FTC
M14004-6246-102904	-1	DAY 1/BASELINE	<40 CP/ML HIV-1 RNA DETECTED	RAL	TDF/FTC
M14004-6246-102904	16	WEEK 2	<40 CP/ML HIV-1 RNA DETECTED	RAL	TDF/FTC
M14004-6246-102904	30	WEEK 4	NO HIV-1 RNA DETECTED	RAL	TDF/FTC
M14004-6246-102904	56	WEEK 8	<40 CP/ML HIV-1 RNA DETECTED	RAL	TDF/FTC
M14004-6246-102904	84	WEEK 12	NO HIV-1 RNA DETECTED	RAL	TDF/FTC

M14004-6246-102904	112	WEEK 16	NO HIV-1 RNA DETECTED	RAL	TDF/FTC
M14004-6246-102904	140	WEEK 20	NO HIV-1 RNA DETECTED	RAL	TDF/FTC
M14004-6246-102904	168	WEEK 24	NO HIV-1 RNA DETECTED	RAL	TDF/FTC
M14004-6246-102904	202	PTW4	2,562	RAL	TDF/FTC
M14004-6246-102904	210	PTW4	158	RAL	TDF/FTC
M14004-6246-102904	232	PTW12	446	RAL	TDF/FTC
M14004-6246-102904	294	PTW12	114	RAL	TDF/FTC

Eight other subjects had at least one study visit with HIV-1 RNA ≥40 copies/mL. Of these 8 subjects, 4 had transient/unconfirmed HIV-1 RNA levels ≥40 copies/mL but <200 copies/mL, 3 subjects had confirmed HIV-1 RNA levels ≥40 copies/mL but <200 copies/mL, and 1 subject had a confirmed HIV-1 RNA level ≥40 copies/mL with a single result ≥200 copies/mL (5,989 copies/mL). All 8 subjects had re-suppression of HIV-1 RNA to <40 copies/mL without changing their ARV regimens.

HIV-1 Resistance Analyses

Only one subject (101903, RAL-based therapy, see Table 6) met the criteria for HIV-1 resistance testing (HIV-1 RNA ≥500 copies/mL on confirmatory testing), which occurred at the anti-HCV Post-Treatment Week 12 visit. Based on the Monogram GenoSure® Prime assay, the subject's virus is susceptible to the drugs in the ARV regimen (RAL, TDF and FTC). The ARV resistance-associated substitutions detected in this subject included V189V/I in reverse transcriptase, and E35D and I62V in protease. None of these substitutions are known to confer resistance to any of the drugs in the subject's ARV regimen.

The presence of ritonavir in the anti-HCV regimen could feasibly select for HIV-1 protease inhibitor resistance-associated substitutions, although HIV-1 RNA levels in this subject were <40 copies/mL (and Target Not Detected) through at least Post-Treatment Week 4. Furthermore, no pre-HCV treatment HIV-1 protease sequence data are available to identify substitutions that emerged as a result of ritonavir selective pressure, and according to the ritonavir prescribing information E35D and I62V are not known to be major resistance-associated substitutions. Therefore, based on the totality of information it is not possible to attribute the detection of HIV-1 protease substitutions E35D and I62V to the use of ritonavir in the anti-HCV treatment regimen.

$CD4^+$ T Cell and Lymphocyte Levels

Overall there was a trend of lower $CD4^+$ T cell counts during the 3-DAA + RBV treatment period in both M14-004 treatment arms, with some of the changes particularly large for individual subjects (Table 7; Report pg. 55). According to the sponsor, 3 subjects (105906, 112901, and 115902; all in 24-week arm) had $CD4^+$ T cell counts <200 cells/mm^3 or <14% during treatment. All 3 subjects achieved HCV SVR12 and continued to have their HIV-1 RNA suppressed <40 copies/mL with no changes to their ARV regimen. None of the subjects had treatment-emergent AIDS-related opportunistic infections, although one subject (105906) received antimicrobial prophylaxis for *Pneumocystis* pneumonia. Based on these summary results complete analysis datasets were requested to conduct an independent review of $CD4^+$ T cell changes in M14-004.

Table 7. Summary of changes in CD4+ T cell levels and percentage in M14-004.

Parameter		Baseline		Change from Baseline		
Timepoint Part 1a Arm	N	Mean	Median	Mean ± SD	Median	Min – Max
CD4+ T-Cell Count (cells/mm^3)						
TW12						
Arm A	27	623.9	609.0	–107.8 ± 181.2	–47.0	–636 – 290
Arm B	32	625.3	575.5	–109.5 ± 213.4	–67.5	–1119 – 144
TW24						
Arm B	29	614.3	561.0	–89.1 ± 129.4	–62.0	–436 – 200
PTW12						
Arm A	30	631.0	611.5	46.7 ± 145.3	25.0	–233 – 488
Arm B	32	625.3	575.5	20.6 ± 107.6	50.5	–283 – 179
CD4+ T-Cell Percentage (%)						
TW12						
Arm A	27	31.1	29.8	1.9 ± 2.8	1.9	–2.1 – 7.7
Arm B	32	29.2	29.5	1.5 ± 3.5	1.0	–7.3 – 7.8
TW24						
Arm B	29	28.7	28.9	3.4 ± 2.6	3.4	–1.1 – 9.5
PTW12						
Arm A	30	31.2	29.9	0.2 ± 2.5	0.2	–5.0 – 4.5
Arm B	32	29.2	29.5	0.7 ± 3.2	0.4	–5.5 – 6.4

Figure 2A illustrates the changes in CD4$^+$ T cell levels over time in M14-004 (cubic spline curve). These results illustrate a modest but clear decline in CD4$^+$ T cell levels during the 3-DAA + RBV treatment period, with a longer period of CD4$^+$ T cell decline in the 24-week treatment arm compared to the 12-week treatment arm. In general, CD4$^+$ T cell levels tended to recover to Baseline levels by approximately 4-weeks post-treatment.

Interestingly, it appears that CD4$^+$ T cell declines during 3-DAA + RBV treatment were non-specific, as CD4$^+$ T cell percentages did not decline during treatment, but in fact tended to increase during the treatment period followed by a return to Baseline levels during the post-treatment period (Figure 2B).

This finding is further illustrated in Figure 3, which shows that treatment with the 3-DAA + RBV regimen was associated with a modest decline in total lymphocyte levels. Consistent with the CD4$^+$ T cell dynamics, a longer period of total lymphocyte decline was apparent in the 24-week treatment arm compared to the 12-week treatment arm.

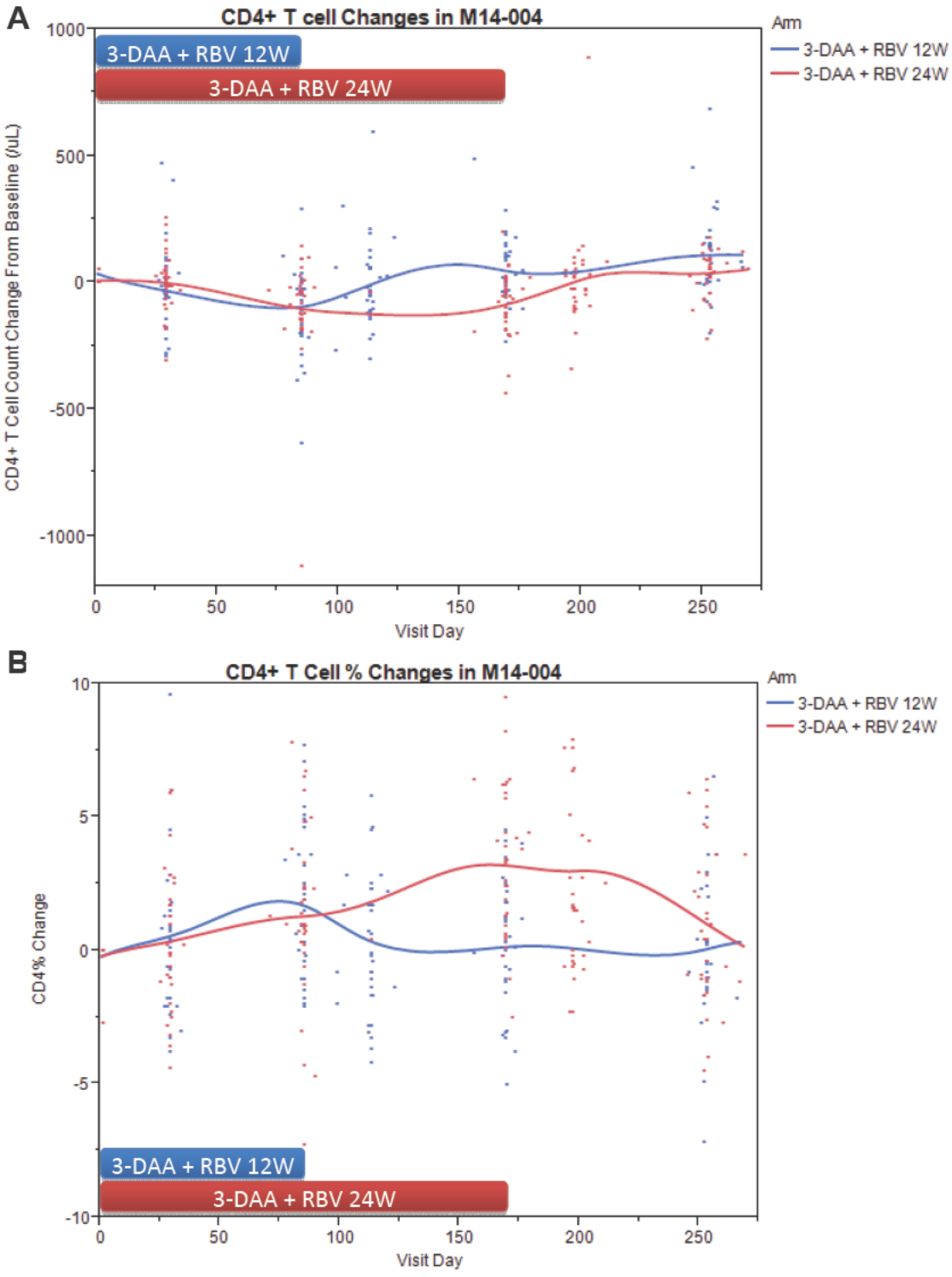

Figure 2. Changes in $CD4^+$ T cell levels (Panel A) and percentage (Panel B) over time in M14-004. Subjects who prematurely discontinued treatment (by >1 week) were censored from this analysis. One $CD4^+$ % result of +31.1% (24-week Arm, Post-Treatment Week 4) is not shown in the figure with the y-axis cutoff of +/-10%.

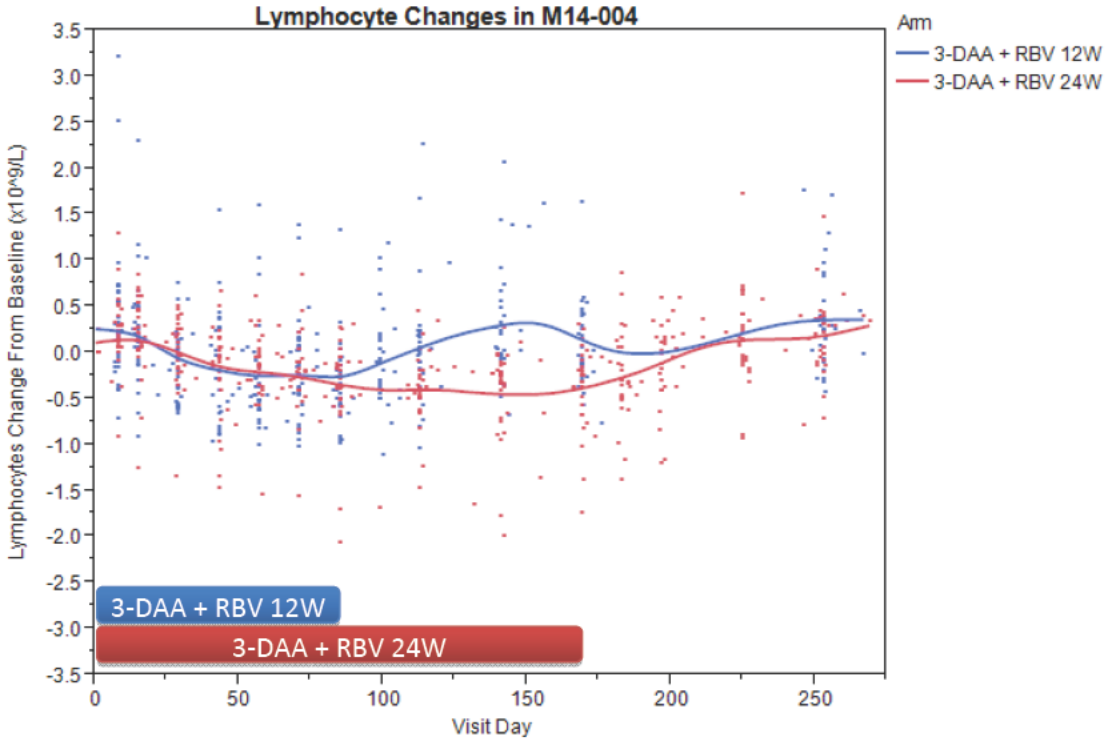

Figure 3. Changes in lymphocyte levels over time in M14-004. Subjects who prematurely discontinued treatment (by >1 week) were censored from this analysis.

Table 8 summarizes the changes in $CD4^+$ T cell levels, $CD4^+$ T cell percentages, and lymphocyte levels at key timepoints in clinical trial M14-004.

Table 8. Summary of changes in $CD4^+$ T cell levels, $CD4^+$ T cell percentages, and lymphocyte levels in M14-004. n/a, not applicable; PTW, post-treatment week; TW, treatment week

	3-DAA + RBV 12W					3-DAA + RBV 24W				
	N	**Median**	**Mean**	**Min**	**Max**	**N**	**Median**	**Mean**	**Min**	**Max**
$CD4^+$ T Cells (/uL)										
TW 12	27	-47.0	-107.8	-636	290	32	-67.5	-109.5	-1119	144
TW 24	0	n/a	n/a	n/a	n/a	29	-62.0	-89.1	-436	200
Last Tx Visit	31	-47.0	-107.6	-636	290	32	-59.5	-93.8	-436	200
PTW 4	30	-35.5	-2.5	-304	596	32	-4.0	2.1	-375	888
$CD4^+$ T Cell Percentage (%)										
TW 12	27	1.9	1.9	-2.1	7.7	32	1.0	1.5	-7.3	7.8
TW 24	0	n/a	n/a	n/a	n/a	29	3.4	3.4	-1.1	9.5
Last Tx Visit	31	1.5	1.6	-3.3	7.7	32	3.4	3.3	-4.7	9.5
PTW 4	30	0.3	0.3	-4.2	5.8	32	1.6	2.7	-4.4	31.1
Total Lymphocytes (x10^9/L)										
TW 12	28	-0.44	-0.35	-0.98	1.32	32	-0.27	-0.35	-2.07	0.31
TW 24	0	n/a	n/a	n/a	n/a	30	-0.29	-0.39	-1.74	0.31
Last Tx Visit	31	-0.42	-0.35	-0.98	1.32	32	-0.26	-0.37	-1.74	0.31
PTW 4	30	0.03	0.08	-1.04	2.26	32	0	-0.10	-1.20	0.60

The sponsor speculated that the decrease in lymphocyte levels during treatment in clinical trial M14-004 was likely due to the inclusion of RBV in the treatment regimen. The sponsor noted that a mean decrease in absolute lymphocyte levels of ~400 cells/µL was observed in the RBV-containing arm but not the RBV-free arm in clinical trial M13-961 (3-DAA +/- RBV in HCV genotype 1b infected subjects).

Independent analyses of clinical trials of combination DAA +/- RBV regimens were conducted to assess the impact RBV on lymphocyte levels; $CD4^+$ T cell levels are not monitored routinely in subjects without HIV-1 coinfection. As shown in Figure 4, across two different AbbVie 3-DAA +/- RBV clinical trials, lymphocyte levels declined by an average of ~400 cells/µL in treatment arms that included RBV but not in RBV-free arms.

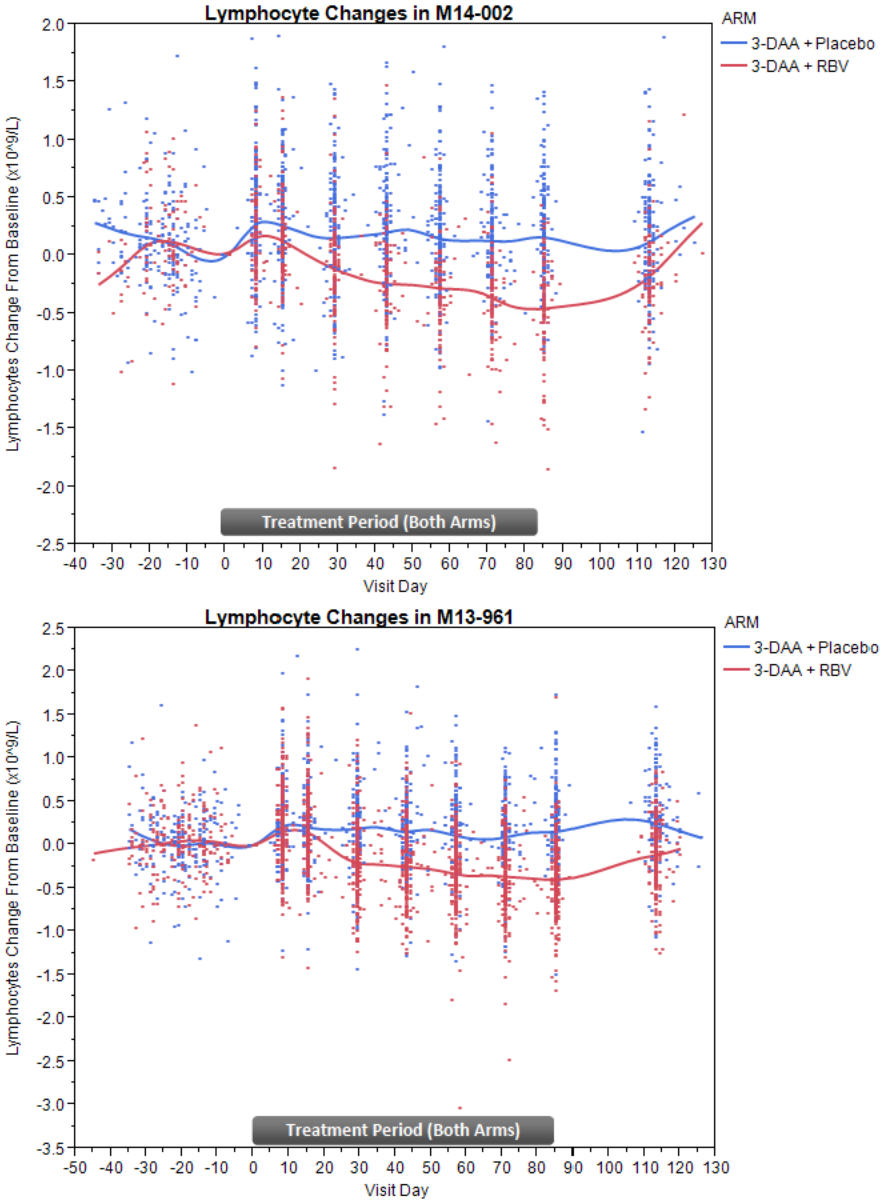

Figure 4. Changes in lymphocyte levels over time in AbbVie clinical trials M14-002 (HCV genotype 1a subjects) and M13-961 (HCV genotype 1b subjects). Subjects who prematurely discontinued treatment (by >1 week) were censored from this analysis.

Similar declines in lymphocyte levels during treatment with an RBV-containing DAA regimen was observed in a Phase 3 trial of Harvoni™ (sofosbuvir {uridine nucleotide analogue NS5B polymerase inhibitor} plus ledipasvir {NS5A inhibitor}) (Figure 5).

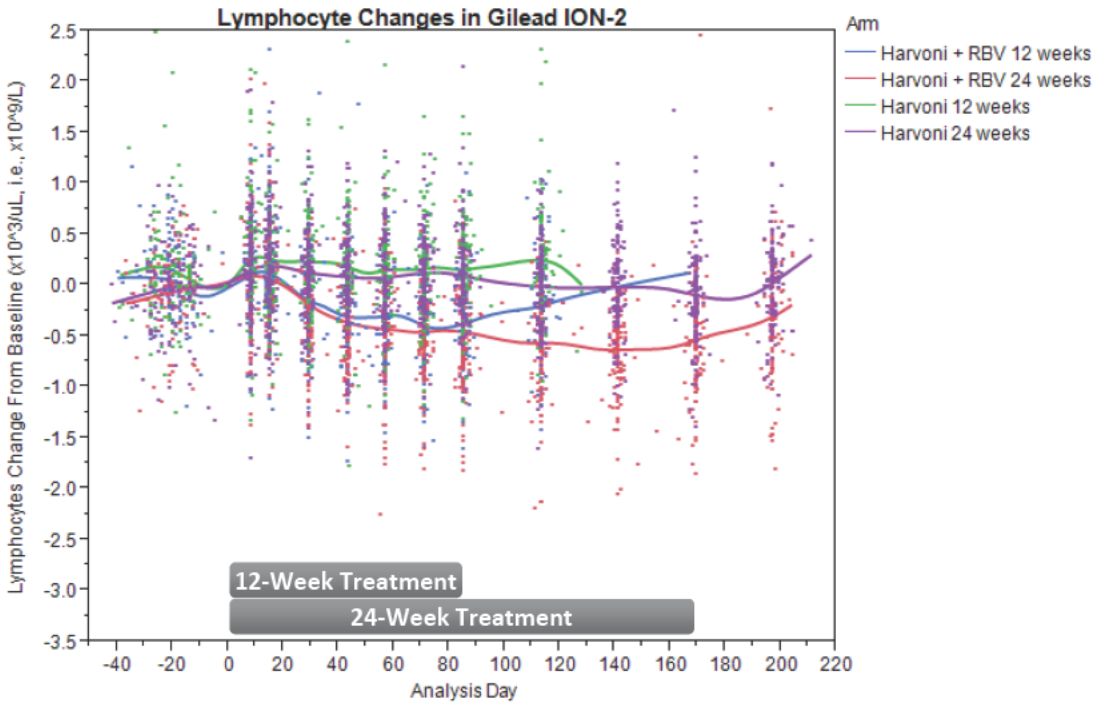

Figure 5. Changes in lymphocyte levels over time in the Gilead ION-2 trial, which studied Harvoni™ (sofosbuvir plus ledipasvir) dosed with or without RBV for 12 or 24 weeks. Subjects who prematurely discontinued treatment (by >1 week) were censored from this analysis.

Table 9 summarizes the median changes in lymphocyte levels at key timepoints in these AbbVie and Gilead DAA +/- RBV trials. Within each of the four trial/duration groups shown, the end-of-treatment changes in lymphocyte levels were significantly different between the DAA-only and DAA+RBV groups ($p<0.0001$, Wilcoxon test).

Table 9. Median changes in lymphocyte levels ($x10^9$/L) from baseline in AbbVie and Gilead DAA +/- RBV trials.

DAA +/- RBV Clinical Trial	Last Treatment Visit				Post-Treatment Week 4			
	DAAs Only (No RBV)		DAAs + RBV		DAAs Only (No RBV)		DAAs + RBV	
	N	Median	N	Median	N	Median	N	Median
AbbVie M14-002 (12W)	205	0.15	100	-0.42	193	0.11	96	-0.17
AbbVie M13-961 (12W)	209	0.11	210	-0.41	204	0.195	202	-0.115
Gilead ION-2 (12W)	109	0.13	111	-0.39	107	0.2	111	-0.2
Gilead ION-2 (24W)	109	-0.07	111	-0.54	107	0.07	109	-0.29

The totality of data from DAA +/- RBV trials in HCV mono-infected and HCV/HIV-1 co-infected subjects indicate that inclusion of RBV in a DAA treatment regimen causes a decline in absolute

lymphocyte levels. The safety implications of this observation are unclear to this reviewer, but at minimum care providers in the setting of HCV/HIV-1 co-infection should be aware that treatment with an RBV-containing DAA regimen can cause a decline in $CD4^+$ T cell levels that reflects a decline in absolute lymphocyte levels and not specific $CD4^+$ T cell loss.

3. ADDITIONAL EXPLORATORY ANALYSIS OF M13-099 (subjects with cirrhosis)

Extensive discussions have been held between the review team and the sponsor regarding the optimal duration of 3-DAA + RBV treatment for HCV genotype 1a patients with cirrhosis based on clinical trial M13-099. Previous analyses documented in the parent Clinical Virology review showed that among HCV genotype 1a subjects treated for 12 weeks in M13-099, all relapses occurred in subjects who were either prior P/R partial and null responders, or treatment-naïve with the IL28B rs12979860 CT or TT genotype, indicating these groups likely benefit from 24 weeks of treatment duration and perhaps all other subjects could be successfully treated with a 12 week duration. Nevertheless, these analyses were not necessarily statistically rigorous, and the review team did not feel that these factors (e.g., IL28B genotyping) would be used in standard clinical practice to guide treatment decisions. Furthermore, treatment failure with the 3-DAA + RBV regimen is often associated with the emergence of drug resistance-associated substitutions in NS3 and NS5A that could make re-treatment particularly challenging, supporting the intent of optimizing treatment efficacy for all subjects. Please see the biometrics review by Joy Mele, M.S., and the clinical review by Russ Fleischer, PA-C, MPH, for additional details.

An additional exploratory analysis was conducted to evaluate the relationship between on-treatment HCV RNA level and treatment outcome for HCV genotype 1a subjects in the 12-week treatment arm of M13-099. As shown in Table 10, HCV RNA levels were quantifiable (≥25 IU/mL) at Treatment Week 1 for 11/11 (100%) subjects who experienced virologic relapse with 12 weeks of treatment; one relapser did not have data available at Week 1. Most relapsers had HCV RNA target detected (TD; above or below LLOQ) at Treatment Week 2. Nearly all subjects had HCV RNA <25 IU/mL at Treatment Week 4, with HCV RNA levels for eventual relapsers falling across all three categories of Week 4 response. Given the results based on Week 1 response, and the fact that LLOQ is a more reproducible cutoff than TD versus TND, it seems like HCV RNA <LLOQ at Week 1 could be one criterion to identify a subset of cirrhotic HCV genotype 1a subjects who are unlikely to benefit from a treatment duration longer than 12 weeks, although again it is unclear if this approach would be consistently and appropriately applied in clinical practice. Furthermore, in this analysis ~88% of subjects with quantifiable HCV RNA at Treatment Week 1 achieved SVR with only 12 weeks of treatment.

Table 10. Relapse rate in M13-099 GT1a 12-week arm according to on-treatment viral RNA level at early timepoints. Censored analysis excluding: premature discontinuation, on-treatment failure, and non-virologic failure. TND, target not detected; TD, target detected

	Relapse rate according to HCV RNA level @ the following timepoints		
On-Tx HCV RNA	**Week 1**	**Week 2**	**Week 4**
<25 IU/mL TND	0/7 (0%)	2/35 (6%)	7/103 (7%)
<25 IU/mL TD	0/32 (0%)	7/71 (10%)	4/28 (14%)
≥25 IU/mL	11/95 (12%)	3/27 (11%)	1/3 (33%)
GT1a 24-week arm: single relapser with HCV RNA ≥25 at WK1, TND at WK2 and WK4			

4. FINAL AGREED LABELING: SECTION 12.4 MICROBIOLOGY

The final agreed Microbiology section of the label (12.4 Microbiology) is pasted below. Note that additional Clinical Virology-related edits have been suggested and incorporated into other sections of the label as appropriate.

12.4 Microbiology

Mechanism of Action

VIEKIRA PAK combines three direct-acting antiviral agents with distinct mechanisms of action and non-overlapping resistance profiles to target HCV at multiple steps in the viral lifecycle.

Ombitasvir

Ombitasvir is an inhibitor of HCV NS5A, which is essential for viral RNA replication and virion assembly. The mechanism of action of ombitasvir has been characterized based on cell culture antiviral activity and drug resistance mapping studies.

Paritaprevir

Paritaprevir is an inhibitor of the HCV NS3/4A protease which is necessary for the proteolytic cleavage of the HCV encoded polyprotein (into mature forms of the NS3, NS4A, NS4B, NS5A, and NS5B proteins) and is essential for viral replication. In a biochemical assay, paritaprevir inhibited the proteolytic activity of recombinant HCV genotype 1a and 1b NS3/4A protease enzymes with IC_{50} values of 0.18 nM and 0.43 nM, respectively. Paritaprevir inhibited the activity of NS3/4A enzymes from single isolates of genotypes 2a, 2b, 3a, and 4a with IC_{50} values of 2.4 nM, 6.3 nM, 14.5 nM, and 0.16 nM, respectively.

Dasabuvir

Dasabuvir is a non-nucleoside inhibitor of the HCV RNA-dependent RNA polymerase encoded by the NS5B gene, which is essential for replication of the viral genome. In a biochemical assay, dasabuvir inhibited a panel of genotype 1a and 1b NS5B polymerases with median IC_{50} values of 2.8 nM (range 2.4 nM to 4.2 nM; n = 3) and 3.7 nM (range 2.2 nM to 10.7 nM; n = 4), respectively. Based on drug resistance mapping studies of HCV genotypes 1a and 1b, dasabuvir targets the palm domain of the NS5B polymerase, and is therefore referred to as a non-nucleoside NS5B-palm polymerase inhibitor. Dasabuvir had reduced activity in biochemical assays against NS5B polymerases from HCV genotypes 2a, 2b, 3a and 4a (IC_{50} values ranging from 900 nM to >20 μM).

Antiviral Activity

Ombitasvir

The EC_{50} values of ombitasvir against genotype 1a-H77 and 1b-Con1 strains in HCV replicon cell culture assays were 14.1 pM and 5 pM, respectively. The median EC_{50} values of ombitasvir against HCV replicons containing NS5A genes from a panel of genotype 1a and 1b isolates from treatment-naïve subjects were 0.68 pM (range 0.35 to 0.88 pM; n = 11) and 0.94 pM (range 0.74 to 1.5 pM; n = 11), respectively. Ombitasvir had EC_{50} values of 12 pM, 4.3 pM, 19 pM, 1.7 pM, 3.2

pM, and 366 pM against chimeric replicons constructed with NS5A from single isolates representing genotypes 2a, 2b, 3a, 4a, 5a, and 6a, respectively.

Paritaprevir

The EC_{50} values of paritaprevir against genotype 1a-H77 and 1b-Con1 strains in the HCV replicon cell culture assay were 1.0 nM and 0.21 nM, respectively. The median EC_{50} values of paritaprevir against HCV replicons containing NS3 genes from a panel of genotype 1a and 1b isolates from treatment-naïve subjects were 0.68 nM (range 0.43 nM to 1.87 nM; n = 11) and 0.06 nM (range 0.03 nM to 0.09 nM; n = 9), respectively. Paritaprevir had an EC_{50} value of 5.3 nM against the HCV genotype 2a-JFH-1 replicon cell line, and EC_{50} values of 19 nM, 0.09 nM, and 0.68 nM against replicon cell lines containing NS3 from a single isolate each of genotype 3a, 4a, and 6a, respectively.

In HCV replicon cell culture assays, ritonavir did not exhibit a direct antiviral effect and the presence of ritonavir did not affect the antiviral activity of paritaprevir.

Dasabuvir

The EC_{50} values of dasabuvir against genotype 1a-H77 and 1b-Con1 strains in HCV replicon cell culture assays were 7.7 nM and 1.8 nM, respectively. The median EC_{50} values of dasabuvir against HCV replicons containing NS5B genes from a panel of genotype 1a and 1b isolates from treatment-naïve subjects were 0.6 nM (range 0.4 nM to 2.1 nM; n = 11) and 0.3 nM (range 0.2 nM to 2 nM; n = 10), respectively.

Combination Antiviral Activity

Evaluation of pairwise combinations of ombitasvir, paritaprevir, dasabuvir and ribavirin in HCV genotype 1 replicon cell culture assays showed no evidence of antagonism in antiviral activity.

Resistance

In Cell Culture

Exposure of HCV genotype 1a and 1b replicons to ombitasvir, paritaprevir or dasabuvir resulted in the emergence of drug resistant replicons carrying amino acid substitutions in NS5A, NS3, or NS5B, respectively. Amino acid substitutions in NS5A, NS3, or NS5B selected in cell culture or identified in Phase 2b and 3 clinical trials were phenotypically characterized in genotype 1a or 1b replicons.

For ombitasvir, in HCV genotype 1a replicons single NS5A substitutions M28T/V, Q30E/R, L31V, H58D, and Y93C/H/L/N reduced ombitasvir antiviral activity by 58- to 67,000-fold. In genotype 1b replicons, single NS5A substitutions L28T, L31F/V, and Y93H reduced ombitasvir antiviral activity by 8- to 661-fold. In general, combinations of ombitasvir resistance-associated substitutions in HCV genotype 1a or 1b replicons further reduced ombitasvir antiviral activity.

For paritaprevir, in HCV genotype 1a replicons single NS3 substitutions F43L, R155G/K/S, A156T, and D168A/E/F/H/N/V/Y reduced paritaprevir antiviral activity by 7- to 219-fold. An NS3 Q80K substitution in a genotype 1a replicon reduced paritaprevir antiviral activity by 3-fold. Combinations of V36M, Y56H, or E357K with R155K or D168 substitutions reduced the activity of paritaprevir by an additional 2- to 7-fold relative to the single R155K or D168 substitutions in

genotype 1a replicons. In genotype 1b replicons single NS3 substitutions A156T and D168A/H/V reduced paritaprevir antiviral activity by 7- to 159-fold. The combination of Y56H with D168 substitutions reduced the activity of paritaprevir by an additional 16- to 26-fold relative to the single D168 substitutions in genotype 1b replicons.

For dasabuvir, in HCV genotype 1a replicons single NS5B substitutions C316Y, M414I/T, E446K/Q, Y448C/H, A553T, G554S, S556G/R, and Y561H reduced dasabuvir antiviral activity by 8- to 1,472-fold. In genotype 1b replicons, single NS5B substitutions C316H/N/Y, S368T, N411S, M414I/T, Y448C/H, A553V, S556G and D559G reduced dasabuvir antiviral activity by 5- to 1,569-fold.

In Clinical Studies

In a pooled analysis of subjects treated with regimens containing ombitasvir, paritaprevir, and dasabuvir with or without ribavirin (for 12 or 24 weeks) in Phase 2b and Phase 3 clinical trials, resistance analyses were conducted for 64 subjects who experienced virologic failure (20 with on-treatment virologic failure, 44 with post-treatment relapse). Treatment-emergent substitutions observed in the viral populations of these subjects are shown in Table 8. Treatment-emergent substitutions were detected in all 3 HCV drug targets in 30/57 (53%) HCV genotype 1a infected subjects, and 1/6 (17%) HCV genotype 1b infected subjects.

Table 8. Treatment-Emergent Amino Acid Substitutions in the Pooled Analysis of VIEKIRA PAK with and without Ribavirin Regimens (12- or 24-week durations) in Phase 2b and Phase 3 Clinical Trials

Target	Emergent Amino Acid Substitutions	Genotype 1a N = 58[a] % (n)	Genotype 1b N = 6 % (n)
NS3	Any of the following NS3 substitutions: V36A/M/T, F43L, V55I, Y56H, Q80L, I132V, R155K, A156G, D168(any), P334S, S342P, E357K, V406A/I, T449I, P470S, V23A (NS4A)	88 (51)	67 (4)
	V36A/M/T[b]	7 (4)	--
	V55I[b]	7 (4)	--
	Y56H[b]	10 (6)	50 (3)
	I132V[b]	7 (4)	--
	R155K	16 (9)	--
	D168 (any)[d]	72 (42)	67 (4)
	D168V	59 (34)	50 (3)
	P334S[b,c]	7 (4)	--
	E357K[b,c]	5 (3)	17 (1)
	V406A/I[b,c]	5 (3)	--
	T449I[b,c]	5 (3)	--
	P470S[b,c]	5 (3)	--
	NS4A V23A[b]	--	17 (1)

	F43L[b], Q80L[b], A156G, S342P[b,c]	<5%	--
NS5A	Any of the following NS5A substitutions: K24R, M28A/T/V, Q30E/K/R, H/Q54Y, H58D/P/R, Y93C/H/N	78 (45)	33 (2)
	K24R	5 (3)	--
	M28A/T/V	33 (19)	--
	Q30E/K/R	47 (27)	--
	H/Q54Y	--	17(1)
	H58D/P/R	7 (4)	--
	Y93C/N	5 (3)	--
	Y93H	--	33 (2)
NS5B	Any of the following NS5B substitutions: G307R, C316Y, M414I/T, E446K/Q, A450V, A553I/T/V, G554S, S556G/R, G558R, D559G/I/N/V, Y561H	67 (38)	33 (2)
	C316Y	4(2)	17 (1)
	M414I	--	17 (1)
	M414T	5 (3)	17 (1)
	A553I/T/V	7 (4)	--
	S556G/R	39 (22)	17 (1)
	D559G/I/N/V	7 (4)	--
	Y561H	5 (3)	--
	G307R, E446K/Q, A450V, G554S, G558R	<5%	--

a. N = 57 for the NS5B target.
b. Substitutions were observed in combination with other emergent substitutions at NS3 position R155 or D168.
c. Position located in NS3 helicase domain.
d. D168A/F/H/I/L/N/T/V/Y.

Persistence of Resistance-Associated Substitutions

The persistence of ombitasvir, paritaprevir, and dasabuvir treatment-emergent amino acid substitutions in NS5A, NS3, and NS5B, respectively, was assessed in HCV genotype 1a-infected subjects in Phase 2 trials whose virus had at least 1 treatment-emergent resistance-associated substitution in the drug target, and with available data through at least 24 weeks post-treatment. Population and clonal nucleotide sequence analyses (assay sensitivity approximately 5-10%) were conducted to detect the persistence of viral populations with treatment-emergent substitutions.

For ombitasvir, viral populations with 1 or more resistance-associated treatment-emergent substitutions in NS5A persisted at detectable levels through at least Post-Treatment Week 24 in 24/24 (100%) subjects, and through Post-Treatment Week 48 in 18/18 (100%) subjects with available data.

For paritaprevir, viral populations with 1 or more treatment-emergent substitutions in NS3 persisted at detectable levels through at least Post-Treatment Week 24 in 17/29 (59%) subjects, and through Post-Treatment Week 48 in 5/22 (23%) subjects with available data. Resistance-associated variant R155K

remained detectable in 5/8 (63%) subjects through Post-Treatment Week 24, and in 1/5 (20%) subjects through Post-Treatment Week 48. Resistance-associated D168 substitutions remained detectable in 6/22 (27%) subjects through Post-Treatment Week 24, and were no longer detectable through Post-Treatment Week 48.

For dasabuvir, viral populations with 1 or more treatment-emergent substitutions in NS5B persisted at detectable levels through at least Post-Treatment Week 24 in 11/16 (69%) subjects, and through Post-Treatment Week 48 in 8/15 (53%) subjects with available data. Treatment-emergent S556G persisted through Post-Treatment Week 48 in 6/9 (67%) subjects.

Due to (b) (4) subjects infected with HCV genotype 1b, trends in persistence of treatment-emergent substitutions in this genotype could not be established.

The lack of detection of virus containing a resistance-associated substitution does not indicate that the resistant virus is no longer present at clinically significant levels. The long-term clinical impact of the emergence or persistence of virus containing VIEKIRA PAK-resistance-associated substitutions is unknown.

Effect of Baseline HCV Polymorphisms on Treatment Response

A pooled analysis of subjects in the Phase 3 clinical trials of ombitasvir, paritaprevir, and dasabuvir with or without ribavirin was conducted to explore the association between baseline HCV NS5A, NS3, or NS5B resistance-associated polymorphisms and treatment outcome. Baseline samples from HCV genotype 1a infected subjects who experienced virologic failure (n=47), as well as samples from a subset of demographically matched subjects who achieved SVR (n=94), were analyzed to compare the frequencies of resistance-associated polymorphisms in these two populations. The NS3 Q80K polymorphism was detected in approximately 38% of subjects in this analysis and was enriched approximately 2-fold in virologic failure subjects compared to SVR-achieving subjects. Ombitasvir resistance-associated polymorphisms in NS5A (pooling data from all resistance-associated amino acid positions) were detected in approximately 22% of subjects in this analysis and similarly were enriched approximately 2-fold in virologic failure subjects. Dasabuvir resistance-associated polymorphisms in NS5B were detected in approximately 5% of subjects in this analysis and were not enriched in virologic failure subjects.

In contrast to the Phase 3 subset analysis, no association of NS3 or NS5A polymorphisms and treatment outcome was seen in an analysis of noncirrhotic HCV genotype 1a-infected subjects (n=174 for NS3 and n=183 for NS5A) who received ombitasvir, paritaprevir, and dasabuvir with or without ribavirin (for 12 or 24 weeks) in a Phase 2b trial.

(b) (4)

Cross-resistance

Cross-resistance is expected among NS5A inhibitors, NS3/4A protease inhibitors, and non-nucleoside NS5B-palm inhibitors by class. Dasabuvir retained full activity against HCV replicons containing a single NS5B S282T substitution, which is associated with resistance to nucleos(t)ide analogue NS5B polymerase inhibitors. In clinical trials of VIEKIRA PAK, no subjects who experienced virologic

failure had treatment-emergent substitutions potentially associated with resistance to nucleot(s)ide analogue NS5B polymerase inhibitors.

The impact of prior ombitasvir, paritaprevir, or dasabuvir treatment experience on the efficacy of other NS5A inhibitors, NS3/4A protease inhibitors, or NS5B inhibitors has not been studied. Similarly, the efficacy of VIEKIRA PAK has not been studied in subjects who have failed prior treatment with another NS5A inhibitor, NS3/4A protease inhibitor, or NS5B inhibitor.

5. FINAL AGREED PMRs/PMCs

The sponsor has agreed to the following virology-related PMR:

Conduct the following site-directed mutant HCV replicon phenotype analyses:

- Sofosbuvir activity against HCV replicons carrying NS5B substitutions associated with dasabuvir resistance: C316Y (GT1a and GT1b) and S556G (GT1a).
- Dasabuvir activity against HCV replicons carrying the following NS5B substitutions: L159F (GT1a and GT1b), V321A (GT1a and GT1b), M423I (GT1a), I482T (GT1a) and A486V (GT1b).
- Paritaprevir activity against HCV replicons carrying substitutions in the NS3 helicase (e.g., P334S, S342P, V406A/I, T449I, P470S) that emerged in virologic failure subjects treated with the 3-DAA ± RBV regimen; evaluate the impact of these substitutions alone and in combination with other key resistance-associated substitutions (e.g., R155K or D168x) that were often detected in combination.

Final Report Submission: 2/2/2015

In addition, the sponsor has agreed to the following PMC:

Submit a complete report for ongoing clinical Study M13-102, “A Follow-up Study to Assess Resistance and Durability of Response to AbbVie Direct-Acting Antiviral Agent (DAA) Therapy in Subjects Who Participated in Phase 2 or 3 Clinical Studies for the Treatment of Chronic Hepatitis C Virus (HCV) Infection.”

Study Completion: 10/31/2016, Final Report Submission: 10/31/2017

6. ADMINISTRATIVE

6.1 Reviewer’s Signature

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Patrick R. Harrington, Ph.D.
Clinical Virology Reviewer, Division of Antiviral Products

6.2 Concurrence

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/s/

PATRICK R HARRINGTON
11/18/2014

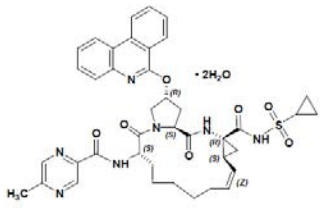
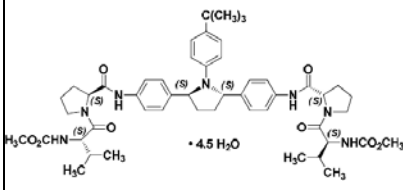
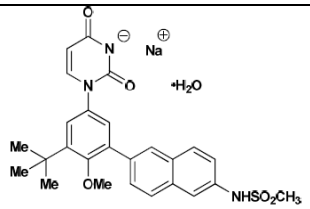
JULIAN J O REAR
11/18/2014

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
CLINICAL VIROLOGY REVIEW
NDA: 206619 **SDN**: 000 (Original NDA) **REVIEW COMPLETED:** 09/12/2014
Clinical Virology Reviewer: Patrick R. Harrington, Ph.D.

NDA#: 206619 **SDN:** 000 (Original NDA)
Reviewer's Name(s): Patrick R. Harrington, Ph.D.

Sponsor: AbbVie, Inc.
1 N. Waukegan Road, Dp. PA77/Bldg. AP30
North Chicago, IL 60064
Troy ZumBrunnen, PharmD
Director, Regulatory Affairs

Initial Complete Submission Dates:
Correspondence Date: 4/21/2014
CDER Receipt Date: 4/21/2014
Assigned Date: 4/22/2014
Review Complete Date: 09/12/2014
PDUFA Date: 12/21/2014

Proprietary Name	**Viekira Pak™ (combination product: paritaprevir/ritonavir, ombitasvir and dasabuvir)**		
Individual Drug Names [class]	paritaprevir (PTV, ABT-450) [NS3/4A protease inhibitor]	ombitasvir (OMB, ABT-267) [NS5A inhibitor]	dasabuvir (DBV, ABT-333) [non-nucleoside NS5B-palm polymerase inhibitor]
Individual IND #s	103526	108434	101636
Chemical Names	(2R,6S,12Z,13aS,14aR,16aS)-N-(Cyclopropylsulfonyl)-6-{[(5-methylpyrazin-2-yl)carbonyl] amino}-5,16-dioxo-2-(phenanthridin-6-yloxy)-1,2,3,6,7,8,9,10,11,13a,14,15,16,16atetradecahydrocyclopropa[e]pyrrolo[1,2-a][1,4] diazacyclopentadecine-14a(5H)-carboxamide dihydrate	Dimethyl ([(2S,5S)-1-(4-tert-butylphenyl)pyrrolidine-2,5-diyl]bis{benzene-4,1-diylcarbamoyl(2S)pyrrolidine-2,1-diyl[(2S)-3-methyl-1-oxobutane-1,2-diyl]}) biscarbamate hydrate	Sodium 3-(3-tert-butyl-4-methoxy-5-{6-[(methylsulfonyl)amino] naphthalen-2-yl}phenyl)-2,6-dioxo-3,6-dihydro-2H-pyrimidin-1-ide hydrate (1:1:1)
Structures	 **paritaprevir**	 **ombitasvir**	 **dasabuvir**
Molecular Formulas	$C_{40}H_{43}N_7O_7S \cdot 2H_2O$	$C_{50}H_{67}N_7O_8 \cdot 4.5H_2O$	$C_{26}H_{26}N_3O_5S{\bullet}Na{\bullet}H_2O$ (salt); $C_{26}H_{27}N_3O_5S$ (acid)
Molecular Weights	765.88 (anhydrate), 801.91 (hydrate)	894.11 (anhydrate); 975.20 (hydrate)	533.57 (salt); 493.57 (acid)

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Amendments: none
Related/Supporting Documents:

- NDA 206619 SDNs/eCTDs (received as of 8/29/2014): 001/000, 002/001 (Presubmission), 003/002 (Presubmission), 004/003 (Completed NDA), 007/006 (Response to 5/7/2014 request), 008/007 (Response to 5/12/2014 request), 010/009 (Preliminary data from liver transplant trial M12-999), 018/017 (Updated SVR data), 021/020 (Response to 7/17/2014 request), 022/021 (Sponsor's mid-cycle meeting minutes), 024/023 (Updated report and datasets from liver transplant trial M12-999), 027/026 (Updated draft labeling), 030/029 (120-day safety update), 032/031 (Response to initial labeling edits), 033/032 (Response to 8/27/2014 request), 034/033 (Response to 9/11/2014 request)

Dosage Form and Route of Administration: 12.5 mg/75 mg/50 mg (ombitasvir/paritaprevir/ritonavir) fixed-dose combination tablet, 250 mg dasabuvir tablet; Oral
Dispensed: Rx **x** OTC__

Proposed Indication(s): Treatment of chronic HCV GT1 infection in adults, including those with compensated cirrhosis, who are either treatment-naïve or previously treated with pegylated interferon (pegIFN) and ribavirin

Abbreviations: ALT, alanine amino transferase; BOC, boceprevir; CC, cytotoxicity concentration; CLIA, Clinical Laboratories Improvement Amendments; DAA, direct acting antiviral agent; DBV, dasabuvir; EC, effective concentration; EOT, end of treatment; HCV, hepatitis C virus; FDC, fixed-dose combination; GLP, good laboratory practice; GT, genotype; HIV(-1), human immunodeficiency virus (type 1); IC, inhibitory concentration; IFN(α), interferon (alfa); ITT, intent-to-teat; LiPA, line-probe assay; LLOQ, lower limit of quantification; LOD, limit of detection; OMB, ombitasvir; Peg-IFNα, pegylated interferon alfa; PK, pharmacokinetic; PM, polymorphism; P/R, pegylated interferon alfa plus ribavirin; PTV, paritaprevir; PTW, post-treatment week; rtv or /r, ritonavir; RBV, ribavirin; RT-PCR, reverse transcription polymerase chain reaction; SMV, simeprevir; SOF, sofosbuvir; SVR, sustained virologic response; TND, target not detected; TVR, telaprevir; UTR, untranslated region; VF, virologic failure;

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DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
CLINICAL VIROLOGY REVIEW
NDA: 206619 **SDN**: 000 (Original NDA) **REVIEW COMPLETED:** 09/12/2014
Clinical Virology Reviewer: Patrick R. Harrington, Ph.D.

EXECUTIVE SUMMARY

1. RECOMMENDATIONS

1.1 Recommendation and Conclusion on Approvability

This Original NDA is approvable from a Clinical Virology perspective for the treatment of chronic HCV genotype 1 infected patients who are either naïve to prior anti-HCV therapy or who have failed prior therapy with Peg-IFNα/RBV, including patients with compensated cirrhosis.

1.2 Recommendation on Phase 4 (Post-Marketing) Commitments, Agreements, and/or Risk Management Steps, if Approvable.

This reviewer recommends the following post-marketing commitments or requirements, as appropriate:

1) Please conduct site-directed mutant phenotype analyses of sofosbuvir against HCV replicons carrying the following NS5B substitutions associated with dasabuvir resistance: C316Y (GT1a and GT1b), S556G (GT1a).

2) Please conduct site-directed mutant phenotype analyses of dasabuvir against HCV genotype 1a and 1b replicons carrying the following sofosbuvir treatment-emergent substitutions: L159F and V321A.

3) Please conduct site-directed mutant phenotype analyses of dasabuvir against HCV replicons carrying the following substitutions that are associated with resistance to certain non-nucleoside NS5B-thumb2 polymerase inhibitors: M423I (GT1a), I482T (GT1a) and A486V (GT1b).

4) Please conduct paritaprevir phenotypic analyses of substitutions in the NS3 helicase (e.g., P334S, S342P, V406A/I, T449I, P470S) that emerged in virologic failure subjects treated with the 3-DAA +/- RBV regimen. Evaluate the impact of these substitutions alone and in combination with other key resistance-associated substitutions (e.g., R155K or D168x) that were often detected in combination.

5) Please submit the complete study reports from Phase 3 trials, including complete data from later follow-up timepoints for HCV RNA levels and persistence of resistance-associated substitutions.

2. SUMMARY OF OND VIROLOGY ASSESSMENTS

2.1 Nonclinical Virology

Paritaprevir
Paritaprevir (PTV, ABT-450) inhibits the HCV NS3/4A protease, which results in inhibition of HCV replication. In a biochemical assay, PTV at sub-nanomolar concentrations inhibited the activity of recombinant NS3/4A protease enzymes from genotype 1a and genotype 1b HCV laboratory strains. In cell culture studies, PTV inhibited the replication of stable HCV genotype 1a (H77) and 1b (Con1)

replicons with 50% effective concentration (EC_{50}) values of 0.94 nM and 0.32 nM, respectively. PTV had consistent, sub- to low nanomolar cell culture anti-HCV activity against panels of transient HCV replicons carrying NS3 genes from genotype 1a or 1b clinical isolates. The cell culture anti-HCV activity of PTV against genotype 1a and 1b stable replicons was reduced in the presence of 40% human plasma by 24- and 27-fold, respectively. The PTV 50% cytotoxicity concentration (CC_{50}) value for HCV genotype 1b replicon cells was 37 µM, reflecting a therapeutic index of 39,000-116,000 for HCV genotype 1a and 1b replicons. In cell culture HCV replicon assays, PTV generally had non-antagonistic combination anti-HCV activity relationships with ombitasvir (OMB, ABT-267), dasabuvir (DBV, ABT-333), ribavirin (RBV), RBV + ritonavir, and interferon-α. Ritonavir, an HIV-1 protease inhibitor that is used as a PK enhancer for PTV and has no anti-HCV activity, had no impact on the PTV EC_{50} value against an HCV genotype 1b replicon.

Selection of HCV genotype 1a and 1b replicon-harboring cells with PTV resulted in the emergence of HCV replicon cell colonies with reduced susceptibilities to PTV. The predominant NS3 treatment-emergent substitutions detected in genotype 1a replicons were Q41R, R155K, D168E/N and I170T/V. The predominant NS3 treatment-emergent substitutions in genotype 1b replicons were R155Q, A156T/V and D168H/V. In HCV genotype 1a replicons, PTV anti-HCV activity was reduced >3-fold by the following single amino acid substitutions: F43L, R155G/K/S/T/V/W, A156T, and D168A/E/F/H/N/V/Y. The NS3 substitutions V36L/M, Y56H and E357K in combination with R155K or a D168 substitution further reduced HCV susceptibility to PTV by 2- to 7-fold relative to the R155K or D168x single substitutions. An NS3 Q80K substitution, which is a common polymorphism in HCV genotype 1a, conferred ~3-fold reduced HCV genotype 1a susceptibility to PTV. In HCV genotype 1b replicons, PTV anti-HCV activity was reduced by the following single amino acid substitutions: R155K, A156T, and D168A/E/H/K/T/V/Y. Based on overlapping resistance pathways, some degree of cross-resistance between PTV and other approved NS3/4A protease inhibitors is expected. Cross-resistance between PTV and IFNα, RBV and other classes of HCV direct-acting antivirals (DAAs) is not expected.

Ombitasvir

Ombitasvir (OMB, ABT-267) is an NS5A inhibitor. The mechanism of action of OMB has been characterized in cell culture HCV replicon activity and drug resistance selection studies. NS5A inhibitors like OMB inhibit HCV RNA replication and also viral assembly/release. In cell culture studies, OMB inhibited the replication of stable HCV genotype 1a (H77) and 1b (Con1) replicons with EC_{50} values of 14.1 pM and 5.0 pM, respectively. OMB generally had low- to sub-picomolar cell culture anti-HCV activity against panels of transient HCV replicons carrying NS5A genes (encoding amino acids 1-214) from genotype 1a and 1b clinical isolates from DAA treatment-naïve subjects. The cell culture anti-HCV activity of OMB against genotype 1a and 1b stable replicons was reduced in the presence of 40% human plasma by 11- to 13-fold. The OMB CC_{50} value for HCV genotype 1b replicon cells was >32 µM, reflecting a therapeutic index of >2,000,000 for HCV genotype 1a and 1b replicons. OMB generally had non-antagonistic combination anti-HCV activity relationships with PTV, DBV, RBV and interferon-α.

Selection of HCV genotype 1a and 1b replicon-harboring cells with OMB resulted in the emergence of HCV replicon cell colonies with reduced susceptibilities to OMB. Predominant OMB treatment-emergent substitutions in NS5A included M28T/V, Q30R and Y93C/H in genotype 1a replicons, and L28T, L31F/V, Y93H, L28M/V+L31F, L28M+Y93H, R30Q+Y93H, L31F/V+Y93H, and P58A+Y93H in genotype 1b replicons. These substitutions and others associated with NS5A inhibitor resistance conferred reduced HCV replicon susceptibility to OMB. Based on overlapping resistance pathways, some degree of cross-resistance between OMB and other NS5A inhibitors is expected. Cross-resistance between OMB and IFNα, RBV and other classes of HCV DAAs is not expected.

Dasabuvir (DBV, ABT-333, A-998821)
Dasabuvir (DBV, ABT-333) is a first-in-class non-nucleoside inhibitor of the HCV NS5B RNA-dependent, RNA polymerase, targeting the "palm" domain of the NS5B enzyme. In a biochemical assay, DBV at low nanomolar concentrations inhibited the activity of recombinant NS5B polymerase enzymes from genotype 1a and genotype 1b HCV isolates. DBV inhibited the replication of stable HCV genotype 1a (H77) and 1b (Con1 and N) replicons with EC_{50} values of 7.7 nM and 1.8-8.7 nM, respectively. DBV had low to sub-nanomolar cell culture anti-HCV activity against panels of transient HCV replicons carrying NS5B genes from genotype 1a or 1b clinical isolates from DAA treatment-naïve subjects. The cell culture anti-HCV activity of DBV against genotype 1a and 1b stable replicons was reduced in the presence of 40% human plasma by approximately 12- to 17-fold. The DBV CC_{50} value for HCV genotype 1a (H77) and 1b (Con1) replicon cells was approximately 10.3 µM, reflecting a therapeutic index of at least 1,300. In cell culture HCV replicon assays, DBV generally had non-antagonistic combination anti-HCV activity relationships with PTV, OMB, RBV and interferon-α.

Selection of HCV genotype 1a and 1b replicon-harboring cells with DBV resulted in the emergence of HCV replicon cell colonies with reduced susceptibilities to DBV. Several different NS5B amino acid substitutions emerged in the DBV-selected replicons and were associated with reduced HCV replicon susceptibility to DBV. The DBV resistance pathways observed in cell culture, including NS5B substitutions C316Y, M414T, Y448C/H and S556G, are consistent with the targeting of the "palm" domain of the NS5B polymerase. Cross-resistance is expected between DBV and other non-nucleoside NS5B-palm polymerase inhibitors in development. Based on the totality of available evidence from non-clinical virology studies and resistance analyses of clinical trials, cross-resistance is generally not expected between DBV and IFNα, RBV or other classes of HCV DAAs, including other classes of NS5B polymerase inhibitors such as the FDA-approved uridine nucleotide analogue sofosbuvir. Additional phenotype analyses will be requested to further investigate the potential for DBV cross-resistance with other classes of NS5B polymerase inhibitors.

2.2 Clinical Virology

Clinical Virology Review of Efficacy
The efficacy of Viekira Pak™ dosed with or without RBV (referred to throughout this review as "3-DAA +/- RBV") was evaluated in six Phase 3 trials and three Phase 2 trials. With the exception of Phase 2b trial M11-652, the 3-DAA regimen was administered QD as PTV/r/OMB 150 mg/100 mg/25 mg in fixed-dose combination (FDC) tablets dosed QD, plus DBV 250 mg BID. RBV dosing (as appropriate) was weight-based, either 1,000 mg or 1,200 mg daily divided BID per local label. All subjects in these trials were to be chronically infected with HCV genotype 1 (with certain trials subtype-specific) and naïve to HCV DAAs. Efficacy analyses for these trials were primarily based on SVR12, defined as HCV RNA <LLOQ 12 weeks following therapy.

Phase 3 clinical trials M11-646 and M13-098 were placebo-controlled studies that evaluated the 3-DAA + RBV regimen dosed for 12 weeks in noncirrhotic, HCV genotype 1 infected subjects who were either treatment-naïve (M11-646) or pegylated recombinant human interferon alfa plus ribavirin (P/R) treatment-experienced. The overall intent-to-treat (ITT) SVR12 rates for active arms in both trials were approximately 96%. Virologic failure, including on-treatment virologic breakthrough and post-treatment virologic relapse, occurred in approximately 2% of subjects in both trials, with most virologic failures observed in HCV genotype 1a infected subjects.

In Phase 3 trial M13-099, the 3-DAA + RBV regimen was dosed for 12 or 24 weeks in treatment-naïve and P/R treatment-experienced adults with chronic HCV genotype 1 infection and compensated

cirrhosis. The overall intent-to-treat (ITT) SVR12 rates in the 12- and 24-week arms were 91.8% and 95.9%, respectively. Virologic relapse occurred more often in the 12-week arm compared to the 24-week arm (6.3% versus 0.6%), and almost exclusively in HCV genotype 1a infected subjects. In the 12-week duration arm, most occurrences of virologic relapse occurred among HCV genotype 1a infected subjects who were prior P/R null responders, although relapse also occurred in small numbers of subjects in other subgroups, including treatment-naïve IL28B non-CC genotype (rs12979860) subjects and a prior P/R partial responder.

Phase 3 trial M14-002 compared the efficacy of the 3-DAA regimen dosed with or without RBV for 12 weeks, noncirrhotic, treatment-naïve subjects with chronic HCV genotype 1a infection. Intent-to-treat SVR12 rates were 89.7% and 96% for the 3-DAA + Placebo and 3-DAA + RBV groups, respectively. Virologic failure, including virologic breakthrough and relapse, was observed in 16 (7.8%) subjects in the 3-DAA + Placebo group and 2 (2%) subjects in the 3-DAA + RBV group. The 4-month safety update report noted 3 additional subjects in the 3-DAA + Placebo group who experienced relapse between the SVR12 and SVR24 assessments. Virologic failure was more common in the 3-DAA + Placebo group across all IL28B rs12979860 genotypes. Results from this trial clearly indicate that adding RBV to the 3-DAA regimen provides an efficacy benefit in HCV genotype 1a infected patients.

Phase 3 trials M13-961 and M13-389 compared the efficacy of the 3-DAA regimen dosed with or without RBV for 12 weeks in noncirrhotic, HCV genotype 1b infected subjects who were either treatment-naïve (M13-961) or P/R treatment-experienced (M13-389). Across both of these trials virologic failure was not observed in any HCV genotype 1b infected subject treated with the 3-DAA regimen without RBV, indicating that RBV does not provide an efficacy benefit in noncirrhotic HCV genotype 1b infected patients.

Across all Phase 2b/3 trials evaluating the efficacy of the 3-DAA +/- RBV regimen administered for 8-24 weeks, 81/2498 (3.2%) subjects experienced virologic failure, including on-treatment virologic failure and post-treatment virologic relapse (including all known post-SVR12 relapsers). Virologic failure rates differed according to HCV subtype, with rates of 74/1363 (5.4%) and 7/1131 (0.6%) in HCV genotype 1a and 1b infected subjects, respectively, and were slightly lower if considering only subjects enrolled in arms evaluating treatment durations of 12 or 24 weeks. Based on the totality of efficacy data from the sponsor's Phase 2b/3 program, it is clear that the treatment for HCV genotype 1a infected subjects was optimal when all three HCV DAAs were dosed together with RBV for ≥12 weeks. For HCV genotype 1b infected subjects, including RBV with the 3-DAA regimen was not necessary for optimal efficacy in noncirrhotic subjects. However, according to the sponsor's regimen justification, in two ongoing Phase 2 trials virologic failure occurred in 5/118 (4.2%) HCV genotype 1b infected subjects treated with a 2-DAA regimen of PTV/r + OMB (no DBV or RBV), indicating the 2-DAA regimen likely has suboptimal efficacy for HCV genotype 1b compared to the full 3-DAA regimen. No studies have been conducted to determine if HCV genotype 1b cirrhotic patients can receive the 3-DAA regimen without RBV without an impact on treatment efficacy.

SVR12 was generally durable across all clinical trials. Pooling available data from all 6 Phase 3 trials, of those who achieved SVR12 (observed) and had available SVR24 results, 453/454 (99.8%) achieved SVR24. In Phase 2b clinical trial M11-652, among subjects who achieved SVR12 (observed) with any combination DAA +/- RBV regimen in M11-652, SVR24 and SVR48 were achieved in 501/504 (99.4%) and 490/494 (99.1%) subjects with available data, respectively. Based on summary data provided in the 4-month safety update, across all Phase 2 and Phase 3 trials that studied at least 2 of the DAAs included in the 3-DAA combination, a total of 11/2943 (0.4%) SVR12-achieving subjects have experienced confirmed (based on repeated HCV RNA analysis) virologic relapse in the SVR24 window, and 2/2581(0.08%) subjects have experienced confirmed virologic

relapse after achieving SVR24. Complete nucleotide sequence analysis data were not reported for all of these late relapsers to distinguish between late relapse and re-infection; however, at least one of the post-SVR24 relapses reflected true virologic relapse based on >97% nucleotide sequence identity of viral populations at baseline and relapse.

Drug Resistance

For each HCV DAA/target, an analysis of treatment-emergent amino acid substitutions was conducted pooling data from treatment failure subjects who received the 3-DAA +/- RBV regimen in Phase 2 and Phase 3 trials. Treatment-emergent substitutions included (most common substitutions in bold):

NS3/4A:

- Genotype 1a subjects (n=75): V36A/M/T, F43L, V55I, Y56H, Q80L, I132V, **R155K**, A156G, **D168x** (including A, F, H, I, L, N, T, V, Y), P334S, S342P, E357K, V406A/I, T449I, P470S
- Genotype 1b subjects (n=10): **Y56H**, **D168A/V**, E357K, NS4A V23A

NS5A:

- Genotype 1a subjects (n=75): K24R, **M28A/T/V**, **Q30E/K/R**, H58D/P/R, E62D, Y93C/N
- Genotype 1b subjects (n=10): H54Y, **Y93H**

NS5B:

- Genotype 1a subjects (n=74): G307R, C316Y, M414T, E446K/Q, A450V, A553T, G554S, **S556G/R**, G558R, D559G/I/N/V, Y561H, L588F
- Genotype 1b subjects (n=10): C316Y, M414I, S556G (substitutions emerged in only 2 subjects)

Overall, considering subjects who were to receive the 3-DAA +/- RBV regimen for 12 or 24 weeks (8-week duration excluded), NS3/4A, NS5A and NS5B resistance-associated substitutions emerged in viral populations from 87.9%, 77.6% and 66.7% genotype 1a virologic failure subjects, respectively, and in viral populations from 66.7%, 33.3% and 33.3% genotype 1b virologic failure subjects, respectively. Among genotype 1a virologic failure subjects, 93.1% had viral populations with at least 1 treatment-emergent substitution in any drug target, 86.2% with treatment-emergent substitutions in at least 2 viral drug targets, and 52.6% with treatment-emergent substitutions in all 3 viral drug targets.

Analyses of virologic failure subjects from Phase 2 trials of 2- or 3-DAA +/- RBV combination regimens were conducted to evaluate the persistence of viruses with resistance-associated substitutions in NS3, NS5A and NS5B. Among subjects with available data:

- 17/29 (59%) subjects still had viral populations with at least 1 treatment-emergent NS3 substitution detected through at least Post-Treatment Week 24, and 5/22 (23%) through at least Post-Treatment Week 48.
- 24/24 (100%) subjects had viral populations with treatment-emergent NS5A substitutions detected through at least Post-Treatment Week 24, and 18/18 (100%) subjects had viral populations with substitutions detected through at least Post-Treatment Week 48.
- 11/16 (69%) subjects still had viral populations with at least 1 treatment-emergent NS5B substitution detected through at least Post-Treatment Week 24, and 8/15 (53%) through at least Post-Treatment Week 48.

Two sets of analyses were conducted to determine if Baseline polymorphisms in NS3, NS5A or NS5B impacted the efficacy of the 3-DAA +/- RBV regimen in HCV genotype 1a subjects. The first set of

analyses evaluated the proportion Baseline polymorphisms in virologic failure subjects compared to a subset of SVR subjects with available data from the pooled Phase 3 trials. The second set of analyses compared the SVR rates for subjects with or without specific Baseline polymorphisms in a large Phase 2b trial, M11-652. In the pooled Phase 3 analysis, Baseline resistance-associated polymorphisms in NS3 (primarily driven by a Q80K polymorphism) and NS5A were enriched ~2-fold among subjects who experienced virologic failure relative to those who achieved SVR. Polymorphisms in NS5B known to potentially impact ABT-333 anti-HCV activity were rare and were not enriched in virologic failure subjects relative to SVR subjects. In M11-652, NS3 K80 was detected in viral populations from ~41% of subjects and associated with a ~5% overall lower SVR rate for subjects treated with the 3-DAA +/- RBV regimen for 8-24 weeks, although differences in SVR rates were not necessarily consistent across arms. Approximately 20% of HCV genotype 1a subjects treated in M11-652 with the 3-DAA +/- RBV regimen for 8-24 weeks had viral populations detected with 1 or more NS5A resistance-associated polymorphisms, but there was no clear indication that the polymorphisms impacted treatment efficacy. DBV resistance-associated polymorphisms in NS5B were uncommon and not associated with treatment efficacy in M11-652.

3. ADMINISTRATIVE

3.1 Reviewer's Signature

_____
Patrick R. Harrington, Ph.D.
Clinical Virology Reviewer, Division of Antiviral Products

3.2 Concurrence

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Julian J. O'Rear, Ph.D.
Clinical Virology Team Leader, Division of Antiviral Products

OND CLINICAL VIROLOGY REVIEW

1. INTRODUCTION AND BACKGROUND

1.1 Important milestones in product development

Initial Pre-IND and IND submissions for the NS3/4A protease inhibitor paritaprevir (PTV, ABT-450), the NS5A inhibitor ombitasvir (OMB, ABT-267) and the non-nucleoside analogue NS5B-palm polymerase inhibitor dasabuvir (DBV, ABT-333) were as follows:

- Paritaprevir (IND 103526): Pre-IND submission 9/15/2008, Original IND submission 12/19/2008
- Ombitasvir (IND 108434): Original IND submission 6/28/2010
- Dasabuvir (IND 101636): Pre-IND submission 2/8/2008, Original IND submission 5/2/2008

An End-of-Phase 2 meeting to discuss the Phase 3 development of the PTV/r/OMB/DBV combination was held with the sponsor on 10/1/2012 (see Clinical Virology review of IND 103526 SDN 217). This combination regimen dosed with or without ribavirin (RBV) was granted FDA Breakthrough Therapy Designation on 5/1/2013 (see Clinical Virology review of IND 103526 SDN 294). A Pre-NDA face-to-face meeting to discuss the current application was held with the sponsor on 1/29/2014 (see Clinical Virology review of IND 103526 SDN 475).

Note that the generic name for ABT-450, paritaprevir, was accepted during the NDA review cycle near the time of completion of this review. In some instances ABT-450 was used as the drug name in this review.

1.2 Methodology

This section summarizes the key clinical virology procedures that were used for Phase 3 clinical trials to support the Viekira Pak™ NDA. Additional methodologies for other nonclinical and clinical virology analyses are summarized as needed throughout this review.

HCV genotype/subtype determination
Hepatitis C virus (HCV) genotype/subtype determination was based on the Siemens Versant HCV genotype 2.0 line-probe assay (LiPA). This assay identifies different HCV genotypes and some subtypes, including 1a and 1b, based on RT-PCR amplification and reverse-hybridization targeting the HCV 5' untranslated region (UTR) and core region of the HCV genome.

HCV genotypes/subtypes in clinical trials were further characterized by nucleotide sequence and phylogenetic analyses of NS3, NS5A, and/or NS5B sequences to assess for concordance with the clinical laboratory assays noted above.

HCV viral load assessments
HCV RNA levels were determined using the FDA-approved Roche COBAS® TaqMan® HCV v2.0 test, which has a reported lower limit of quantification (LLOQ) of 25 IU/mL, and a limit of detection (LOD) of approximately 15 IU/mL. These analyses were conducted (b) (4)

Resistance-related Assessments
Genotypic resistance assessments included Sanger population nucleotide sequence analysis of HCV NS3/4A, NS5A and NS5B genome regions. For these analyses HCV RNA was purified from plasma or serum samples, and target genes were amplified by RT-PCR followed by nested PCR using primers appropriate for genotype 1a or 1b. Only samples with ≥1,000 IU/mL HCV RNA were amplified to reduce the chances of sampling bias. Nested PCR products were sequenced across both strands under Good Laboratory Practice (GLP) conditions in a Clinical Laboratories Improvement Amendments (CLIA)-certified laboratory. At least two sequencing reads were performed in each direction, providing a minimum of four sequencing reads (i.e., 4X coverage of each identified region). All DNA sequence data were analyzed by two analysts and mixed populations were reported if the minor species accounted for at least 25% of the peak area of the electropherogram. Minor species accounting for lower proportions were reported at the discretion of the analyst if the signal to background ratio permitted an unambiguous call.

Clonal nucleotide sequence analyses were also to be conducted but only limited data were included in the integrated resistance analysis for this initial NDA. This review only considered clonal sequencing data from Phase 2 trials for analyses of persistence of resistance-associated substitutions; see Section 5.6 and Appendix D for additional details. Additional clonal sequencing data will be included in a final integrated resistance report for the 3-DAA +/- RBV regimens, which will be provided in a future submission after all subjects in the Phase 3 trials complete Post-Treatment Week 48 (PTW48).

For the 6 Phase 3 trials, resistance analyses were conducted for subjects who did not achieve a sustained virologic response (SVR12). Baseline samples and samples collected near the time of virologic failure were analyzed. In addition, Baseline samples were analyzed from a subset of subjects who achieved SVR, with 2 SVR-achieving subjects analyzed for every 1 virologic failure subject, with subjects matched by HCV subtype, IL28B genotype, Baseline HCV RNA, and sex to the extent possible. In addition to virologic failure samples, Baseline samples were analyzed for all subjects in Phase 2 trials.

When reading the resistance analysis summaries it is important to be aware that mathematical modeling studies of HCV population dynamics predict that viral subpopulations harboring nearly all combinations of single- and double-nucleotide substitutions are generated every day in HCV-infected subjects. Based on these predictions, it should be assumed that nearly every amino acid substitution possible in the HCV genome is present in at least a small minority of circulating HCV variants within a given subject, even prior to any drug exposure. Therefore, for the purposes of this review, the "presence" or "detection" of an HCV amino acid substitution in a clinical specimen by population nucleotide sequence analysis implies that the substitution was highly enriched in the patient's mixed HCV population. Conversely, because population-based sequence analyses typically cannot detect minority populations that contribute <20% of the total population, the absence or lack of detection of a given substitution does not necessarily mean it does not exist or was not enriched in the subject. Rather, the substitution may be present but at a level below the limit of detection of the assay.

1.3 Prior FDA Virology reviews

This is the original NDA submission and initial Clinical Virology review of Viekira Pak™, covering the combination of PTV, OMB and DBV. The sponsor has not requested regulatory approval of the individual components of this combination product, with the exception of the pharmacokinetic (PK) enhancer, ritonavir (rtv), which is an approved HIV-1 protease inhibitor. Clinical Virology reviews of IND submissions for PTV (IND 103526), OMB (IND 108434) and DBV (IND 101636) were primarily

conducted by Patrick Harrington, Ph.D. Clinical Virology Reviewers Takashi Komatsu, Ph.D., Damon Deming, Ph.D. and Lisa Naeger, Ph.D. also reviewed earlier IND submissions for PTV, OMB and DBV, respectively. Pre-IND submissions for PTV and DBV were reviewed by Jules O'Rear, Ph.D., Clinical Virology Team Leader.

1.4 Major virology issues that arose during product development

Evolving Treatment Paradigm

The rapidly evolving HCV treatment paradigm has impacted all HCV treatments in development in recent years. With the emergence of tolerable, short duration, highly effective, interferon-free regimens, the acceptability standards for efficacy and safety of HCV treatment have changed dramatically. For example, treatment guidelines released in early 2014 (HCV Treatment Guidelines) explicitly no longer recommend use of boceprevir- or telaprevir-based regimens, which were only first approved in 2011 and at the time rapidly became the standard-of-care for the treatment of chronic HCV genotype 1 infection. It is now reasonable to expect that the standard-of-care for the treatment of chronic HCV genotype 1 infection should be >90% effective for most patient groups. Furthermore, there is little justification for using a treatment regimen that is sub-optimally effective, particularly if treatment failure is associated with drug resistance that may impact the efficacy of subsequent treatment.

HCV Drug Resistance

Although HCV drug resistance is not necessarily a new issue in HCV drug development, it has become clear throughout the development of the individual direct-acting antiviral (DAA) components of Viekira Pak™ that treatment failure is usually associated with the emergence of viral populations with reduced susceptibility to the individual DAAs that were used. Furthermore, based on data from both the sponsor's program as well as others', many resistance-associated substitutions can predominate (i.e., detected by population nucleotide sequence analysis) in the viral population for months or longer after stopping treatment, particularly for NS5A inhibitor and some NS3/4A protease inhibitor resistance-associated substitutions. In general, amino acid substitutions associated with resistance to one HCV DAA often confer cross-resistance to other HCV DAAs in the same class, which could impact the efficacy of subsequent treatment. Therefore from this reviewer's perspective, it is highly desired that only the most effective combination DAA regimens are used, as a high likelihood of SVR significantly reduces drug resistance-related risks.

It has also become evident that certain natural amino acid polymorphisms in DAA target proteins, referred to as resistance-associated polymorphisms, can impact the efficacy of some DAA-based treatments. The most striking current example is the NS3 Q80K polymorphism that is predominant in ~40% of HCV genotype 1a infected subjects and significantly reduces the efficacy of the NS3/4A protease inhibitor simeprevir (SMV, Olysio™) in combination with pegylated interferon alpha (Peg-IFNα) plus RBV (P/R). Resistance-associated polymorphisms in NS5A are also detected at varying frequencies (b) (4) The natural presence of DAA resistance-associated polymorphisms emphasizes the need for high resistance barrier treatment regimens to ensure optimal treatment efficacy.

Impact of IL28B Genotype and Prior P/R Treatment History

It is well established that an unfavorable IL28B genotype or a history of poor responsiveness to interferon-based therapy is associated with reduced treatment efficacy of P/R and certain P/R + DAA therapies, which has generally been attributed to a sub-optimal response to the interferon component

of these regimens. As combination DAA regimens began to be studied in clinical trials it was initially unclear if IL28B genotype or interferon-responsiveness would impact the efficacy of an interferon-free treatment regimen. Results from early combination DAA clinical trials eventually indicated that, indeed, both an unfavorable IL28B genotype and a history of poor responsiveness to interferon-based therapy can influence the efficacy of interferon-free treatment regimens.

One of the first examples demonstrating the impact of IL28B genotype on the efficacy of an interferon-free treatment was the Boehringer Ingelheim SOUND-C2 trial, which evaluated faldaprevir (NS3/4A protease inhibitor) and deleobuvir (nonnucleoside NS5B-thumb1 polymerase inhibitor) with or without RBV in treatment-naïve HCV genotype 1 infected subjects (Zeuzem et al., 2013). Similarly, an early AbbVie trial of PTV/r, DBV and RBV demonstrated lower SVR rates among prior P/R null and partial responders compared to previously untreated subjects (Poordad et al., 2013). These studies clearly indicate that interferon responsiveness, presumably a reflection of innate immune system function in general, can play an important role in augmenting the efficacy of interferon-free regimens. Nevertheless, with reports of >90% efficacy for multiple different interferon-free, combination DAA regimens in both treatment-naïve and P/R-based treatment failure subjects (Kowdley et al., 2014; Sulkowski et al., 2014; Lawitz et al., 2014), it has also become clear that certain DAA combination regimens can successfully overcome these negative treatment response predictors.

HCV Genotype/Subtype and Commercially Available Assays

HCV is an extremely genetically diverse virus, with at least 7 confirmed genotypes and 67 subtypes (Smith et al., 2014). For many years it has been demonstrated that HCV genotype can impact treatment efficacy, and therefore determination of HCV genotype has been a part of standard clinical practice since at least 2004 (Strader et al., 2004; Ghany et al., 2009; Ghany et al., 2011). In general, commercially available assays have been reliable for determination of HCV genotype to help guide treatment decision making (Ghany et al., 2009).

In recent years it has been demonstrated that not just HCV genotype, but also HCV subtype (e.g., 1a versus 1b) can impact the efficacy of certain HCV DAA-containing regimens (Lok et al., 2012; Zeuzem et al., 2013), including regimens developed by the sponsor of this NDA. The reference method for determination of HCV genotype and subtype has been based on nucleotide sequencing and phylogenetic analysis of an NS5B genome region, although core/E1 nucleotide sequences can also be considered (Simmonds et al., 1993; Simmonds et al., 2005; Smith et al., 2014). Importantly, there has been a history of performance problems with certain earlier genotyping/subtyping methods when compared to the reference NS5B nucleotide sequencing approach. In general, the best performing and most commonly used commercial assays have been the Siemens Versant HCV Genotype 2.0 assay (LiPA) and the Abbott RealTime HCV Genotype II assay. Unlike earlier commercial assays such as the Trugene™ HCV 5'NC genotyping assay, both the Siemens and Abbott assays can reliably distinguish between HCV subtypes 1a and 1b (Chevaliez et al., 2009; Abbott assay label). The Siemens assay, although not currently FDA approved, has by far been the most commonly used assay in HCV clinical trials. The Abbott assay was approved in 2013.

Contribution of Individual Drug Components

A highly effective anti-HCV regimen needs to have exceptional anti-HCV activity and durability to result in a high rate of patients achieving SVR. While many single anti-HCV agents have been shown to have highly potent anti-HCV activity when dosed as monotherapy, resulting in declines of plasma HCV RNA levels by multiple orders of magnitude in just a few days, this activity usually is not durable. For most classes of HCV DAAs, including those in this NDA, DAA monotherapy eventually leads to a plateau or rebound in viral RNA levels, reflecting the rapid selection of viral populations that are resistant to the DAA. To date, no single anti-HCV agent administered as monotherapy has

demonstrated a high rate of treatment efficacy (i.e., SVR). Therefore, virtually all HCV drug development has focused on identifying combinations of at least two anti-HCV agents that result in optimal treatment efficacy.

This NDA is for a combination of 3 different HCV DAAs, representing 3 different drug classes, which in some patient populations are to be dosed in combination with RBV as a fourth anti-HCV agent to maximize treatment efficacy. Furthermore, low dose ritonavir (rtv) is included as a PK enhancer for PTV, resulting in a regimen that includes up to 5 different drug components. While this complex combination regimen has demonstrated exceptional treatment efficacy in many patient populations, it has been challenging to determine the precise contribution of each anti-HCV component in certain patient populations.

An ideal method to demonstrate the contribution of each component of a combination anti-HCV regimen would be to conduct a fully powered clinical trial that randomizes study subjects across several different treatment arms that include either partial or complete combination regimens, such as in a factorial or modified factorial design. However, such an approach is arguably not ethical if there is other evidence that removal of one or multiple components from the regimen could lead to a higher rate of treatment failure, particularly when treatment failure is associated with the emergence of drug resistance that could impact future treatment and there are few or no serious safety signals. Also, it would not be practical and in the best interest of patients to require separate fully powered factorial studies for all patient populations in question, which could significantly delay the availability of important treatment regimens with demonstrated efficacy and safety. Recognizing these challenges, the Division of Antiviral Products has written in draft guidance that alternatives to factorial clinical trial designs can be used to demonstrate or further support the contribution of individual drug components towards the efficacy of a combination anti-HCV drug regimen. The contribution of all drugs in Viekira Pak™ is discussed further in Section 4.13.

1.5 State of Antivirals Used for the Indication(s) Sought

According to the U.S. Centers for Disease Control and Prevention (CDC), an estimated 3.2 million people in the U.S. have chronic HCV infection. The virus is transmitted primarily by the use of contaminated needles, but may also be transmitted by exposure to contaminated blood in healthcare settings, by mother-to-child transmission, or less commonly through sexual contact. Prior to the implementation of screening of the blood supply in the 1990s, HCV was commonly transmitted through blood transfusions and organ transplants. Approximately 15-30% of acute HCV infections are resolved without treatment, while the infection becomes chronic in ~70-85% of cases. Most patients with chronic HCV develop chronic liver disease, ~5-25% of patients eventually develop cirrhosis over a period of ~20-30 years, and a subset of these patients will eventually die due to liver cancer or other complications. Chronic HCV infection is the leading indication for liver transplantation in the U.S., and the CDC reported that in 2007 there were >15,000 deaths in the U.S. attributable to chronic HCV infection, surpassing the number of U.S. deaths attributed to human immunodeficiency virus (HIV) infection (Ly et al., 2012).

Hepatitis C viruses that circulate in the general population are extremely diverse. As noted above, there are at least 7 confirmed genotypes and 67 subtypes of HCV, which are assigned based on nucleotide sequence relatedness in the core/E1 and NS5B genome regions (Smith et al., 2014). The most common HCV genotype in the U.S. is genotype 1, with subtype 1a being relatively more common than subtype 1b. To put the extensive HCV genetic diversity in perspective, the genetic

diversity just within HCV genotype 1 is comparable to the genetic diversity among all known HIV-1 clades.

The goal of treatment for chronic HCV is to obtain a sustained virologic response (SVR), which is defined as a lack of detection of HCV RNA in blood several months after completing a course of treatment. Specifically, FDA recommends the use of SVR12 as the primary efficacy endpoint in clinical trials of HCV treatments (FDA HCV DAA draft guidance), which is usually defined as HCV RNA below the lower limit of quantification (<LLOQ, based on a sensitive assay) 12 weeks following the end of treatment. The SVR12 endpoint predicts long term durability of virologic response, is generally considered a virologic cure, and numerous studies have demonstrated correlations between SVR and improvements in clinical outcomes (reviewed in FDA HCV DAA draft guidance).

The treatment of chronic HCV infection has been evolving rapidly in recent years. Prior to 2011 the standard-of-care of chronic HCV infection was a challenging course of treatment with pegylated interferon alfa plus ribavirin (P/R) for a duration of 24-48 weeks depending on HCV genotype. Both Peg-IFNα and RBV are often poorly tolerated. Use of one or both agents is also contraindicated in many HCV infected patients, for example patients with autoimmune disorders, depression, organ transplant, or one of several other concurrent medial diseases, and also for women who are pregnant or unwilling to use contraception. Furthermore, use of IFNα or Peg-IFNα is associated with poor weight gain and impaired growth in children.

A major focus in HCV drug discovery and development in the past ~10-15 years has been in the area HCV direct-acting antivirals (DAAs), which are designed to target specific steps in the HCV replication cycle that are catalyzed by viral encoded functions. Note that RBV is also believed to have some direct-acting anti-HCV activity but is generally not classified in the category of HCV DAAs. The first FDA-approved HCV DAAs were the NS3/4A protease inhibitors boceprevir (BOC, Victrelis™) and telaprevir (TVR, Incivek™), both approved in 2011. Each of these DAAs was approved for use in combination with P/R for the treatment of HCV genotype 1 infected patients. The addition of BOC or TVR to a P/R background regimen resulted in improved efficacy, and in many patients a reduced treatment duration, and these single DAA + P/R combination regimens quickly became the new standard-of-care for HCV genotype 1 (Ghany et al., 2011). However, these regimens were not ideal for HCV-infected patients as they still required use of P/R, with a total treatment duration of 24 to 48 weeks, and both DAAs added toxicities and complexities to treatment. Furthermore, virologic failure was common among patients who are poorly responsive to the P/R background therapy, and was associated with the emergence of HCV viral populations that are resistant to BOC, TVR and other NS3/4A protease inhibitors in development.

Two other HCV DAAs were recently approved in late 2013: simeprevir (SMV, Olysio™) and sofosbuvir (SOF, Sovaldi™). SMV is an HCV NS3/4A protease inhibitor, which is the same DAA class as BOC and TVR, and also has overlapping resistance pathways with BOC and TVR. While SMV has some advantages over BOC and TVR, it has many of the same limitations of BOC and TVR in that its efficacy is established only for HCV genotype 1 and it should be administered with P/R for a total treatment duration of 24 to 48 weeks. Furthermore, it is strongly recommended that alternative treatments are considered for HCV genotype 1a subjects who carry a predominant HCV NS3 Q80K polymorphism, which is naturally present in ~40% of HCV genotype 1a subjects and was associated with significantly reduced SMV + P/R treatment efficacy (Olysio™ label).

SOF is a first-in-class uridine nucleotide analogue prodrug HCV NS5B polymerase inhibitor, and the first FDA-approved HCV DAA with efficacy established for HCV genotypes beyond genotype 1 (Sovaldi™ label). The approval of SOF represented a major advancement in the treatment of chronic

HCV infection. For HCV genotypes 2 or 3, SOF is highly effective when dosed in combination with RBV for 12 or 24 weeks, representing the first FDA-approved regimen for chronic HCV that does not include interferon. In addition, SOF in combination with P/R for 12 weeks is approved for HCV genotype 1 and genotype 4 infected patients. Although this regimen still includes P/R, the shorter treatment duration and improved efficacy over BOC + P/R, TVR + P/R and SMV + P/R represent significant improvements in the treatment of chronic HCV genotype 1 infection. Furthermore, SOF + RBV for 24 weeks can be considered for HCV genotype 1 patients who cannot use interferon, although efficacy of this regimen is suboptimal for HCV genotype 1 (SVR rates of 76% in an HCV/HIV-1 coinfected trial). Finally, since SOF represents a distinct HCV DAA class with resistance pathways that do not overlap with NS3/4A protease inhibitors, it potentially can be combined with an NS3/4A protease inhibitor to create an alternative interferon-free, combination DAA treatment regimen. Along these lines, SOF has been studied in combination with SMV, with evidence of favorable efficacy (HCV Treatment Guidelines). Although the number of subjects studied with SOF/SMV has been small, the efficacy results were sufficient for the regimen to be recommended in treatment guidelines as a first-line therapy for HCV genotype 1 patients who cannot use interferon.

(b) (4), (b) (5)

Despite the major advances in the treatment of chronic HCV infection with the approval of four different HCV DAAs since 2011, there is still a major public health need for better optimized treatment regimens. In particular, the development of more highly efficacious, short duration, interferon-free regimens for HCV genotype 1 remains a significant goal of HCV drug development. Furthermore, a regimen that does not require RBV, has limited drug-drug interactions, and is highly effective across various patient populations regardless of treatment history (including NS3/4A protease inhibitor experience), disease status, or HCV genotype or subtype, would also be a significant improvement over currently available HCV therapies.

The present NDA represents several important milestones in the treatment of chronic HCV infection. The drugs covered in this application include HCV DAAs from 3 different classes. One of the drugs (paritaprevir) is a ritonavir-boosted NS3/4A protease inhibitor which is expected to have enhanced activity and durability over currently approved NS3/4A protease inhibitors. The other two drugs represent novel DAA classes, including an NS5A inhibitor (ombitasvir) and a non-nucleoside NS5B-palm polymerase inhibitor (dasabuvir). The combination regimen including all 3 HCV DAAs, in some cases also dosed with RBV, was found to be highly effective in HCV genotype 1 infected subjects, including in populations that are generally considered difficult-to-treat such as genotype 1a subjects, prior P/R treatment failure subjects, and subjects with compensated cirrhosis. Importantly, the regimen does not require interferon, and most patients require only a 12-week duration of treatment. One of the challenges with the use of this combination drug regimen will be the prediction and management of drug-drug interactions. Furthermore, at this time efficacy is established only for HCV genotype 1, and in subjects who are naïve to HCV DAAs. Nevertheless, if approved, this NDA will provide important new treatment options for many HCV infected patients in the U.S.

2. NONCLINICAL VIROLOGY

2.1 Introduction

Section 2 of this review covers the key non-clinical virology characteristics of PTV, OMB, and DBV, including each drug's mechanism of action, antiviral activity in cell culture, antiviral activity in the

presence of serum proteins, combination antiviral activity with other HCV DAAs, cytotoxicity, drug resistance mechanisms, and the potential for cross-resistance with other anti-HCV agents.

To understand the mechanisms of action of the 3 different HCV DAAs covered in this application, it is important first to understand some of the fundamental characteristics of the HCV genome and its replication. HCV is a positive-strand RNA virus in the *Flaviviridae* family. The genome is a single-stranded, positive-sense RNA of about 9.6 kilobases in length, and codes for a single polyprotein that is processed into 10 different structural and nonstructural proteins (Figure 1; from Scheel and Rice, 2013).

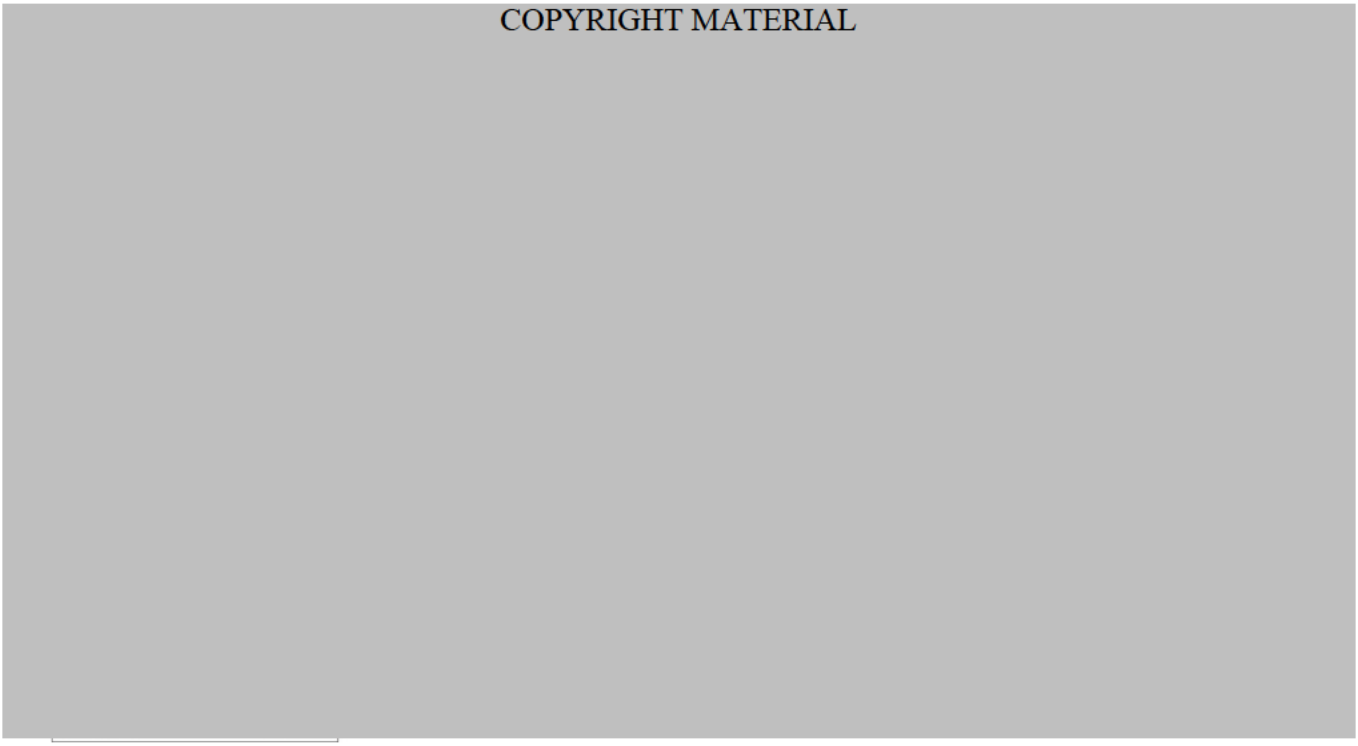


Figure 1. HCV genome organization, translation and polyprotein cleavage sites (Scheel and Rice, 2013).

Following viral attachment, entry, fusion of the viral and cellular envelopes, and uncoating, the RNA genome is released into the cytoplasm of an infected cell where it is first translated within endoplasmic reticulum-derived membranous structures. The HCV polyprotein is co- and post-translationally processed in the cytoplasm by cellular and viral proteases to release the 10 individual viral proteins. Following proteolytic processing of the HCV polyprotein, HCV RNA replication occurs in the cytoplasm in association with endoplasmic reticulum-derived membrane structures. The resulting negative-sense RNAs are used as templates for the production of positive-sense HCV RNA genomes, and these positive-sense RNAs can be translated to produce new viral proteins, transcribed to produce new negative-sense RNA templates, or packaged into newly formed virions in association with lipid droplet particles. In a complex and not fully understood process, the newly formed lipid-associated viral particles are finally released from cells and are available to initiate new rounds of infection.

The key HCV proteins targeted by PTV, OMB, and DBV are the NS3/4A serine protease, NS5A protein, and NS5B RNA-dependent RNA polymerase, respectively. The NS3/4A serine protease

(includes NS3 protease and NS4A, which serves as a co-factor in this process) is responsible for releasing mature forms of the viral nonstructural proteins NS3, NS4A, NS4B, NS5A and NS5B from the polyprotein. The NS5A protein is an unusual antiviral drug target in that it is not known to have enzymatic activity, but it is an essential multi-functional protein with key activities in HCV replication, virus assembly, and modulation of cellular signaling pathways. The NS5B protein is an RNA-dependent RNA polymerase and is the key viral protein responsible for HCV RNA genome replication.

2.2 Paritaprevir (PTV, ABT-450, A-1043422.0)

Mechanism of Action

Paritaprevir (PTV, ABT-450) inhibits the HCV NS3/4A protease, which results in inhibition of HCV replication.

In a biochemical assay, PTV inhibited the activity of recombinant NS3/4A protease enzymes from genotype 1a (H77) and genotype 1b (N) HCV laboratory strains with 50% inhibitory concentration (IC_{50}) values of 0.043 nM and 0.054 nM, respectively (Report R&D/08/1559). The activity of PTV was specific for the HCV NS3/4A protease based on the lack of measurable activity (IC_{50} values of >200,000 nM) against a panel of human serine and cysteine proteases, reflecting selectivity indices of >4,000,000 for HCV genotype 1a and 1b NS3/4A proteases.

In a more recent study characterizing PTV activity and resistance profiles for different HCV genotypes/subtypes (Report R&D/13/636), PTV inhibited the activity of recombinant NS3/4A protease enzymes from genotype 1a (H77) and genotype 1b (Con1) HCV prototype laboratory strains with IC_{50} values of 0.18 nM and 0.43 nM, respectively. The higher IC_{50} values reported from this study presumably are due to a shorter (30 minute) enzyme + inhibitor pre-incubation period compared to the 08/1559 study (5 hours).

The mechanism of action of PTV is also supported by drug resistance selection and characterization studies (see below).

Antiviral Activity in Cell Culture

PTV inhibited the replication of stable HCV genotype 1a (H77) and 1b (Con1) replicons in Huh-7 cells with 50% effective concentration (EC_{50}) values of 0.94 nM and 0.32 nM, respectively (Report R&D/08/1559). In these assays the replicon-harboring cells were exposed to PTV for 3 days, and replicon replication was measured based on luciferase reporter gene expression.

PTV had consistent cell culture anti-HCV activity against panels of transient HCV replicons carrying NS3 genes from genotype 1a or 1b clinical isolates from DAA treatment-naïve subjects. In one study, PTV had a median EC_{50} value of 0.95 nM, and a range of 0.5-4.2 nM, against replicons derived from 15 HCV genotype 1a clinical isolates (Report R&D/08/1559). In a later study using an optimized replicon shuttle vector, PTV had a median EC_{50} value of 0.68 nM (range 0.43-1.87 nM) against replicons derived from 11 other HCV genotype 1a clinical isolates, and a median EC_{50} value of 0.058 nM (range 0.033-0.087 nM) against replicons derived from 9 different HCV genotype 1b clinical isolates (Report R&D/13/1064).

The breadth of PTV activity across different HCV genotypes/subtypes was evaluated in cell culture HCV stable replicon assays and biochemical assays (Report R&D/08/1559, Report R&D/13/636). PTV had comparable anti-HCV activities against single isolates representative of HCV genotypes 1a, 1b, 4a and 6a, with >5-fold reduced activity against HCV genotypes 2a, 2b and 3a relative to

genotype 1a (Table 1; derived from Report R&D/13/636). The reduced activity against the HCV genotype 3a isolate is likely due, at least in part, to an NS3 D168Q sequence (relative to genotype 1) that is naturally present in HCV genotype 3. Substitutions at this position represent key PTV resistance pathways, and the Q168 sequence in genotype 3 has been shown to reduce the activities of other HCV NS3/4A protease inhibitors such as simeprevir (Lenz et al., 2013). The specific amino acid change(s) responsible for the reduced activity of PTV against HCV genotype 2a and 2b isolates is not clear; NS3 amino acid variants in genotype 2 relative to genotype 1 include V36L (also in genotype 3), Q80G, S122R and A166S (also in genotype 3).

Table 1. PTV activity against various HCV genotypes/subtypes in cell culture HCV stable replicon and biochemical assays. Results shown are mean EC_{50} or IC_{50} values for single isolates. NS3 or NS3/4A genes from genotypes 3b, 4a and 6a were engineered into a genotype 1b (Con1) replicon backbone. The NS3/4A gene for the genotype 6a isolate was synthesized based on a consensus of 15 published sequences. Data for the genotype 2a (JFH-1) replicon were generated at (b) (4)

HCV genotype/ subtype	Replicon assay EC_{50} value (nM)	Biochemical assay IC_{50} value (nM)
1a-H77	1.0	0.18
1b-Con1	0.21	0.43
2a-JFH-1	5.3	2.4
2b	Not Available	6.3
3a	19	14.5
4a	0.09	0.16
6a	0.68	Not Available

In a PK study, two main metabolites of PTV were identified in healthy adult male subjects following a single oral dose of 200 mg PTV co-administered with 100 mg ritonavir: M2 (A-1231059) and M29 (R&D/12/1024). These metabolites represented approximately 5.6% and 2.5% of the total drug related material in plasma, respectively. To this reviewer's knowledge no cell culture studies of these metabolites were reported in the NDA.

Since low dose ritonavir is used as a PK enhancer for PTV and was developed as an inhibitor of HIV-1 protease, the sponsor conducted a study to evaluate if ritonavir has anti-HCV activity. In an HCV genotype 1b (Con1) replicon cell culture system, the EC_{50} value for ritonavir was 15 µM and the 50% cytotoxicity concentration (CC_{50}) value was 27 µM, indicating a lack of specific anti-HCV activity (Report R&D/09/931).

PTV had no measurable antiviral activity against human immunodeficiency virus (HIV-1) or hepatitis B virus (HBV) in cell culture assays (Report R&D/13/637).

Antiviral Activity in Cell Culture in the Presence of Serum and Serum Proteins

The cell culture anti-HCV activity of PTV against genotype 1a and 1b stable replicons was reduced in the presence of 40% human plasma by 24- and 27-fold, respectively (Report R&D/08/1559). The PTV EC_{50} values against HCV genotype 1a (H77) and 1b (Con1) replicons in the presence of 40% human plasma were 23 nM and 8.7 nM, respectively. Note that 5% fetal bovine serum was also included in the cell culture media for these studies.

Cytotoxicity/Therapeutic Index

The PTV CC_{50} value for HCV genotype 1b replicon cells was 37 µM, reflecting a therapeutic index of 39,000-116,000 for HCV genotype 1a and 1b replicons (Report R&D/08/1559).

Combination Antiviral Activity in Cell Culture

In cell culture HCV replicon assays, PTV generally had non-antagonistic combination anti-HCV activity relationships with OMB, DBV, RBV, RBV + ritonavir, and interferon-α (Reports R&D/11/049, R&D/09/930, R&D/09/931, R&D/08/1559). In these studies, HCV replicons were exposed to combinations of test compounds at concentrations that spanned the EC_{50} values of the individual compounds, and synergy, additivity and antagonism were calculated according to the Prichard and Shipman 1990 method. Some evidence of antagonism was observed between PTV and interferon-α in one genotype 1a replicon study, although two other replicon studies did not indicate an antagonistic relationship (Report R&D/08/1559). Slight antagonism was also observed between PTV and OMB, but this only occurred at the lowest PTV concentrations tested and not at concentrations at or above the PTV EC_{50} value (Report R&D/11/049).

Ritonavir concentrations of 0.012 to 3 µM had no impact on the PTV EC_{50} value against the genotype 1b (Con1) replicon (Report R&D/09/931). According to the sponsor the ritonavir concentrations expected in subjects treated with a 100 mg QD dose are within the range of concentrations tested in this study.

Resistance Development in Cell Culture

Treatment of HCV genotype 1a and 1b replicon-harboring cells with PTV (10- or 100-fold above EC_{50} value) in the presence of G418 for 3 weeks resulted in the emergence of HCV replicon cell colonies with reduced susceptibilities to PTV (Report R&D/08/1559). At PTV concentrations that were 10- and 100-fold above the EC_{50} value, approximately 0.1% and <0.015% of input cells survived PTV treatment, respectively. Drug resistant replicon cell colonies were not observed when cells were exposed to PTV at a concentration 500-fold above the EC_{50} value. Ten to twelve colonies from each treatment condition were harvested and expanded for genotypic characterization. Treatment-emergent substitution patterns differed somewhat by the HCV replicon subtype. The predominant NS3 treatment-emergent substitutions (detected in ≥2 replicon isolates) for genotype 1a PTV-selected replicons were Q41R, R155K, D168E/N and I170T/V. The predominant NS3 treatment-emergent substitutions for genotype 1b PTV-selected replicons were R155Q, A156T/V and D168H/V. In four instances when Q41R and I170V were detected they were always detected with D168E, with an additional replicon population carrying only D168E. Certain subtype-specific differences in NS3 treatment-emergent resistance patterns, particularly for R155K, previously have been attributed to the differences in the number or type of nucleotide changes required to generate an amino acid substitution (Sarrazin et al., 2007; McCown et al., 2009; Powdrill et al., 2011), which may explain some of the differences in PTV treatment-emergent resistance patterns between genotype 1a and 1b replicons. In genotype 1a only 1 nucleotide change is required to generate the R155K substitution, whereas 2 nucleotide changes are required in genotype 1b.

In another study (Report R&D/13/636), selection of PTV-resistant, HCV genotype 3a- or 4a-derived replicons resulted in the emergence of amino acid substitutions at NS3 positions associated with resistance to PTV or other NS3/4A protease inhibitors. Treatment-emergent NS3 substitutions in genotype 3a-derived replicons included Q80R and Q168R, both of which conferred reduced HCV susceptibility to PTV when re-introduced by site-directed mutagenesis. Treatment-emergent NS3 substitutions in genotype 4a-derived replicons included R155C, A156T/V and D168H/V; again, these substitutions conferred reduced HCV susceptibility to PTV when re-introduced by site-directed mutagenesis.

Effect of Specific Amino Acid Substitutions on PTV Anti-HCV Activity

Appendix A includes a comprehensive list of specific NS3/4A amino acid substitutions evaluated for their impact on PTV anti-HCV activity in genotype 1a and 1b replicon cell culture studies (R&D/14/0224). The specific amino acid substitutions evaluated include substitutions identified in PTV cell culture resistance selection studies, treatment-emergent substitutions in PTV-treated subjects who experienced virologic failure, and substitutions associated with resistance to other NS3/4A protease inhibitors.

In HCV genotype 1a replicons, PTV anti-HCV activity was reduced by >3-fold by the following single amino acid substitutions: F43L (19-fold), R155G/K/S/T/V/W (5- to 36-fold), A156T (17-fold), and D168A/E/F/H/N/V/Y (14- to 206-fold). The following single substitutions conferred no or small (≤3-fold) reductions in PTV anti-HCV activity: V36A/L/M, T54S, V55I, Y56H, Q80K/L/R, R155M, E357K and NS4A V23A. The substitutions V36L/M, Y56H and E357K in combination with R155K or a D168 substitution further reduced HCV susceptibility to PTV by 2- to 7-fold relative to the R155K or D168x single substitutions. The Q80K substitution, which is a common polymorphism in HCV genotype 1a, did not further reduce HCV susceptibility to PTV when evaluated in combination with R155K, but had a modest (up to 2.5-fold) impact on PTV activity when combined with D168 substitutions. In clinical trials, the most commonly observed treatment-emergent substitutions in NS3/4A were D168A/V/Y and R155K in HCV genotype 1a subjects (see Section 5.2), which as single substitutions conferred ≥27-fold reductions in HCV genotype 1a replicon susceptibility to PTV.

In HCV genotype 1b replicons, PTV anti-HCV activity was reduced by >3-fold by the following single amino acid substitutions: R155K (40-fold), A156T (7-fold), and D168A/E/H/K/T/V/Y (4- to 873-fold). The impact of the Y56H substitution by itself could not be evaluated due to poor replication capacity, although it enhanced the resistance of HCV replicons carrying D168A/V/Y substitutions by 12- to 26-fold. In clinical trials of genotype 1b subjects, the most commonly observed treatment-emergent substitutions in NS3/4A were D168A/V and Y56H (see Section 5.2). All 3 HCV genotype 1b subjects with treatment-emergent Y56H also had treatment-emergent D168A or D168V.

Cross-Resistance

Cross-resistance between PTV and most other NS3/4A protease inhibitors is expected. PTV resistance-associated substitutions were most commonly observed at NS3 positions R155 and D168 in both PTV cell culture resistance selection studies and also in subjects who experienced virologic failure in clinical trials. Amino acid substitutions at these positions have been shown to emerge frequently in patients failing treatment with other NS3/4A protease inhibitors such as boceprevir, simeprevir and telaprevir, and reduce the antiviral activity of all of these compounds in HCV replicon assays. Some newer generation NS3/4A protease inhibitors that are currently in clinical development may have improved antiviral activity against HCV populations carrying substitutions at these positions, although to this reviewer's knowledge efficacy of these compounds in subjects who previously failed treatment with an NS3/4A protease inhibitor-containing regimen has not been established. Furthermore, although the activities of some newer generation NS3/4A protease inhibitors may not be impacted by R155K or D168x single substitutions, it will be important to determine if their activities are impacted by combinations of substitutions (e.g., D168x with Y56H or NS3 helicase substitutions) that emerge in subjects who have failed treatment with a PTV-containing regimen.

Cross-resistance between PTV and IFNα, RBV and other classes of HCV DAAs is not expected. However, cross-resistance must also be considered for OMB with NS5A inhibitors and DBV with non-nucleoside NS5B-palm inhibitors, given that all 3 drugs are to be dosed in combination (See sections 2.3 and 2.4, respectively).

2.3 Ombitasvir (OMB, ABT-267, A-1233617.0)

Mechanism of Action

Ombitasvir (OMB, ABT-267) is an NS5A inhibitor. The mechanism of action of OMB has been characterized in HCV replicon activity and drug resistance selection studies, although the precise mechanism of NS5A inhibition and the resulting inhibition of HCV replication is unclear. Based on drug resistance mapping, NS5A inhibitors like OMB appear to target primarily the N-terminus of the protein. Inhibition of HCV replicons with picomolar EC_{50} values indicates that OMB targeting of NS5A results in inhibition of HCV RNA replication. Based on cell culture studies and viral RNA kinetic modeling in subjects treated with the prototypic NS5A inhibitor daclatasvir, inhibition of NS5A may also impair HCV assembly or release (Guedj et al., 2013; McGivern et al., 2014).

Antiviral Activity in Cell Culture

OMB inhibited the replication of stable HCV genotype 1a (H77) and 1b (Con1) replicons in Huh-7 cells with EC_{50} values of 14.1 pM and 5.0 pM, respectively (Report R&D/10/430). In these assays the replicon-harboring cells were exposed to drug for 4 days, and replicon replication was measured based on luciferase reporter gene expression.

OMB had relatively broad anti-HCV activity across different HCV genotypes/subtypes. OMB EC_{50} values were 2-19 pM against stable HCV replicons carrying NS5A genes (encoding amino acids 1-214 or 1-125) from HCV genotype 2a, 2b, 3a, 4a and 5a single isolates (Report R&D/10/430, Report R&D/13/635). OMB activity against a stable HCV genotype 6a-based replicon was relatively lower, with an EC_{50} value of 366 pM (Report R&D/13/635).

OMB generally had low- to sub-picomolar cell culture anti-HCV activity against panels of transient HCV replicons carrying NS5A genes (encoding amino acids 1-214) from genotype 1a, 1b, 2a, 2b, 3a and 4a clinical isolates from DAA treatment-naïve subjects (Table 2; Report R&D/13/635, pg. 15). Note that only single isolates from genotypes 5a and 6a were analyzed, and the sponsor excluded certain "outliers" from the mean EC_{50} value calculations. An NS5A L31M polymorphism is common in HCV genotype 2a and 2b isolates, and in one of the genotype 2b isolates an L28F polymorphism was detected in combination with L31M, which according to the sponsor conferred a 75-fold reduction in OMB anti-HCV activity. This isolate was excluded from the mean EC_{50} value (1.1 pM) for HCV genotype 2b isolates shown in **Table 2**; presumably the EC_{50} value for this isolate was 45 pM. Also, a single genotype 3a isolate carrying an A30K polymorphism was excluded from the mean EC_{50} value (4.5 pM) for HCV genotype 3a isolates; this isolate had an EC_{50} value of 55 pM. The "two" genotype 6a isolates reported in **Table 2** reflect data obtained from a single isolate with and without an introduced NS5A L28F substitution. According to the sponsor there is approximately 50% variability between leucine and phenylalanine at NS5A amino acid position 28 in genotype 6a isolates in the European HCV database.

Table 2. Anti-HCV activity of OMB against panels of transient HCV replicons carrying NS5A genes from clinical isolates representing different HCV genotypes/subtypes. Median EC_{50} values were not provided, and results from two "outlier" samples (1 genotype 2b, 1 genotype 3a) were excluded for calculations of mean EC_{50} values. The genotype 6a NS5A replicons were based a single isolate with or without an introduced L28M substitution.

HCV Replicon Subtype	Number of Samples	EC_{50}, pM Range	Mean EC_{50}, pM, ± Std. Dev.[a]
GT 1a	11	0.35 - 0.88	0.66 ± 0.14
GT 1b	11	0.74 - 1.5	1.0 ± 0.23
GT 2a	9	0.87 - 11	3.9 ± 3.0
GT 2b	14	0.54 - 45	1.1 ± 1.2
GT 3a	13	0.90 - 55	4.5 ± 4.3
GT 4a	9	0.10 - 0.36	0.24 ± 0.08
GT 5a	1	-	0.67
GT 6a	2	42 - 68	55 ± 18

a. Outliers due to presence of known resistance-associated variants were excluded when calculating the mean EC_{50} values.

In a PK study, four major OMB metabolites were identified in healthy adult male subjects following a single 25 mg oral dose of OMB: M23 (A-1242846), M29 (A-1538855), M36 (A-1548255) and M37 (A-1543706) (Report R&D/13/057). Based on a cell culture study conducted using the HCV genotype 1a and 1b replicon systems, these metabolites had ≥78,000-fold reduced anti-HCV activities relative to OMB (Report R&D/13/686). A second study (Report R&D/13/1037) similarly found that the M29 and M36 metabolites were ≥278,266-fold less active against HCV genotype 1-6 replicons relative to OMB.

OMB had no measurable antiviral activity against HIV-1 or HBV in cell culture assays (Report R&D/13/637).

Antiviral Activity in Cell Culture in the Presence of Serum and Serum Proteins

The cell culture anti-HCV activity of OMB against genotype 1a and 1b stable replicons was reduced in the presence of 40% human plasma by 11- to 13-fold (Report R&D/10/430). The OMB EC_{50} values against HCV genotype 1a (H77) and 1b (Con1) replicons in the presence of 40% human plasma were 186 pM and 56 pM, respectively. Note that 5% fetal bovine serum was also included in the cell culture media for these studies.

Cytotoxicity/Therapeutic Index

The OMB CC_{50} value for HCV genotype 1b replicon cells was >32 µM, reflecting a therapeutic index of >2,000,000 for HCV genotype 1a and 1b replicons (Report R&D/10/430).

Combination Antiviral Activity in Cell Culture

In cell culture HCV replicon assays, OMB generally had non-antagonistic combination anti-HCV activity relationships with PTV, DBV, RBV and interferon-α (Reports R&D/11/049, R&D/11/050, R&D/10/430). In these studies, HCV replicons were exposed to combinations of test compounds at concentrations that spanned the EC_{50} values of the individual compounds, and synergy, additivity and antagonism were calculated according to the Prichard and Shipman 1990 method. Slight antagonism between OMB and PTV was observed at the lowest PTV concentrations tested, but not at concentrations at or above the PTV EC_{50} value (Report R&D/11/049). Similarly, slight antagonism

between OMB and DBV was observed only at the lowest drug concentrations tested (Report R&D/11/050).

Resistance Development in Cell Culture

Treatment of HCV genotype 1a (H77) and 1b (Con1) replicon-harboring cells with OMB (10-, 100-, or 1,000-fold above EC_{50} value) in the presence of G418 for 3 weeks resulted in the emergence of HCV replicon cell colonies with reduced susceptibilities to OMB (Report R&D/10/430). In general, more drug resistant replicon colonies emerged in the genotype 1a replicons compared to the genotype 1b replicons at each of the OMB resistance selection conditions, indicating that the barrier to OMB resistance was lower for the genotype 1a-H77 replicon strain.

The predominant NS5A treatment-emergent substitutions for genotype 1a OMB-selected replicon clones were M28T/V, Q30R and Y93C/H (Report R&D/10/430). M28V was detected only in the 10x EC_{50} value selections, whereas M28T was detected in the 100x and 1,000x EC_{50} value selections. The Q30R and Y93H substitutions were also detected in a greater proportion of replicons in the 1,000x EC_{50} value selections compared to the 10x EC_{50} value selections. Combinations of multiple NS5A treatment-emergent substitutions were not commonly detected in the genotype 1a OMB-selected replicon clones, indicating that certain single NS5A amino acid substitutions are likely sufficient to confer significant HCV genotype 1a resistance to OMB.

Treatment-emergent genotypic resistance patterns for genotype 1b OMB-selected replicon clones differed from those observed in the genotype 1a replicon clones, and also included a larger proportion of replicons with multiple NS5A substitutions. Single and combination NS5A treatment-emergent substitutions included L28T, L31F/V, Y93H, L28M/V+L31F, L28M+Y93H, R30Q+Y93H, L31F/V+Y93H, and P58A+Y93H (Report R&D/10/430).

Resistance-associated substitutions also emerged in OMB-treated HCV replicons carrying NS5A genes (encoding amino acids 1-214 or 1-125) from HCV genotypes 2a, 2b, 3a, 4a, 5a and 6a (Report R&D/13/635). Predominant treatment-emergent NS5A substitutions included T24A and F28S for GT 2a; L28F, L31V and Y93H for GT2b; M28T, L31F, and Y93H for GT 3a; L28V for GT 4a; L28I, L31F and L31V for GT 5a; and L31V and T58A/N/S for GT 6a. Taken together, these results indicate that there are multiple possible OMB resistance pathways, which are likely influenced by the HCV genotype/subtype background.

Given the low pM EC_{50} value of OMB, the low genetic barrier to resistance, and a serum half-life of approximately 24 hours (Clinical Pharmacology Summary), in treated patients OMB likely exerts antiviral drug pressure for an extended period of time after cessation of dosing, which can select for drug resistant viral populations.

Effect of Specific Amino Acid Substitutions on OMB Anti-HCV Activity

Appendix B includes a comprehensive list of specific NS5A amino acid substitutions evaluated for their impact on OMB anti-HCV activity in replicon cell culture studies (R&D/14/0224). The specific amino acid substitutions evaluated include substitutions identified in OMB cell culture resistance selection studies, treatment-emergent substitutions in OMB-treated subjects who experienced virologic failure, and substitutions associated with resistance to other NS5A inhibitors.

In genotype 1a replicons, substitutions at positions M28, Q30, L31, H58 and Y93 conferred reduced HCV susceptibility to OMB. Several different single NS5A amino acid substitutions conferred large (>500-fold) reductions in OMB anti-HCV activity, including M28T, Q30E/R and Y93C/H/L/N/S, and combinations of substitutions at positions M28, Q30, L31, H58 or Y93 conferred even larger

reductions (some >100,000-fold) in OMB anti-HCV activity. In clinical trials in genotype 1a subjects, the most commonly observed treatment-emergent substitutions in NS5A were M28A/T/V and Q30E/K/R (see Section 5.3). In HCV genotype 1a replicons the M28T, M28V, Q30E, Q30R single substitutions each conferred 9,065-, 59-, 1,340-, and 809-fold reductions in OMB activity, respectively (no data available for M28A or Q30K).

In genotype 1b replicons, single NS5A substitutions L28T and Y93H conferred 661- and 76-fold reductions in OMB anti-HCV activity. Combinations of substitutions at positions L28, R30, L31, P58 and Y93 conferred 142- to >10,000-fold reductions in OMB anti-HCV activity. In clinical trials in genotype 1b subjects, the most commonly observed treatment-emergent substitution was Y93H (see Section 5.3).

Cross-Resistance

Some degree of cross-resistance between OMB and most other NS5A inhibitors is expected. Substitutions at NS5A positions M/L28, Q/R30, H/P58 or Y93 were associated with OMB resistance in cell culture studies and in clinical trials, and substitutions at these positions have been shown to emerge frequently in patients failing treatment with other NS5A inhibitors in development. Certain newer generation NS5A inhibitors that are currently in clinical development may have improved antiviral activity against HCV populations carrying substitutions at these positions, although to this reviewer's knowledge efficacy of these compounds in subjects who previously failed treatment with an NS5A inhibitor-containing regimen has not been established.

Cross-resistance between OMB and IFNα, RBV and other classes of HCV DAAs is not expected. Amino acid substitutions that are known to confer reduced HCV susceptibility to other classes of HCV DAAs, including NS3/4A protease inhibitors and non-nucleoside NS5B-palm polymerase inhibitors, had no impact (≤2.1-fold-changes in EC_{50} values) on the cell culture anti-HCV activity of OMB (Report R&D/10/430). However, cross-resistance must also be considered for PTV with NS3/4A protease inhibitors and DBV with non-nucleoside NS5B-palm inhibitors, given that all 3 drugs are to be dosed in combination (See sections 2.2 and 2.4, respectively).

2.4 Dasabuvir (DBV, ABT-333, A-998821)

Mechanism of Action

Dasabuvir (DBV, ABT-333) is a non-nucleoside inhibitor of the HCV NS5B RNA-dependent, RNA polymerase, targeting the "palm" domain of the NS5B enzyme.

In a biochemical assay, DBV inhibited the activity of recombinant NS5B polymerase enzymes from genotype 1a (n=3) and genotype 1b (n=4) clinical or laboratory isolates with IC_{50} values of 2.2-10.7 nM (Report R&D/08/212). The DBV IC_{50} values for genotype 2a, 2b, 3a or 4a recombinant NS5B polymerase enzymes ranged from 900 nM to >20 µM, indicating DBV activity in this assay was specific for HCV genotype 1. DBV had IC_{50} values of ≥76 µM against a panel of mammalian DNA and RNA polymerases, reflecting selectivity indices of >7,000 for HCV genotype 1a or 1b NS5B polymerases.

The mechanism of action of DBV is also supported by drug resistance selection and characterization studies (see below).

Antiviral Activity in Cell Culture

DBV inhibited the replication of stable HCV genotype 1a (H77) and 1b (Con1 and N) replicons in Huh-7 cells with EC_{50} values of 7.7 nM and 1.8-8.7 nM, respectively. In these assays the replicon-harboring cells were exposed to DBV for 3 days, and replicon replication was measured based on HCV RNA levels as measured by real-time RT-PCR (Report R&D/08/212).

DBV had low to sub-nanomolar cell culture anti-HCV activity against panels of transient HCV replicons carrying NS5B genes from genotype 1a or 1b clinical isolates from DAA treatment-naïve subjects. The median (range) DBV EC_{50} values were 0.6 nM (0.4-2.1 nM) and 0.3 nM (0.2-2.0 nM) for genotype 1a and genotype 1b isolates, respectively (Report R&D/08/212).

In a PK study, the M1 metabolite (A-1041392.0) of DBV was identified as an abundant metabolite in human plasma, feces and urine of male subjects who received a single 400 mg dose of DBV (PK study report R&D/12/843). The metabolite represented approximately 23% of the drug-related radioactivity in circulation, with a maximum plasma concentration approximately 40% lower than the parent drug. The structure of A-1041392.0 is nearly identical with that of DBV, with an additional hydroxyl group (Figure 2; modified from R&D/12/843 pg. 35).

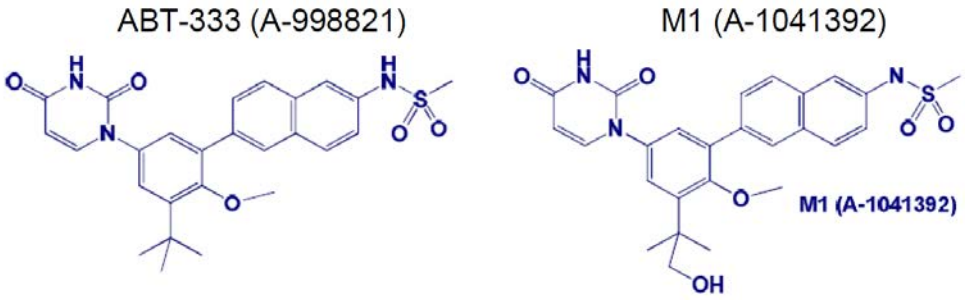


Figure 2. Chemical structures of DBV (ABT-333) and the M1 metabolite, A-1041392.0.

DBV and the A-1041392.0 metabolite have comparable anti-HCV activity in cell culture based on a study conducted using the HCV genotype 1a and 1b replicon systems. In the absence of human plasma, the EC_{50} values of A-1041392.0 were ~5-6 fold higher than those for DBV (Table 3; Report R&D/13/633 pg. 6). However, because DBV anti-HCV activity was reduced to a greater degree in the presence of 40% human plasma, DBV and A-1041392.0 had similar (within 2-fold) protein binding-adjusted EC_{50} values. Given the presence of A-1041392.0 in circulation and similarities in structure and anti-HCV activity with DBV, any treatment-emergent resistance-associated substitutions in DBV-treated subjects could feasibly be attributed to DBV, A-1041392.0, or both.

Table 3. Cell culture anti-HCV activities of DBV (ABT-333) and the M1 metabolite, A-1041392.0, against HCV genotype 1a (H77) and 1b (Con1) subgenomic replicons.

			Genotype 1a		Genotype 1b	
			EC_{50} (nM)			
A-Number	Name	N	0% Human Plasma	40% Human Plasma	0% Human Plasma	40% Human Plasma
A-998821.0	ABT-333	3	6	103	1	15
A-1041392.0	M1 metabolite	3	39	143	8	26

DBV had no measurable antiviral activity against HIV-1 or HBV in cell culture assays (Report R&D/13/637).

Antiviral Activity in Cell Culture in the Presence of Serum and Serum Proteins
The cell culture anti-HCV activity of DBV against genotype 1a and 1b stable replicons was reduced in the presence of 40% human plasma by approximately 12- to 17-fold (Report R&D/08/212, Report R&D/13/633). The DBV EC_{50} values against HCV genotype 1a (H77) and 1b (Con1) replicons in the presence of 40% human plasma were approximately 100 nM and 21 nM, respectively. Note that 5% fetal bovine serum was also included in the cell culture media for these studies.

Cytotoxicity/Therapeutic Index
The DBV CC_{50} value for HCV genotype 1a (H77) and 1b (Con1) replicon cells was approximately 10.3 µM, reflecting a therapeutic index of at least 1,300 for these HCV genotype 1a and 1b replicons (Report R&D/08/212).

Combination Antiviral Activity in Cell Culture
In cell culture HCV replicon assays, DBV generally had non-antagonistic combination anti-HCV activity relationships with PTV, OMB, RBV and interferon-α (Reports R&D/09/930, R&D/11/050, R&D/13/1065,R&D/08/212). In these studies, HCV replicons were exposed to combinations of test compounds at concentrations that spanned the EC_{50} values of the individual compounds, and synergy, additivity and antagonism were calculated according to the Prichard and Shipman 1990, Lowe Additivity or Bliss Independence (Greco et al., 1995) methods. Slight antagonism between DBV and OMB was observed only at the lowest drug concentrations tested (Report R&D/11/050).

Resistance Development in Cell Culture
Treatment of HCV genotype 1a and 1b replicon-harboring cells with DBV (10- or 100-fold above EC_{50} value) in the presence of G418 for 3 weeks resulted in the emergence of HCV replicon cell colonies with reduced susceptibilities to DBV. Several different NS5B amino acid substitutions emerged in the DBV-selected replicons and were associated with reduced HCV replicon susceptibility to DBV, indicating the potential for multiple independent DBV resistance pathways (Table 4; adapted from Report R&D/08/212 pgs. 35-37).

The DBV resistance pathways observed in cell culture are consistent with the targeting of the "palm" domain of the NS5B polymerase (reviewed by Sofia et al., 2012). For the genotype 1a (H77) replicons, the most common substitutions selected by the 10x and 100x EC_{50} value treatments were S556G/N and C316Y, respectively. For the genotype 1b (Con1) replicons, the most common substitutions selected by the 10x and 100x EC_{50} value treatments were M414T and C316Y, respectively. For the genotype 1b (N) replicons, the most common substitutions selected by the 10x and 100x EC_{50} value treatments were M414T and Y448C/H, respectively.

Interestingly, no substitutions at NS5B position 316 were detected in the genotype 1b (N) replicons, despite this position being a key site for resistance-associated substitutions in the genotype 1a (H77) and genotype 1b (Con1) replicons. The sponsor speculated that the lack of NS5B 316 substitutions in the genotype 1b (N) replicons may be attributed to the presence of an asparagine (N) at position 316 in the genotype 1b-N strain, whereas the genotype 1a-H77 and genotype 1b-Con1 strains have a cysteine (C) at this position. The sponsor did not explain why the N316-harboring replicon was less likely to have substitutions emerge at this position. It does not appear that the genotype 1b C316N polymorphism has a major impact on DBV anti-HCV activity based on a site-directed mutant phenotype assay (5-fold reduction in activity, see Appendix C), and also based on similar DBV activities against the genotype 1b-Con1 and -N strains in both enzymatic and replicon assays. This

reviewer speculates that the genetic barrier to generating the tyrosine (Y) substitution may be greater if starting from N316 compared to C316. The C to Y change requires only a single G to A transition at the second position in the codon, whereas an N to Y change requires an A to U transversion in the first position in the codon, which likely occurs less frequently during HCV replication (Powdrill et al., 2011).

Table 4. Genotypic and phenotypic analyses of HCV replicon colonies (not site-directed mutants) selected for resistance to DBV.

NS5B Substitution	DBV EC_{50} value (nM)	Fold-Change in EC_{50} value
Genotype 1a (H77 strain)		
C316Y	1,250	190
C316Y + C110S	1,740	270
C316Y + S431G	1,060	160
A395G	65	10
M414T	400	61
N444K	150	23
Y448C	2,600	400
Y448H	1,630	250
S556G	550	84
S556N + V405I	190	29
D559G + Q19E	780	150
S565F + V405I	110	17
Genotype 1b (Con1 strain)		
C316Y	980	1,200
C316Y + K69R + N110S	1,600	2,000
C316Y + M71I + S189P	1,500	1,900
C316Y + N117S	3,900	4,800
C316Y + A376C	870	1,100
C316Y + Y586C	1,500	1,900
C316Y + L588F	2,600	3,300
S368T	52	65
N411S	9.9	12
M414T	26	33
M414T + F162T	5.1	6
C445F + C451S + I585V	12	16
A553V	19	24
S556G	10	12
Genotype 1b (N strain)		
F193Y + Y586C	320	39
I392F + Y586C	82	10
M414T	210	26
M414T + A435T	240	29
M414T + L587V	140	17
Y448C	1,800	220
Y448C + K81R	2,900	350
Y448C + A442T + Y586C	3,900	480

Y448C + S506N + Y586C	1,040	130
Y448H	89	11
Y448H + E440G	200	24
S556G	260	32
S556G + F551L	1,100	140
S556R	21	3
D559G + H95R + S19G	89	11

Effect of Specific Amino Acid Substitutions on OMB Anti-HCV Activity

Appendix C includes a comprehensive list of specific NS5B amino acid substitutions evaluated for their impact on DBV anti-HCV activity in replicon cell culture studies and biochemical studies (Report R&D/14/0224, Report R&D/08/212). The specific amino acid substitutions evaluated include substitutions identified in DBV cell culture resistance selection studies, treatment-emergent substitutions in DBV-treated subjects who experienced virologic failure, and substitutions associated with resistance to other NS5B polymerase inhibitors.

In genotype 1a replicons, the following single substitutions conferred >3-fold reductions in DBV anti-HCV activity: C316Y, M414I/T/V, E446K/Q, Y448C/H, A553T, G554S, S556G/R and Y561H. Combinations of substitutions at positions C316, M414 and S556 conferred large reductions (>100-fold) in DBV anti-HCV activity. In clinical trials in genotype 1a subjects, the most commonly observed treatment-emergent substitution in NS5B was S556G, which confers a 30-fold reduction in DBV anti-HCV activity.

In genotype 1b replicons, single NS5B substitutions C316H/N/Y, S368T, N411S, M414I/T/V, Y448C/H, A553V, S556G and D559G conferred ≥5-fold-reductions in DBV anti-HCV activity. In Phase 2b/3 clinical trials in genotype 1b subjects, clear DBV treatment-emergent resistance substitutions were detected in only two subjects, including C316Y+M414I and S556G (see Section 5.4). In genotype 1b replicons these substitutions conferred ~2,600- and 11-fold reductions in DBV anti-HCV activity, respectively.

Cross-Resistance

DBV is a non-nucleoside polymerase inhibitor targeting the NS5B “palm” domain. No non-nucleoside NS5B polymerase inhibitors of any class are currently FDA-approved. Cross-resistance is expected between DBV and other non-nucleoside NS5B-palm polymerase inhibitors in development.

In phenotype assays (Appendix C) DBV anti-HCV activity was not reduced (≤2-fold) by the NS5B S282T substitution that is associated with resistance to nucleos(t)ide analogue NS5B polymerase inhibitors (e.g., sofosbuvir). DBV anti-HCV activity was not reduced by NS5B substitutions A421T/V, P495A/S, P496S or V499A, which are associated with resistance to non-nucleoside NS5B-thumb1 polymerase inhibitors. DBV anti-HCV activity also was not reduced by an M423T substitution that is associated with resistance to non-nucleoside NS5B-thumb2 polymerase inhibitors.

In clinical trials, the emergence of substitutions at NS5B positions associated with resistance to other NS5B polymerase inhibitors was uncommon. A small number of subjects had single substitutions that are associated with resistance to certain non-nucleoside NS5B-thumb2 polymerase inhibitors, including M423I (GT1a), I482T (GT1a) and A486V (GT1b) (1 subject for each substitution, see Section 5.4); for examples of published reports on these resistance-associated substitutions/positions see Shi et al., 2009 and Jiang et al., 2014. In addition, an NS5B C316Y substitution emerged in 3 subjects. A C316N substitution/polymorphism in HCV genotype 1b has been shown to be potentially

associated with reduced sofosbuvir efficacy (Sovaldi™ label, Donaldson et al., 2014; also see Clinical Virology reviews of NDA 204671 by Eric Donaldson, Ph.D., and Lisa Naeger, Ph.D.). Although the C316Y substitution is not known to be associated with sofosbuvir resistance, the change is predicted to add bulk to the active site of the NS5B polymerase, and therefore in theory could potentially impact sofosbuvir anti-HCV activity. This reviewer is not aware of any cell culture phenotype data generated by the sponsor on the impact of C316Y on sofosbuvir anti-HCV activity. (b) (4)

The most commonly observed DBV treatment-emergent substitution in clinical trials was NS5B S556G (see Section 5.4), which is not known to impact the activity of other classes of NS5B polymerase inhibitors, including sofosbuvir. According to Eric Donaldson, Ph.D., in a Phase 3 trial of sofosbuvir in combination with P/R (NEUTRINO, Study 110), S556G was detected at baseline in 7/54 HCV genotype 1b infected subjects who achieved SVR12, and in 1/9 who failed treatment. Similarly, 0/6 (0%) HCV genotype 1a infected subjects with baseline S556G failed treatment, indicating that the emergence of S556G in subjects who fail treatment with the AbbVie 3-DAA +/- RBV regimen is unlikely to impact sofosbuvir efficacy. This reviewer is not aware of any cell culture phenotype data on the impact of S556G on sofosbuvir anti-HCV activity.

Based on the totality of evidence from non-clinical virology studies and resistance analyses of clinical trials, cross-resistance is generally not expected between DBV and IFNα, RBV or other classes of HCV DAAs. However, additional phenotypic analyses should be conducted to further investigate the potential for DBV cross-resistance with certain classes of NS5B polymerase inhibitors. The activity of sofosbuvir should be evaluated against HCV replicons carrying the NS5B C316Y or S556G substitutions. In addition, DBV phenotypic analyses of NS5B substitutions that are associated with resistance to certain non-nucleoside NS5B-thumb2 polymerase inhibitors and emerged in some DBV-treated subjects should be conducted: M423I (GT1a), I482T (GT1a) and A486V (GT1b).

Because DBV is to be dosed with PTV and OMB, cross-resistance must also be considered for PTV with NS3/4A protease inhibitors and OMB with NS5A inhibitors (See sections 2.2 and 2.3, respectively).

2.5 Animal Studies

No Clinical Virology-related animal studies were reported.

3. RELEVANT CLINICAL FINDINGS FROM OTHER DISCIPLINES

3.1 Summary of Clinical Efficacy (Biometrics Review)

Data from 6 Phase 3 trials and the large Phase 2b trial, M11-652, were analyzed by the Statistical Reviewer, Joy Mele, M.S. Overall, the results submitted by AbbVie demonstrate the efficacy of the Viekira Pak™ (3-DAA) regimen with or without RBV for the treatment of HCV genotype 1 infected patients with or without cirrhosis. The primary efficacy comparison in all 6 trials was to the historical SVR rate from trials of telaprevir plus P/R, with predefined thresholds for non-inferiority and

superiority. In all trials the SVR12 rates observed in 3-DAA +/- RBV treatment arms were shown be superior to the historical control.

Key subgroup efficacy analyses focused on HCV genotype 1a infected subjects. In clinical trial M14-002, SVR12 rates for noncirrhotic, treatment-naïve HCV genotype 1a infected subjects were statistically significantly higher (about 7% higher) among subjects who received the 3-DAA + RBV regimen compared to those who received the 3-DAA regimen without RBV. In clinical trial M13-099, SVR12 rates for cirrhotic HCV genotype 1a infected subjects were higher among those treated for 24 weeks compared to 12 weeks; although efficacy was not statistically different between these two treatment durations this difference was observed across multiple subgroups and there were no serious safety issues recommending against longer treatment. In contrast to HCV genotype 1a, HCV genotype 1b infected subjects required less aggressive treatment for optimal efficacy, with SVR12 rates of 99-100% among noncirrhotic HCV genotype 1b infected subjects treated with 3-DAA without RBV, and cirrhotic subjects treated with 3-DAA + RBV for 12 weeks.

For a more detailed FDA review of efficacy please see the review by the Statistical Reviewer, Joy Mele, M.S.

3.2 Summary of Clinical Safety (Clinical Review)

The primary safety analysis conducted by the Clinical Reviewer, Russ Fleischer, PA-C, MPH, considered data from approximately 3,000 subjects across Phase 3 and key Phase 2 trials that studied various 2-DAA and 3-DAA +/- RBV combination regimens. The most important clinically relevant treatment-emergent adverse effect related to treatment was elevated transaminase levels. Treatment generally resulted in a rapid decrease from baseline in alanine amino transferase (ALT) levels consistent with the reduction in viral load and hepatic inflammation caused by HCV infection. However, approximately 1% of 3-DAA + RBV-treated subjects experienced ≥Grade 3 post-baseline ALT elevations. These ALT elevations were generally asymptomatic and occurred during the first 28 days of study drug treatment and usually resolved with continued DAA treatment. Concomitant systemic estrogen-containing medication was associated with an increased risk of ALT elevations.

Other safety events of interest included transient elevations of total and indirect bilirubin, as well as hemolytic anemia and other RBV-related adverse events such as fatigue, headache, asthenia, nausea, dyspnea, dry skin, and pruritus. RBV dose modifications due to anemia and other causes did not negatively impact SVR rates. When RBV was not included in the regimen the frequencies of nausea, insomnia, pruritus and anemia were decreased, although there were only small numeric increases in the frequency of most adverse events among subjects who did and did not receive RBV. Furthermore, there were no clinically relevant safety differences between cirrhotic subjects treated for 12 or 24 weeks and the majority of treatment-emergent adverse events occurred within the first 85 days of dosing.

For a more detailed FDA review of safety please see the clinical review by Russ Fleischer, PA-C, M.P.H.

4. CLINICAL VIROLOGY REVIEW OF EFFICACY

4.1 Summary of Key Efficacy Trials

Section 4 of this review summarizes independent efficacy analyses of 6 Phase 3 trials and 3 Phase 2 trials conducted to support the efficacy of the AbbVie 3-DAA +/- RBV regimen. Table 5 briefly summarizes the key design aspects of these trials, and additional study design details are included in the following summaries of efficacy for each trial.

With the exception of M11-652, in all of these trials the 3-DAA regimen was administered QD as PTV/r/OMB 150 mg/100 mg/25 mg in fixed-dose combination (FDC) tablets, with 2 tablets administered per QD dose, plus DBV 250 mg BID. RBV dosing (as appropriate) was weight-based, either 1,000 mg or 1,200 mg daily divided BID per local label.

Table 5. Overview of key Phase 2 and Phase 3 trials of the AbbVie 3-DAA +/- RBV regimen.

Trial	Population	Regimen	Duration
M11-646 (Phase 3)	GT1, Tx-naïve, noncirrhotic	3-DAA + RBV (vs. placebo)	12 weeks
M13-098 (Phase 3)	GT1, Tx-exp., noncirrhotic	3-DAA + RBV (vs. placebo)	12 weeks
M13-099 (Phase 3)	GT1, Compensated cirrhosis (Tx-naïve or -exp.)	3-DAA + RBV	12 vs. 24 weeks
M13-961 (Phase 3)	GT1b, Tx-naïve, noncirrhotic	3-DAA +/- RBV	12 weeks
M13-389 (Phase 3)	GT1b, Tx-exp., noncirrhotic	3-DAA +/- RBV	12 weeks
M14-002 (Phase 3)	GT1a, Tx-naïve, noncirrhotic	3-DAA +/- RBV	12 weeks
M11-652 (Phase 2)	GT1, Tx-naïve and Tx-exp., noncirrhotic	2- or 3-DAA +/- RBV	8, 12 or 24 weeks
M14-103 (Phase 2)	GT1, noncirrhotic, on opioid replacement therapy	3-DAA + RBV	12 weeks
M12-999 (Phase 2)	GT1, post-liver transplant	3-DAA +/- RBV	24 weeks

4.2 Efficacy in Phase 3 Trial M11-646 (3-DAA+RBV, Naïve)

Summary of Trial Design

Clinical trial M11-646 was a Phase 3, randomized, double-blind, placebo-controlled study of the 3-DAA + RBV regimen in noncirrhotic, treatment-naïve adults with chronic HCV genotype 1 infection. Approximately 600 subjects were to be randomized in a 3:1 ratio to receive either the active 3-DAA +

RBV regimen or placebo for 12 weeks. Following 12 weeks of double-blind treatment, subjects initially randomized to receive placebo were administered open-label 3-DAA + RBV treatment for 12 weeks.

The primary objectives of this study were to show the noninferiority in SVR12 rates (defined as HCV RNA <LLOQ 12 weeks following therapy) after 12 weeks of treatment with the 3-DAA + RBV regimen to the historical SVR rate of telaprevir plus P/R therapy, and to assess the safety of the active treatment regimen versus placebo for 12 weeks.

The primary analysis occurred after subjects initially randomized to active drug completed through Post-Treatment Week 12 or prematurely discontinued the study, and subjects who were initially randomized to placebo completed 12 weeks of open-label active treatment or prematurely discontinued study drug. Additional follow-up analyses are planned. This reviewer's efficacy analysis focused only on the active arm, although resistance analyses included 2 placebo arm subjects who received open-label 3-DAA + RBV treatment and experienced on-treatment virologic failure.

Reviewer's Efficacy Analysis

The overall intent-to-treat (ITT) SVR12 rate for subjects in the active 3-DAA + RBV arm was 456/473 (96.4%), with SVR12 rates of 95.7% and 98.0% for genotype 1a and genotype 1b subjects, respectively (Table 6). Virologic failure was observed in 7 (2.1%) HCV genotype 1a subjects and 1 (0.6%) genotype 1b subject, with 7/8 failures specifically observed as virologic relapse.

Table 6. SVR12 (ITT) and virologic failure rates in clinical trial M11-646.

Efficacy Outcome	All subjects, active arm (n=473)	HCV Genotype 1 Subtype	
		1A (n=322)	1B (n=151)
SVR12 (ITT)[1]	**456/473 (96.4%)**	**308/322 (95.7%)**	**148/151 (98.0%)**
Any VF[2]	8/473 (1.7%)	7/322 (2.2%)	1/151 (0.6%)
On-Tx VF (vBT)	1/473 (0.2%)	1/322 (0.3%)	0/151 (0%)
Relapse[3]	7/473 (1.5%)	6/322 (1.9%)	1/151 (0.6%)
Among EOT <LLOQ[4]	7/462 (1.5%)	6/313 (1.9%)	1/149 (0.7%)
Other Failures[5]	9/473 (1.9%)	7/322 (2.2%)	2/151 (1.3%)

[1] Intent-to-treat (ITT) analysis, except subjects with missing SVR12 outcome results could be classified as SVR12 responders based on subsequent follow-up results. Includes updated SVR12 result from Subject 42765-604209 (GT1a, IL28B CT) reported by the sponsor during the review cycle.
[2] Total of On-Treatment Virologic Failure (On-Tx VF) and Relapse results.
[3] Relapse rate excludes subjects who discontinued prematurely (defined by sponsor as <77 days duration).
[4] Excludes "Other Failures." 461/462 of these end-of-treatment (EOT) <LLOQ results were Target Not Detected (TND). One subject (38627-111206) had HCV RNA <LLOQ/Detected at EOT and did not experience virologic relapse.
[5] Includes 7 subjects who discontinued treatment between Days 1 and 15 and were responding virologically or missing HCV RNA data (12338-105203, 38851-130205, 42368-125208, 44365-383207, 45261-121203, 45264-582208, 45610-803211), and 2 subjects who completed treatment but were missing HCV RNA data through Follow-up Week ≥12 (38898-760211, 42420-202208).

Overall, there were no major differences in SVR12 or virologic failure rates according to IL28B rs12979860 genotype (Table 7).

Table 7. SVR12 rates in M11-646 according to HCV subtype and IL28B genotype.

Efficacy Outcome	HCV Genotype 1a			HCV Genotype 1b		
	IL28B CC (n=106)	IL28B CT (n=170)	IL28B TT (n=46)	IL28B CC (n=38)	IL28B CT (n=84)	IL28B TT (n=29)
SVR12*	103 (97.2%)	161 (94.7%)	44 (95.7%)	36 (94.7%)	83 (98.8%)	29 (100%)
Virologic Failure	2 (1.9%)	3 (1.8%)	2 (4.3%)	0 (0%)	1 (1.2%)	0 (0%)

* Includes updated SVR12 result from Subject 42765-604209 (GT1a, IL28B CT) reported by the sponsor during the review cycle.

4.3 Efficacy in Phase 3 Trial M13-098 (3-DAA+RBV, Experienced)

Summary of Trial Design

Clinical trial M13-098 was a Phase 3, randomized, double-blind, placebo-controlled study of the 3-DAA + RBV regimen in noncirrhotic, P/R treatment-experienced adults with chronic HCV genotype 1 infection. Subjects were to be naïve to HCV DAAs, and were classified based on their prior P/R treatment history as follows:

- Null responder: received ≥12 weeks of P/R and failed to achieve a ≥2 $\log_{10}$ IU/mL reduction in HCV RNA at Week 12 (Weeks 10 – 16), or received ≥4 weeks of P/R and achieved a <1 $\log_{10}$ IU/mL reduction in HCV RNA at Week 4 (≥25 days)
- Partial responder: received ≥20 weeks of P/R and achieved a ≥2 $\log_{10}$ IU/mL reduction in HCV RNA at Week 12 (Weeks 10 – 16), but failed to achieve HCV RNA undetectable at the end of treatment
- Relapser: received ≥36 weeks of P/R with undetectable HCV RNA at or after the end of treatment, but HCV RNA was detectable within 52 weeks of treatment follow-up

Approximately 400 subjects were to be randomized in a 3:1 ratio to receive either the active 3-DAA + RBV therapy or placebo for 12 weeks. Following 12 weeks of double-blind treatment, subjects initially randomized to receive placebo were administered open-label 3-DAA + RBV treatment for 12 weeks.

The primary objectives of this study were to compare the SVR12 rate (defined as HCV RNA <LLOQ 12 weeks following therapy) after 12 weeks of treatment with the 3-DAA + RBV regimen to the historical SVR rate of telaprevir plus P/R therapy, and to assess the safety of the active treatment regimen versus placebo for 12 weeks.

As in M11-646, the primary analysis occurred after subjects initially randomized to active drug completed through Post-Treatment Week 12 or prematurely discontinued the study, and subjects who were initially randomized to placebo completed 12 weeks of open-label active treatment or prematurely discontinued study drug. Additional follow-up analyses are planned. Again, this reviewer's efficacy analysis focused only on the active arm.

Reviewer's Efficacy Analysis

The overall intent-to-treat (ITT) SVR12 rate for subjects in the active arm was 286/297 (96.3%), with SVR12 rates of 96.0% and 96.7% for genotype 1a and genotype 1b subjects, respectively (Table 8). Virologic failure was observed in 5 (2.9%) HCV genotype 1a subjects and 2 (1.6%) genotype 1b subjects, with all 7 failures specifically observed as virologic relapse.

Note that the SVR12 rate includes one subject (47651-361303) who was missing data at Post-Treatment Week 12 but had HCV RNA <LLOQ/TND from Treatment Week 1 through Post-Treatment Week 8, with a local Post-Treatment Week ~19 HCV RNA result of "UNQUANTIFIABLE". This subject was classified as an SVR12 responder in my analysis. A request for more information about the local HCV RNA assay that was used was communicated to the sponsor on 5/7/2014. The sponsor responded in SDN 007 noting that the assay name and performance characteristics are unknown, but HCV RNA results of "HCV RNA not detected" were subsequently reported by the central laboratory for Post-Treatment Weeks 24 and 36.

Table 8. SVR12 (ITT) and virologic failure rates in clinical trial M13-098.

Efficacy Outcome	All subjects, active arm (n=297)	HCV Genotype 1 Subtype		
		1A (n=173)	1B (n=123)	1 (n=1)
SVR12 (ITT)[1]	**286/297 (96.3%)**	**166/173 (96%)**	**119/123 (96.7%)**	**1/1 (100%)**
Any VF[2]	7/297 (2.4%)	5/173 (2.9%)	2/123 (1.6%)	0/1 (0%)
On-Tx VF (vBT)	0/297 (0%)	0/173 (0%)	0/123 (0%)	0/1 (0%)
Relapse[3]	7/297 (2.4%)	5/173 (2.9%)	2/123 (1.6%)	0/1 (0%)
Among EOT <LLOQ[4]	7/293 (2.4%)	5/171 (2.9%)	2/121 (1.7%)	0/1 (0%)
Other Failures[5]	4/297 (1.3%)	2/173 (1.2%)	2/123 (1.6%)	0/1 (0%)

[1]Intent-to-treat (ITT) analysis, except subjects with missing SVR12 outcome results could be classified as SVR12 responders based on subsequent follow-up results.
[2]Total of On-Treatment Virologic Failure (On-Tx VF) and Relapse results.
[3]Relapse rate excludes subjects who discontinued prematurely (defined by sponsor as <77 days duration).
[4]Excludes non-SVR12 subjects who prematurely discontinued. 291/293 of these end-of-treatment (EOT) <LLOQ results were Target Not Detected (TND). Two subjects (40766-561303 and 42371-131311) had HCV RNA <LLOQ/Detected at EOT and experienced subsequent virologic relapse.
[5]Includes 4 subjects who discontinued treatment between Days 3 and 72 and were responding virologically or missing HCV RNA data (32887-782303, 44436-563306, 47651-361301, 48391-381311).

There was a numerical trend of a higher virologic failure rate in prior P/R null responders compared to subjects with other categories of prior P/R responsiveness (Table 9). Six (86%) of the 7 virologic failures were observed in prior P/R null responders.

Table 9. SVR12 rates in M13-098 according to HCV genotype 1 subtype and prior P/R response. The group of genotype 1a P/R null responders includes 3 subjects who were classified as "P/R Week 4 Futility" failures (all 3 achieved SVR12). The single subject without a reported HCV genotype 1 subtype was a prior P/R partial responder (achieved SVR12).

Efficacy Outcome	HCV Genotype 1a			HCV Genotype 1b		
	Null (n=87)	Partial (n=36)	Relapser (n=50)	Null (n=59)	Partial (n=28)	Relapser (n=36)
SVR12	83 (95.4%)	36 (100%)	47 (94%)	56 (94.9%)	28 (100%)	35 (97.2%)
Virologic Failure	4 (4.6%)	0 (0%)	1 (2%)	2 (3.4%)	0 (0%)	0 (0%)

4.4 Efficacy in Phase 3 Trial M13-099 (Subjects with Cirrhosis)

Summary of Trial Design

Clinical trial M13-099 was a Phase 3, randomized, open-label study of the 3-DAA + RBV regimen dosed for 12 or 24 weeks in treatment-naïve and P/R treatment-experienced adults with chronic HCV genotype 1 infection and compensated cirrhosis. Approximately 380 subjects were enrolled across the 12- and 24-week treatment arms. All subjects were to be naïve to HCV DAAs. Subjects previously treated with P/R were classified as follows (as in M13-098):

- Null responder: received ≥12 weeks of P/R and failed to achieve a ≥2 $\log_{10}$ IU/mL reduction in HCV RNA at Week 12 (Weeks 10 – 16), or received ≥4 weeks of P/R and achieved a <1 $\log_{10}$ IU/mL reduction in HCV RNA at Week 4 (≥ 25 days)
- Partial responder: received ≥20 weeks of P/R and achieved a ≥2 $\log_{10}$ IU/mL reduction in HCV RNA at Week 12 (Weeks 10 – 16), but failed to achieve HCV RNA undetectable at the end of treatment
- Relapser: received ≥36 weeks of P/R with undetectable HCV RNA at or after the end of treatment, but HCV RNA was detectable within 52 weeks of treatment follow-up

The primary objectives of this study were to assess safety and to compare the SVR12 rates (defined as HCV RNA <LLOQ 12 weeks following therapy) after 12 or 24 weeks of treatment with the 3-DAA + RBV regimen with the historical SVR rate for telaprevir plus P/R therapy in HCV genotype 1 infected adults with compensated cirrhosis. The primary analysis occurred after all randomized subjects completed through Post-Treatment Week 12 or prematurely discontinued from the study.

Reviewer's Efficacy Analysis

The overall intent-to-treat (ITT) SVR12 rates in the 12- and 24-week arms were 91.8% and 95.9%, respectively (Table 10). Relapse rates were higher in the 12-week arm compared to the 24-week arm: 6.3% versus 0.6%. Note that one subject (M13099-47008-606109) in the 24-week arm who was classified as an SVR12 responder was classified as a nonresponder ("Other Failure") in my analysis. The subject did not have HCV RNA results after Post-Treatment Week 8, although HCV RNA levels were <LLOQ by Treatment Week 2 and <LLOQ/TND through Post-Treatment Week 8.

Table 10. SVR12 (ITT) and virologic failure rates in clinical trial M13-099.

Efficacy Outcome	12-Week Arm (n=208)	24-Week Arm (n=172)
SVR12 (ITT)[1]	**191/208 (91.8%)**	**165/172 (95.9%)**
Any VF[2]	14/208 (6.7%)	4/172 (2.3%)
On-Tx VF (vBT)	1/208 (0.5%)	3/172 (1.7%)
Relapse[3]	13/208 (6.3%)	1/172 (0.6%)
Among EOT <LLOQ[4]	13/203 (6.4%)	1/165 (0.6%)
Other Failures[5]	4/208 (1.9%)	3/172 (1.7%)

[1]Intent-to-treat (ITT) analysis, except subjects with missing SVR12 outcome results could be classified as SVR12 responders based on subsequent follow-up results. Includes updated SVR12 result from Subject 12538-117112 (24-week arm, tx-naïve, GT1a, IL28B TT) reported by the sponsor during the review cycle.
[2]Total of On-Treatment Virologic Failure (On-Tx VF) and Relapse results.
[3]Relapse rate is cumulative through all follow-up results but excludes subjects who discontinued prematurely (defined by sponsor as <77 days duration for 12-week arm or <154 days for 24-week arm). Includes one SVR12 responder who experienced post-SVR12 relapse (12538-117105).
[4]Excludes "Other Failures". 368/368 of these end-of-treatment (EOT) <LLOQ results were Target Not Detected (TND).
[5]Includes 7 subjects without evidence of VF who prematurely discontinued treatment or were missing HCV RNA data for analysis (20229-137103, 31549-114106, 38624-111109, 44318-382117, 44318-382123, 47008-606109, 47487-203108).

For previously treatment-naïve subjects, SVR12 rates were lower and virologic failure rates higher among HCV genotype 1a subjects treated for 12 weeks compared to those treated for 24 weeks. The lower SVR12 rate and higher virologic failure rate with the 12 week duration could be attributed to HCV genotype 1a, IL28B non-CC subjects, although the numbers of subjects in these subgroups were small (Table 11). No treatment-naïve, HCV genotype 1b subjects experienced virologic failure regardless of treatment duration, indicating that 12 weeks of 3-DAA + RBV treatment was adequate.

Table 11. SVR12 and virologic failure rates in previously treatment-naïve subjects according to HCV genotype 1 subtype, IL28B genotype (rs12979860), and treatment duration. Note that all virologic failures in this table refer to post-treatment relapse.

HCV Genotype 1a Subjects						
	IL28B CC		IL28B CT		IL28B TT	
Efficacy Outcome	**12 Weeks**	**24 Weeks**	**12 Weeks**	**24 Weeks**	**12 Weeks**	**24 Weeks**
SVR12	19/19 (100%)	14/16 (87.5%)	31/34 (91.2%)	29/31 (93.5%)	9/11 (81.8%)	9/9 (100%)
Virologic Failure	0/19 (0%)	0/16 (0%)	3/34 (8.8%)	1/31 (3.2%)	1/11 (9.1%)	0/9 (0%)
HCV Genotype 1b Subjects						
	IL28B CC		IL28B CT		IL28B TT	
Efficacy Outcome	**12 Weeks**	**24 Weeks**	**12 Weeks**	**24 Weeks**	**12 Weeks**	**24 Weeks**
SVR12	4/4 (100%)	5/5 (100%)	13/13 (100%)	10/10 (100%)	5/5 (100%)	3/3 (100%)
Virologic Failure	0/4 (0%)	0/5 (0%)	0/13 (0%)	0/10 (0%)	0/5 (0%)	0/3 (0%)

For P/R treatment-experienced subjects, again there appeared to be a numeric improvement in SVR12 and virologic failure rates with the extended 24-week treatment duration for HCV genotype 1a subjects (Table 12). Overall virologic failure rates for HCV genotype 1a, prior P/R treatment-experienced subjects were 9/76 (11.8%) and 3/65 (4.6%) for subjects treated for 12 weeks and 24 weeks, respectively. Most (7/9, 78%) of the HCV genotype 1a virologic failures observed in the 12-week duration group were among prior P/R null responders. Again, there was no clear indication that HCV genotype 1b subjects benefited from the 24 week treatment duration, with only a single HCV genotype 1b subject treated for 12 weeks experiencing virologic failure (relapse).

Table 12. SVR12 and virologic failure rates in prior P/R treatment failure subjects according to HCV genotype 1 subtype, prior P/R response, and treatment duration.

HCV Genotype 1a Subjects						
	NULL RESPONDERS		PARTIAL RESPONDERS		RELAPSERS	
Efficacy Outcome	**12 Weeks**	**24 Weeks**	**12 Weeks**	**24 Weeks**	**12 Weeks**	**24 Weeks**
SVR12	40/50 (80%)	39/42 (92.9%)	11/11 (100%)	10/10 (100%)	14/15 (93.3%)	13/13 (100%)
Virologic Failure	7/50 (14%)	3/42 (7.1%)[1]	1/11 (9.1%)[2]	0/10 (0%)	1/15 (6.7%)[3]	0/13 (0%)
HCV Genotype 1b Subjects						
	NULL RESPONDERS		PARTIAL RESPONDERS		RELAPSERS	
Efficacy Outcome	**12 Weeks**	**24 Weeks**	**12 Weeks**	**24 Weeks**	**12 Weeks**	**24 Weeks**
SVR12	25/25 (100%)	20/20 (100%)	6/7 (85.7%)	3/3 (100%)	14/14 (100%)	10/10 (100%)
Virologic Failure	0/25 (0%)	0/20 (0%)	1/7 (14.3%)	0/3 (0%)	0/14 (0%)	0/10 (0%)

[1]All on-treatment virologic failure.
[2]Reflects a subject (12538-117105) who experienced post-SVR12 relapse.
[3]Virologic failure in this subject (31542-101111) was on-treatment, which would not have been impacted by a longer treatment duration.

4.5 Efficacy in Phase 3 Trial M13-961 (3-DAA±RBV, GT1b, Tx-naïve)

Summary of Trial Design

Clinical trial M13-961 was a Phase 3, randomized, double-blind, placebo-controlled study of the 3-DAA regimen dosed with or without RBV for 12 weeks in noncirrhotic, treatment-naïve adults with chronic HCV genotype 1b infection. Approximately 400 subjects were to be randomized 1:1 to receive the 3-DAA regimen with RBV or RBV placebo.

The primary objectives of this study were to compare the safety of the combination regimen dosed with or without RBV, and to show the noninferiority in SVR12 rates (defined as HCV RNA <LLOQ 12 weeks following therapy) in either arm compared with the historical SVR rate for telaprevir plus P/R therapy. Noninferiority of SVR12 rates for the 3-DAA versus 3-DAA + RBV regimen was included as a secondary objective. The primary analysis occurred after all subjects completed through Post-Treatment Week 12 or prematurely discontinued the study.

Reviewer's Efficacy Analysis

SVR12 rates were ≥99% across both arms (Table 13). Only a single subject (47538-232506, 3-DAA + RBV) experienced virologic failure (breakthrough). One other non-SVR12 subject (47502-214509) was missing HCV RNA data at or after Post-Treatment Week 12. This subject had no HCV RNA data after Post-Treatment Week 8, but was classified as an SVR12 responder by the sponsor.

Since there was only a single virologic failure across both arms, no additional subgroup efficacy analyses were conducted for this trial.

Table 13. SVR12 and virologic failure rates in clinical trial M13-961 (Treatment-naïve, noncirrhotic, HCV genotype 1b subjects).

Efficacy Outcome	3-DAA + Placebo (n=209)	3-DAA + RBV (n=210)
SVR12 (ITT)[1]	**209/209 (100%)**	**208/210 (99.0%)**
On-Tx VF (vBT)	0/209 (0%)	1/210 (0.5%)
Other Failures[2]	0/209 (0%)	1/210 (0.5%)

[1]Intent-to-treat (ITT) analysis, except subjects with missing SVR12 outcome results could be classified as SVR12 responders based on subsequent follow-up results. Includes updated SVR12 results from Subjects 47627-215518 and 48934-109502 (both in 3-DAA + Placebo arm) reported by the sponsor during the review cycle.
[2]Includes 1 subject without evidence of virologic failure who was missing adequate HCV RNA data for analysis (47502-214509).

4.6 Efficacy in Phase 3 Trial M13-389 (3-DAA±RBV, GT1b, Tx-experienced)

Summary of Trial Design

Clinical trial M13-389 was a Phase 3, open-label, randomized study of the 3-DAA regimen dosed with or without RBV for 12 weeks in P/R treatment-experienced, noncirrhotic adults with chronic HCV genotype 1b infection. Approximately 210 subjects were to be randomized 1:1 to receive the 3-DAA regimen with or without RBV. All subjects were to be naïve to HCV DAAs. Subjects were classified based on their prior P/R treatment response as follows:

- Null responder: received ≥12 weeks of P/R and failed to achieve a ≥2 $\log_{10}$ IU/mL reduction in HCV RNA at Week 12 (Weeks 10 – 16) (no criteria based on Week 4 response)

- Partial responder: received ≥20 weeks of P/R and achieved a ≥2 $\log_{10}$ IU/mL reduction in HCV RNA at Week 12 (Weeks 10 – 16), but failed to achieve HCV RNA undetectable at the end of treatment
- Relapser: received ≥36 weeks of P/R with undetectable HCV RNA at or after the end of treatment, but HCV RNA was detectable within 52 weeks of treatment follow-up

The primary objectives of this study were to compare the safety of the 3-DAA combination regimen dosed with or without RBV, and to show the noninferiority in SVR12 rates (defined as HCV RNA <LLOQ 12 weeks following therapy) in either arm compared with the historical SVR rate for telaprevir plus P/R therapy. Noninferiority of SVR12 rate for the 3-DAA versus 3-DAA + RBV regimen was included as a secondary objective. The primary analysis occurred after all subjects completed Post-Treatment Week 12 or prematurely discontinued from the study.

Note that clinical trial M13-389 was originally designed as a Phase 2b trial evaluating various combinations of DAAs with or without RBV dosed for 8 or 12 weeks in both HCV genotype 1a and 1b infected subjects, with subjects dosed with the non-co-formulated drug products. During the conduct of the trial the protocol was amended multiple times ultimately resulting in the Phase 3 protocol in which only HCV genotype 1b subjects were enrolled and received the 3-DAA +/- RBV regimen for 12 weeks, with PTV/r/OMB administered as the fixed-dose combination tablet as in the other Phase 3 trials. See the M13-389 protocol history for details. Seven subjects who were treated in M13-389 were excluded from the sponsor's ITT efficacy analysis and were also excluded from this reviewer's efficacy analysis shown below. Six (3 GT1a, 3 GT1b) of the 7 subjects were enrolled before the protocol was amended to a Phase 3 trial, and the other subject did not have an HCV subtype reported by the central laboratory; all 7 subjects achieved SVR12.

Reviewer's Efficacy Analysis

SVR12 rates in both arms were >97%, with no observations of virologic failure in either arm (Table 14). Three non-SVR12 subjects either discontinued treatment early (13 days and 36 days), or were missing HCV RNA data at or after Post-Treatment Week 12. Note that one of these subjects (48812-10223) who had no HCV RNA data after Post-Treatment Week 8 was classified as an SVR12 responder by the sponsor.

Table 14. SVR12 and virologic failure rates in clinical trial M13-389 (P/R treatment-experienced, noncirrhotic, HCV genotype 1b subjects).

Efficacy Outcome	3-DAA (n=91)	3-DAA + RBV (n=88)
SVR12 (ITT)[1]	**90/91 (98.9%)**	**86/88 (97.7%)**
Virologic Failure (vBT or Relapse)	0/91 (0%)	0/88 (0%)
Other Failures[2]	1/91 (1.1%)	2/88 (2.3%)

[1]Intent-to-treat (ITT) analysis, except subjects with missing SVR12 outcome results could be classified as SVR12 responders based on subsequent follow-up results. Includes updated SVR12 result from Subject 48811-10224 (3-DAA + RBV arm) reported by the sponsor during the review cycle.
[2]Includes 3 subjects without evidence of virologic failure who either discontinued treatment after 12-36 days (45122-10040, 45531-10303) or were missing adequate HCV RNA data for analysis (48812-10223).

There was a similar distribution of prior P/R null responders, partial responders and relapsers both within and between treatment arms (28-36% for each subgroup within each arm). Since there were no virologic failures across both arms, no additional subgroup efficacy analyses were conducted.

4.7 Efficacy in Phase 3 Trial M14-002 (3-DAA±RBV, GT1a, Tx-naïve)

Summary of Trial Design

Clinical trial M14-002 was a Phase 3, randomized, double-blind, placebo-controlled study of the 3-DAA regimen dosed with or without RBV for 12 weeks in noncirrhotic, treatment-naïve adults with chronic HCV genotype 1a infection. Approximately 300 subjects were to be randomized 1:2 to receive the 3-DAA regimen with RBV or RBV placebo.

The primary objectives of this study were to compare the safety of the combination regimen dosed with or without RBV, and to show the noninferiority in SVR12 rates (defined as HCV RNA <LLOQ 12 weeks following therapy) in either arm compared with the historical SVR rate for telaprevir plus P/R therapy. Noninferiority of SVR12 rates for the 3-DAA versus 3-DAA + RBV regimen was included as a secondary objective. The primary analysis occurred after all subjects completed through Post-Treatment Week 12 or prematurely discontinued the study.

Reviewer's Efficacy Analysis

Intent-to-treat SVR12 rates were 89.7% and 96% for the 3-DAA + Placebo and 3-DAA + RBV groups, respectively (Table 15). Virologic failure (breakthrough and relapse) was observed in 16 (7.8%) subjects in the 3-DAA + Placebo group and 2 (2%) subjects in the 3-DAA + RBV group. The use of RBV was associated with numerically lower rates of both virologic breakthrough and virologic relapse.

Note that this independent analysis censored one subject who enrolled as a genotype 1a subject based on a local laboratory result but was subsequently found to be infected with HCV genotype 1b (Subject 42419-117414, 3-DAA + placebo arm, IL28B CT, achieved SVR12). Two other subjects (12641-106423 and 40605-148402) classified as SVR12 responders by the sponsor were SVR12 nonresponders in my analysis. Both subjects had HCV RNA TND through Post-Treatment Week 8 without subsequent follow-up and were therefore classified as "Other Failures".

Table 15. SVR12 and virologic failure rates in clinical trial M14-002 (Treatment-naïve, noncirrhotic, HCV genotype 1a subjects).

Efficacy Outcome	3-DAA + Placebo (n=204[6])	3-DAA + RBV (n=100)
SVR12 (ITT)[1]	**183/204 (89.7%)**	**96/100 (96%)**
Any VF[2]	16/204 (7.8%)	2/100 (2%)
On-Tx VF (vBT)	6/204 (2.9%)	1/100 (1%)
Relapse[3]	10/204 (4.9%)	1/100 (1%)
Among EOT <LLOQ[4]	10/193 (5.2%)	1/97 (1%)
Other Failures[5]	5/204 (2.5%)	2/100 (2%)

[1] Intent-to-treat (ITT) analysis, except subjects with missing SVR12 outcome results could be classified as SVR12 responders based on subsequent follow-up results.
[2] Total of On-Treatment Virologic Failure (On-Tx VF) and Relapse results.
[3] Relapse rate excludes subjects who discontinued prematurely (defined by sponsor as <77 days).
[4] Excludes "Other Failures". 288/290 of these end-of-treatment (EOT) <LLOQ results were Target Not Detected (TND). One subject (48575-133402) had a result of <LLOQ/Detected at EOT and experienced relapse. One subject (48574-109409) had a single transient result of <LLOQ/Detected at EOT flanked by TND results.
[5] Includes 7 subjects without evidence of virologic failure who prematurely discontinued treatment or were missing adequate HCV RNA data for analysis (12641-106423, 33471-103407, 37536-124403, 40605-148402, 42368-144405, 48582-108408, 48582-108409).
[6] Excludes one HCV genotype 1b subject (42419-117414) who enrolled due to protocol error (enrolled based on local laboratory result).

The addition of RBV to the 3-DAA regimen resulted in a lower virologic failure rate for IL28B CC, CT, and TT genotype subjects (Table 16 and Figure 3), although the numbers of virologic failure subjects for analysis were relatively small for most subgroups.

Table 16. SVR12 and virologic failure rates according to IL28B status and treatment regimen.

Efficacy Outcome	3-DAA + Placebo			3-DAA + RBV		
	IL28B CC (n=63)	IL28B CT (n=104)	IL28B TT (n=37)	IL28B CC (n=31)	IL28B CT (n=58)	IL28B TT (n=11)
SVR12	60 (95.2%)	91 (87.5%)	32 (86.5%)	30 (96.8%)	55 (94.8%)	11 (100%)
Virologic Failure	2 (3.2%)	11 (10.6%)	3 (8.1%)	0 (0%)	2 (3.4%)	0 (0%)

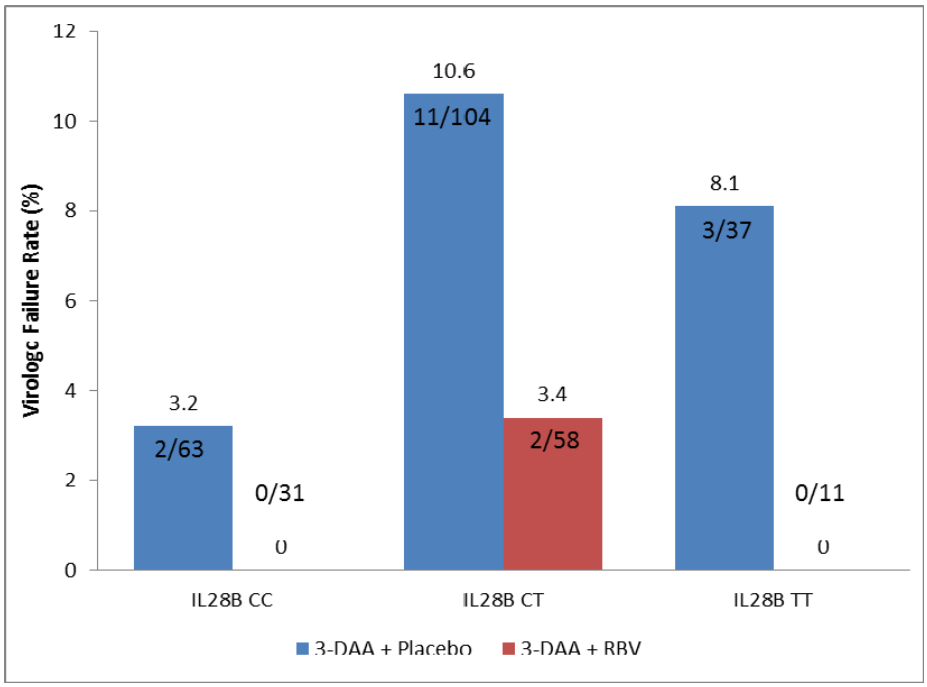


Figure 3. Virologic failure rates according to IL28B status and treatment regimen.

Update to M14-002 efficacy analysis: In the 4-month safety update report for this NDA (SDN 030) it was noted that 3 HCV genotype 1a infected subjects (22887-119403, 33471-103408, and 48574-109402, all IL28B CC genotype) who received the 3-DAA + Placebo regimen and achieved SVR12 experienced virologic relapse at the SVR24 assessment. If considering these 3 late relapses, the SVR and virologic failure rates for the 3-DAA + Placebo arm change to 180/204 (88%) and 19/204 (9%), compared to 96/100 (96%) and 2/100 (2%), respectively, for subjects in the 3-DAA + RBV arm, further illustrating the added efficacy benefit of including RBV for HCV genotype 1a. The updated virologic failure rates for subjects with the IL28B CC genotype are now 5/63 (8%) and 0/31 (0%) for the 3-DAA + Placebo and 3-DAA + RBV arms, respectively, illustrating that RBV provided an efficacy benefit even among subjects with the favorable IL28B genotype.

4.8 Efficacy in Phase 2 Trial M11-652 (Large Phase 2b Trial of Various DAA Combinations)

Summary of Trial Design

Clinical trial M11-652 was a large Phase 2b, open-label, partially randomized study of various combinations of 2- or 3-DAA regimens dosed with or without RBV in treatment-naïve and P/R treatment-experienced adults with chronic HCV genotype 1 infection. All subjects were to be noncirrhotic and naïve to HCV DAAs. All P/R treatment-experienced subjects were to be specifically null responders, defined as previously received P/R for ≥12 weeks with a <2 $\log_{10}$ IU/mL decline in HCV RNA at Week 12.

The overall study design of M11-652 is illustrated in Figure 4 (Protocol pg. 33). The primary efficacy endpoint was SVR24 (defined as HCV RNA <LLOQ 24 weeks following therapy), although SVR12 analyses were also conducted.

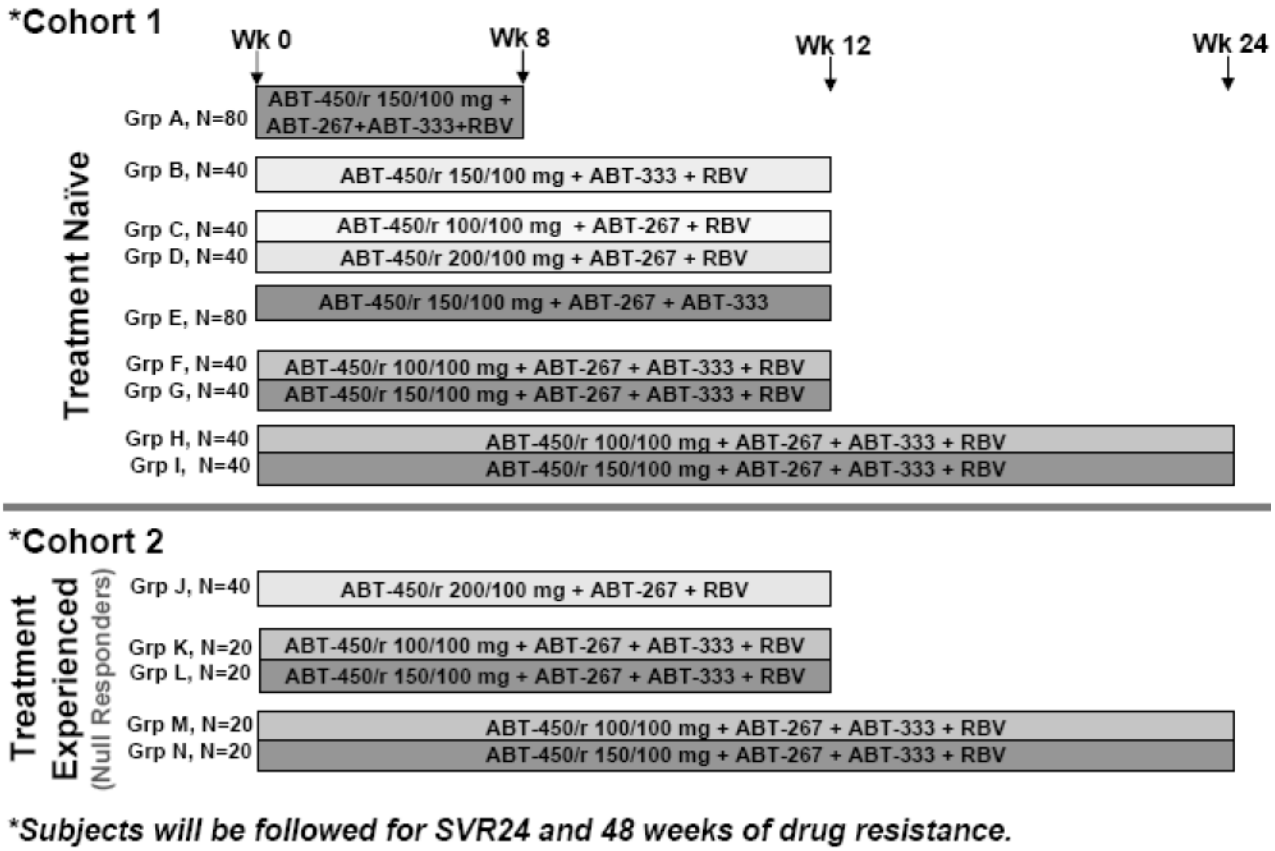


Figure 4. Study design schematic for clinical trial M11-652. ABT-450=PTV, /r=ritonavir, ABT-267=OMB, ABT-333=DBV. ABT-450/r were dosed QD, ABT-267 was dosed at 25 mg QD and ABT-333 was dosed at 400 mg BID; note that these formulations differed from those used in Phase 3 trials.

Reviewer's Efficacy Analysis

Overall efficacy and virologic failure rates are summarized in Table 17. SVR rates were generally highest in the 3-DAA + RBV groups treated for 12 or 24 weeks. In general there did not appear to be an obvious and consistent difference in SVR and virologic failure rates for subjects treated with different dose levels of PTV. SVR rates were clearly higher across all arms in HCV genotype 1b subjects compared to HCV genotype 1a subjects (Figure 5).

Table 17. SVR (ITT) and virologic failure results in M11-652. Arms A-I included treatment-naïve subjects, and Arms J-N included P/R null responders.

Arm	Regimen	Dur. (Wks)	N	SVR12	SVR24	On-Tx Failure	Relapse*
A	PTV(150)/r/OMB/DBV + RBV	8	80	71/80 (88.8%)	70/80 (87.5%)	0 (0%)	10 (12.5%)
B	PTV(150)/r/DBV + RBV	12	41	35/41 (85.4%)	34/41 (82.9%)	1 (2.4%)	4 (9.8%)
C	PTV(100)/r/OMB + RBV	12	39	35/39 (89.7%)	33/39 (84.6%)	0 (0%)	6 (15.4%)
D	PTV(200)/r/OMB + RBV	12	40	37/40 (92.5%)	37/40 (92.5%)	1 (2.5%)	2 (5.0%)
E	PTV(150)/r/OMB/DBV	12	79	70/79 (88.6%)	70/79 (88.6%)	1 (1.3%)	5 (6.3%)
F	PTV(100)/r/OMB/DBV + RBV	12	39	38/39 (97.4%)	38/39 (97.4%)	0 (0%)	0 (0%)
G	PTV(150)/r/OMB/DBV + RBV	12	40	38/40 (95.0%)	38/40 (95.0%)	0 (0%)	1 (2.5%)
H	PTV(100)/r/OMB/DBV + RBV	24	40	37/40 (92.5%)	37/40 (92.5%)	0 (0%)	1 (2.5%)
I	PTV(150)/r/OMB/DBV + RBV	24	40	37/40 (92.5%)	36/40 (90.0%)	0 (0%)	2 (5.0)
J	PTV(200)/r/OMB + RBV	12	45	40/45 (88.9%)	40/45 (88.9%)	0 (0%)	5 (11.1%)
K	PTV(100)/r/OMB/DBV + RBV	12	23	21/23 (91.3%)	21/23 (91.3%)	2 (8.7%)	0 (0%)
L	PTV(150)/r/OMB/DBV + RBV	12	22	21/22 (95.5%)	21/22 (95.5%)	1 (4.5%)	0 (0%)
M	PTV(100)/r/OMB/DBV + RBV	24	23	21/23 (91.3%)	21/23 (91.3%)	1 (4.3%)	0 (0%)
N	PTV(150)/r/OMB/DBV + RBV	24	20	20/20 (100%)	20/20 (100%)	0 (0%)	0 (0%)

*Relapse rates shown are cumulative through end of follow-up and do not exclude subjects who discontinued treatment prematurely.

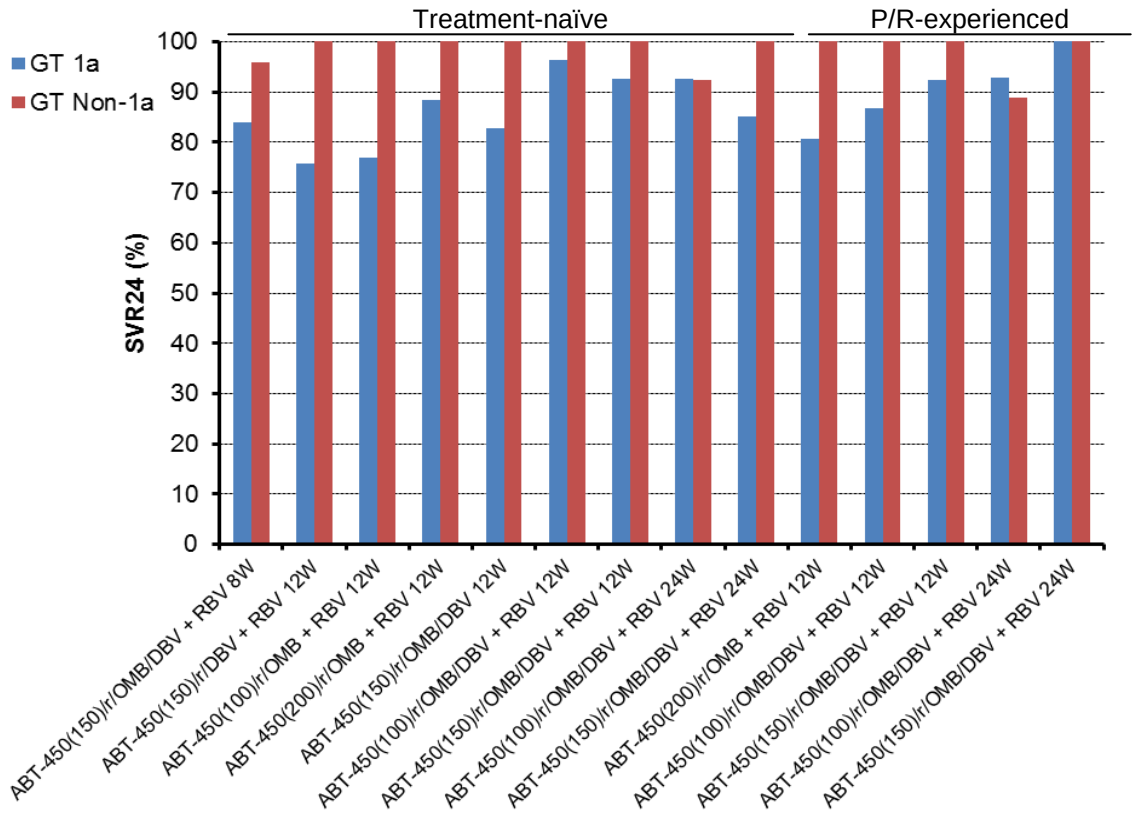


Figure 5. SVR24 rates in M11-652 according to HCV genotype 1 subtype. Genotype "non-1a" refers primarily to HCV genotype 1b infected subjects, but includes 3 subjects with either genotype 1m (n=1) or genotype 1 without a reported subtype (n=2).

All virologic failures in M1-652 occurred in HCV genotype 1a infected subjects except for a single genotype 1b subject in Arm A who experienced virologic relapse after the 8 week treatment duration (Figure 6). Note that the virologic failure rate difference shown in Figure 6 for genotype 1a, P/R-experienced subjects who received the 3-DAA + RBV regimen for 12 versus 24 weeks should not be interpreted as an indication that the 24 week duration was more effective, as all virologic failure in both arms manifested as on-treatment virologic breakthrough; no subjects in either group experienced virologic relapse.

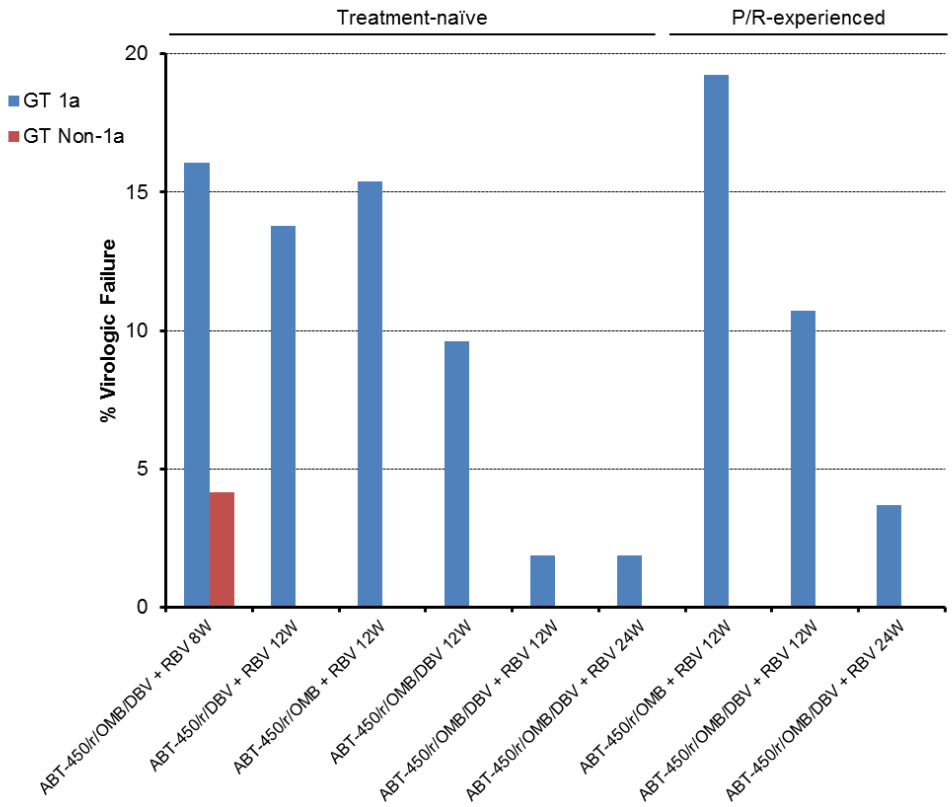


Figure 6. Virologic failure rates (relapse and on-treatment failure) in M11-652 according to HCV genotype 1 subtype, pooling different ABT-450 (PTV) doses. Genotype "non-1a" refers primarily to HCV genotype 1b infected subjects, but includes 3 subjects with either genotype 1m (n=1) or genotype 1 without a reported subtype (n=2). Relapsers include only subjects who completed (within 1 week) planned treatment duration.

The totality of data from M11-652 indicate that the 3-DAA + RBV regimen dosed for ≥12 weeks was optimally effective for treatment-naïve and P/R-experienced HCV genotype 1a subjects. All of the regimens were essentially equally effective in HCV genotype 1b subjects given that only a single genotype 1b subject experienced virologic failure.

4.9 Efficacy in Phase 2 Trial M14-103 (Subjects on Opioid Replacement Therapy)

Summary of Trial Design

Clinical trial M14-103 was a Phase 2, single-arm study evaluating the efficacy of the 3-DAA + RBV regimen dosed for 12 weeks in noncirrhotic HCV genotype 1 infected adults who were on a stable opioid replacement therapy of methadone or buprenorphine ± naloxone. Subjects could have been treatment-naïve or P/R treatment-experienced. Approximately 40 subjects were to be enrolled.

The primary objectives were to assess safety and efficacy based on SVR12 rate (defined as HCV RNA <LLOQ 12 weeks following therapy). The primary analysis occurred after all subjects had completed through Post-Treatment Week 12 or prematurely discontinued the study.

Reviewer's Efficacy Analysis
Thirty-eight subjects were enrolled, of whom 32 were infected with HCV genotype 1a and 6 infected with HCV genotype 1b. All but 2 subjects were previously treatment-naïve.

SVR12 was achieved by 37/38 (97.4%) subjects. The single non-SVR12 subject (12641-12420) prematurely discontinued treatment after 25 days for safety reasons while HCV RNA was <LLOQ/Detected, with no subsequent follow-up results. No subject experienced virologic failure.

4.10 Efficacy in Phase 2 Trial M12-999 (Post-Liver Transplant Subjects, Interim Data)

An interim study report and associated HCV RNA analysis data were submitted during the review cycle for Phase 2 trial M12-999. No drug resistance datasets were provided for independent review.

Summary of Trial Design
Clinical trial M12-999 is an ongoing, open-label study evaluating the 3-DAA regimen dosed with or without RBV in adult liver transplant recipients with recurrent HCV genotype 1 infection. The primary objectives of this study are to assess safety and efficacy based on SVR12 rates (defined as HCV RNA <LLOQ 12 weeks following therapy). The study includes 2 Cohorts, with Cohort 1 consisting of Arm A and Cohort 2 consisting of Arms B and C. As of the time of reporting, enrollment in Cohort 1/Arm A was complete, with 34 subjects enrolled in the U.S. and Spain.

Subjects in Cohort 1/Arm A had fibrosis scores of ≤2 and were to be naïve to P/R treatment post-transplant, although subjects could have received P/R prior to transplant. Subjects were treated with the 3-DAA regimen for 24 weeks, with RBV dosing managed at the discretion of the investigator.

Cohort 2 is currently enrolling and will consist of approximately 40 HCV genotype 1 infected subjects post-liver transplant. In this Cohort enrollment of subjects with more advanced liver fibrosis, but without cirrhosis, is permitted. Subjects in Arm B will receive the 3-DAA regimen for 24 weeks, with RBV dosing managed at the discretion of the investigator. In Arm C, subjects with HCV genotype 1b infection who have not received HCV treatment post-transplant or who are "responders" to P/R therapy (unclear what this means) will receive the 3-DAA regimen without RBV for 24 weeks.

This interim report covers available data from subjects in Cohort 1/Arm A (n=34). In Cohort 1/Arm A, 34 subjects were randomized to receive study drug and all but 1 subject completed study therapy; 1 subject discontinued due to an adverse event. This Arm included 29 HCV genotype 1a infected subjects and 5 HCV genotype 1b infected subjects.

Reviewer's Efficacy Analysis
Thirty-three of 34 subjects (97.1%) achieved SVR12, including 28/29 (96.6%) HCV genotype 1a infected subjects and 5/5 (100%) HCV genotype 1b infected subjects. Of those subjects who achieved SVR12, 20/20 (100%) subjects with available data achieved SVR24.

One subject (105605) with HCV genotype 1a infection experienced virologic relapse by Post-Treatment Day 3. According to the sponsor's summary of resistance data, this subject had an NS3 Q80K polymorphism detected at Baseline without any other resistance-associated polymorphisms in NS5A or NS5B. At failure, the subject had treatment-emergent R155K (with Q80K still detected) in NS3, M28T+Q30R in NS5A, and G554S in NS5B. The overall prevalence of the NS3 Q80K polymorphism in genotype 1a subjects was 11/29 (38%); thus, it appears that 1/11 (9%) HCV genotype 1a subjects with NS3 Q80K experienced virologic failure.

4.11 Pooled Summary of Virologic Failure Rates

Table 18 summarizes the pooled virologic failure rates across all Phase 2/3 trials evaluating the efficacy of the 3-DAA +/- RBV regimen administered for 8-24 weeks. Overall, 73/2498 (2.9%) subjects experienced virologic failure, including on-treatment failure and relapse, with a higher failure rate in subjects infected with HCV genotype 1a compared to those infected with genotype 1b. Virologic failure rates were slightly lower if considering only subjects enrolled in arms evaluating treatment durations of 12 or 24 weeks.

Table 18. Pooled virologic failure rates among subjects treated with 3-DAA +/- RBV in Phase 2/3 trials. "All Subjects" includes 4 HCV genotype 1 subjects who were not identified as having 1a or 1b infection.

	Virologic Failure Rates	
Population	**8-, 12-, or 24-Week Durations**	**12-, or 24-Week Durations**
1a	67/1363 (4.9%)	58/1307 (4.4%)
1b	6/1131 (0.5%)	5/1107 (0.5%)
All Subjects	73/2498 (2.9%)	63/2418 (2.6%)

Note that these pooled virologic failure rates do not include clinical trial M12-999 (post-liver transplant subjects), for which only preliminary data have been provided. These virologic failure rates also do not include subjects who were initially randomized to placebo in clinical trials M11-646 and M13-098 and subsequently received open-label treatment per protocol; complete efficacy data were not available from these subjects for review. However, the sponsor's resistance analyses included 2 subjects from clinical trial M11-646 who experienced on-treatment virologic failure during this open-label treatment period.

Update to virologic failure rates:
The virologic failure rates noted above included any subjects who experienced post-SVR12 virologic relapse based on available data submitted in the original NDA package. This included 3 post-SVR12 relapsers: M13099-12538-117105, M11652-44372-5610 and M11652-37388-5593. The 4-month safety update noted 8 additional post-SVR12 virologic relapsers, all from Phase 3 trials:

- M11646-40282-701212
- M11646-45300-422209
- M11646-47010-605202
- M11646-47142-461202
- M14002-22887-119403
- M14002-33471-103408
- M14002-48574-109402
- M13961-20229-102501

Table 19 summarizes the updated rates of virologic failure in these trials, including all known late virologic relapse subjects, and still excluding subjects who were initially randomized to placebo in clinical trials M11-646 and M13-098 and subsequently received open-label treatment per protocol. The next review section includes a more comprehensive summary of SVR12 durability.

Table 19. Pooled virologic failure rates among subjects treated with 3-DAA +/- RBV in Phase 2/3 trials (updated with late relapser data from NDA safety update). "All Subjects" includes 4 HCV genotype 1 subjects who were not identified as having 1a or 1b infection.

	Virologic Failure Rates	
Population	**8-, 12-, or 24-Week Durations**	**12-, or 24-Week Durations**
All Subjects	81/2498 (3.2%)	71/2418 (2.9%)
Genotype 1a Subjects	74/1363 (5.4%)	65/1307 (5.0%)
On-Tx Failure/Breakthrough	17/1363 (1.2%)	17/1307 (1.3%)
Relapse	57/1363 (4.2%)	48/1307 (3.7%)
Genotype 1b Subjects	7/1131 (0.6%)	6/1107 (0.5%)
On-Tx Failure/Breakthrough	1/1131 (0.1%)	1/1107 (0.1%)
Relapse	6/1131 (0.5%)	5/1107 (0.5%)

4.12 Durability of SVR12

Most subjects in the Phase 3 trials did not have follow-up data reported after the SVR12 assessment in the original NDA submission package. Pooling available data from all 6 Phase 3 trials, of those who achieved SVR12 (observed) and had available SVR24 results, 453/454 (99.8%) achieved SVR24.

In Phase 2b clinical trial M11-652, subjects were to return for visits through 48 weeks of post-treatment follow-up. Among subjects who achieved SVR12 (observed) with any combination DAA +/- RBV regimen in M11-652, SVR24 and SVR48 were achieved in 501/504 (99.4%) and 490/494 (99.2%) of subjects with available data, respectively. Four subjects (37388-5593, 43114-5554, 44366-5520 and 44372-5610) experienced post-SVR12 relapse, 3 between the SVR12 and SVR24 assessment, and 1 between the SVR24 and SVR48 assessment.

The 4-month safety update report for this NDA (SDN 030) included an updated summary of late virologic relapse observed across all Phase 2 and Phase 3 trials that studied at least 2 of the DAAs included in the 3-DAA combination. Consistent with the results summarized above, late relapse after achieving SVR12 has been rare, and even rarer after achieving SVR24. Considering those with available data for analysis, a total of 11/2943 (0.4%) SVR12-achieving subjects have experienced confirmed virologic relapse in the SVR24 window, and 2/2581(0.08%) subjects have experienced confirmed virologic relapse after achieving SVR24 (USUBJIDs: M11652-37388-5593, M13961-20229-102501). Note that while the report summarized "confirmed" late relapses, according to the sponsor's analysis (summarized in SDN 034) if the last available post-treatment HCV RNA value was ≥LLOQ, then the subject would have been considered a relapser (i.e., confirmation not required). Complete nucleotide sequence analysis data were not reported for all of the late relapsers to distinguish between late relapse and re-infection. However, at least one of the post-SVR24 relapsers (M11652-37388-5593) likely experienced true virologic relapse rather than re-infection based on >97% nucleotide sequence similarity between viral populations in samples obtained from this subject at baseline and relapse.

An additional analysis was conducted to assess the frequency of transiently detected HCV RNA levels among subjects who achieved SVR in clinical trial M11-652. Of 521 subjects who achieved SVR12

(or later, if missing), 13 (2.5%) had at least 1 follow-up result that was *not* reported as "HCV RNA NOT DETECTED." This includes the 4 late relapsers noted above. Excluding the 4 clear late relapsers, 9 (1.7%) SVR-achieving subjects had transiently detected HCV RNA results (1 result of 82 IU/mL, all others <LLOQ) between Follow-up Weeks <2 and 12, and all 9 subjects had subsequent results through the end of follow-up reported as "HCV RNA NOT DETECTED." Among the 4 late relapsers, all follow-up HCV RNA results preceding the observation of relapse were reported as "HCV RNA NOT DETECTED," and all initially observed late relapse HCV RNA levels were ≥2,160 IU/mL. Collectively, these results support the use of <LLOQ as an HCV RNA cutoff for SVR12 determination.

4.13 Reviewer's Assessment of Contribution of Individual Drugs

As discussed in Section 1.4, with the Viekira Pak™ regimen 3 different HCV DAAs are dosed, in many cases also with RBV, and therefore it is important to demonstrate that all drugs are contributing towards treatment efficacy. Based on the totality of efficacy data from the sponsor's Phase 2b/3 program, specifically clinical trials M14-002 (see Section 4.7) and M11-652 (see Section 4.8), it is clear that treatment efficacy in HCV genotype 1a infected subjects was optimal when all three HCV DAAs were dosed with RBV for ≥12 weeks.

For HCV genotype 1b subjects, the efficacy contribution of all drug components is less clear. Virologic failure was extremely rare (5/1107, 0.5%) among HCV genotype 1b subjects treated with the 3-DAA +/- RBV regimen for 12 or 24 weeks in Phase 2/3 trials (Table 18). In Phase 3 clinical trials M13-961 and M13-389 (see Sections 4.5 and 4.6, respectively), no noncirrhotic HCV genotype 1b subjects treated with the 3-DAA regimen without RBV experienced virologic failure, clearly indicating that RBV is not necessary for this population. Similarly, across all of the 2-DAA + RBV, 3-DAA, or 3-DAA + RBV treatment groups in clinical trial M11-652, only a single HCV genotype 1b subject experienced virologic failure, which was a relapse observed in the 8-week 3-DAA + RBV treatment group (see Section 4.8). These results raise the question of whether a regimen that includes only 2-DAAs without RBV may be efficacious in HCV genotype 1b infected subjects.

Two ongoing Phase 2 trials, M13-393 and M12-536, are studying a 2-DAA regimen of PTV/r + OMB (no DBV or RBV) in subjects with HCV genotype 1b infection. Clinical trial M13-393 is being conducted in the U.S. and Europe, while M12-536 is being conducted in Japan. As part of the sponsor's regimen justification in the Integrated Summary of Efficacy, the sponsor summarized available virologic failure data from these two trials, and these data were also provided in a summary dataset format. Data were provided for 82 treatment-naïve or P/R treatment-experienced, noncirrhotic subjects who were treated with the 2-DAA regimen for 12 weeks in M13-393, and 36 P/R treatment-experienced subjects treated with the 2-DAA regimen for 12 weeks in M12-536. The sponsor did not comment on the cirrhosis status of subjects in M12-536, although based on the associated summary dataset 17/36 (47%) were considered noncirrhotic, while the remaining 19 subjects did not have their cirrhosis status reported.

In clinical trials M13-393 and M12-536 5/118 (4.2%) HCV genotype 1b subjects have experienced virologic failure (Table 20; 4 relapse and 1 breakthrough). Although this is a relatively low virologic failure rate, it is ~8-fold higher than the virologic failure rate for HCV genotype 1b infected subjects who received the 3-DAA +/- RBV regimen for 12 or 24 weeks in pooled Phase 2/3 trials (5/1107, 0.5%; Table 18).

Table 20. Virologic failure rates in Phase 2 clinical trials M12-536 and M13-393, which studied PTV/r + OMB (no DBV or RBV) in HCV genotype 1b subjects.

Trial	Overall Virologic Failure Rate	Virologic Failure According to P/R Treatment History		
		Tx-Naïve	Null Responders	Partial Responders
M12-536	1/36 (2.8%)	n/a	0/25 (0%)	1/11 (9%)
M13-393	4/82 (4.9%)	0/42 (0%)	4/40 (10%)	n/a

Based on the higher virologic failure rate among HCV genotype 1b subjects treated with the PTV/r + OMB 2-DAA regimen in Phase 2 trials, this reviewer supports the use of the 3-DAA regimen in HCV genotype 1b patients, provided the use of DBV does not raise any major safety concerns. In further support of the 3-DAA regimen over the 2-DAA regimen for HCV genotype 1b patients, the inclusion of a third DAA is likely to reduce the impact of pre-existing resistance-associated polymorphisms in NS5A (b) (4)

In this reviewer's opinion, one question that remains is whether HCV genotype 1b cirrhotic patients can receive the 3-DAA regimen without RBV without an impact on treatment efficacy. In clinical trial M13-099, only a single HCV genotype 1b infected subject treated with the 12- or 24-week 3-DAA + RBV regimen experienced virologic failure (see Section 4.4). The 3-DAA regimen without RBV has not been studied in cirrhotic HCV genotype 1b subjects. Since RBV is not necessary for efficacy in noncirrhotic HCV genotype 1b subjects, the sponsor could consider conducting a trial comparing the efficacy of the 3-DAA regimen dosed with versus without RBV in cirrhotic HCV genotype 1b subjects. Along these lines, the sponsor has recently proposed conducting a trial (M14-490) to evaluate the efficacy of the 3-DAA regimen without RBV in this population; the review is considering a post-marketing commitment for the sponsor to complete this study and submit a study report to the NDA.

5. CLINICAL VIROLOGY REVIEW OF DRUG RESISTANCE

5.1 Overview of Treatment-Emergent Resistance Analyses

For each HCV DAA/target, an analysis of treatment-emergent amino acid substitutions was conducted pooling data from non-SVR subjects who received the 3-DAA +/- RBV regimen in Phase 2 and Phase 3 trials. Baseline and Post-Baseline NS3/4A, NS5A and NS5B population nucleotide sequence analysis data were available from 85 subjects (84 for NS5B), 75 of whom were infected with HCV genotype 1a and 10 were infected with HCV genotype 1b. This list includes 2 placebo arm subjects (noted above) who received open-label rollover 3-DAA + RBV treatment in M11-646 and experienced on-treatment virologic failure. Of these 85 subjects, 20 experienced on-treatment virologic failure, 54 experienced virologic relapse (1 relapser without NS5B sequence data), and 11 failed to achieve SVR for non-virologic reasons and are referred to as "Other" failures throughout this review.

Paired Baseline/Post-Baseline analyses were conducted to identify treatment-emergent substitutions considering known DAA resistance-associated positions. In addition, all amino acid positions across the 3 drug targets were analyzed and potentially novel resistance-associated substitutions were flagged if they were enriched in Post-Baseline samples by at least 2 subjects, and further analyses of these positions were conducted considering other substitutions or common polymorphisms at same position, whether the substitutions emerged in subjects treated with other DAA-containing regimens

(i.e., non-3-DAA regimens) in Phase 2 trials, and also considering the direction of amino acid variants at the position (e.g., whether consistent with drug selection). The following positions were considered by this reviewer as "known" resistance-associated positions based on previous analyses conducted by the sponsor or others:

- NS3/4A (1a/1b): NS3 V36, F43, T54, V55, Y56, Q80, I/V132, R155, A156, D168, I/V170, E357, and NS4A V23 (NS3 F43, T54, V55, I/V132, I/V170, and NS4A V23 not considered "signature" positions by sponsor)
- NS5A (1a/1b): K24, M/L28, P29, Q/R30, L31, P32, H/Q54, H/P58, E/Q62, A92 and Y93 (K24, H/Q54 and E/Q62 not considered "signature" positions by sponsor)
- NS5B: C316, S368, M414, C445, E/Q446, Y448, C451, A553, G554, S556, G558, D559, Y561

5.2 NS3/4A (PTV, ABT-450) Treatment-Emergent Substitutions

Based on paired Baseline/Post-Baseline analyses, the following NS3/4A substitutions emerged in the viruses from non-SVR subjects who received the 3-DAA +/- RBV regimen in Phase 2/3 trials:

- Genotype 1a subjects (n=75): NS3 V36A/M/T, F43L, V55I, Y56H, Q80L, I132V, R155K, A156G, D168x (including A, F, H, I, L, N, T, V, Y, with V most common), P334S, S342P, E357K, V406A/I, T449I, P470S
- Genotype 1b subjects (n=10): NS3 Y56H, D168A/V (V more common), E357K, NS4A V23A

(b) (4) Table 21 summarizes this reviewer's justification for including these substitutions in the list of treatment-emergent substitutions in NS3/4A.

Table 21. NS3/4A substitutions (b) (4) that emerged in viruses from subjects who did not achieve SVR with 3-DAA +/- RBV treatment in pooled Phase 2/3 trials.

GT	Substitution(s)	Known Position?	# Subjs. w/ Emergence	USUBJIDs	Emergence in any subjects in Ph2 non-3-DAA arms?
1a	V36A/M/T	Y	4	M11652-42364-8215, M11652-42808-8035, M11646-47112-384209, M13098-46996-700307	Not Determined
1a	F43L	Y	2	M13099-31542-101102, M14002-14636-139405	Not Determined
1a	Q80L	Y	1	M13098-46996-700307	Not Determined
1a	A156G	Y	1	M14002-31542-100408	Not Determined
1a	P334S	N	4	M11652-12649-5316, M11652-42420-8192, M13099-42808-105111, M14002-16471-105401	Yes (M11-652)
1a	S342P	N	2	M11652-36975-5308, M11646-37572-300203	Yes (M11-652)
1a	V406A/I	N	3	M11652-37388-5593, M13098-42371-131311, M14002-38853-101405	Yes (M12-746, V406I)
1a	T449I	N	3	M11652-42368-5174, M11646-43114-405206, M11646-47112-384209	Yes (M11-652)
1a	P470S	N	3	M11652-42420-8192, M13099-31542-101102, M13099-37869-103101	No
1b	NS4A V23A	Y	1	M11646-39547-302202	Yes (M11-652 and M12-746, GT 1a)

In general, these substitutions either occurred at known NS3/4A protease inhibitor resistance-associated positions, or appear to be potentially novel resistance-associated substitutions that emerged in the viruses from multiple subjects who did not achieve SVR with 3-DAA +/- RBV treatment. Furthermore, some of these "novel" substitutions also emerged in the viruses from subjects who received 2-DAA +/- RBV regimens in Phase 2 studies. Of note, several subjects' viruses had treatment-emergent substitutions in the NS3 helicase domain (NS3 positions after amino acid 181), which is not known to be a common site for the emergence of resistance-associated substitutions; nearly all of these subjects also had treatment-emergent substitutions R155K or D168x.

Table 22 summarizes the numbers of non-SVR subjects who received the 3-DAA +/- RBV regimen in Phase 2/3 trials with virus having treatment-emergent NS3/4A substitutions, considering the list indicated above. Three sets of analyses were conducted with the data subgrouped by genotype 1a and 1b: (a) considering available data from all subjects who did not achieve SVR including those who failed treatment for non-virologic reasons, (b) considering available data from subjects who experienced virologic failure (on-treatment failure or relapse), and (c) considering available data from subjects who experienced virologic failure (on-treatment failure or relapse) but excluding those who were included in the 8-week treatment duration arm in Phase 2 trial M11-652 since 8-weeks is not going to be a recommended treatment duration for this regimen.

Table 22. Treatment-emergent NS3/4A substitutions in viruses from subjects who did not achieve SVR in Phase 2/3 trials of the 3-DAA +/- RBV regimen. Bolded positions summarize all data for the position (b) (4)

		All Subjects w/ Available Data		All Virologic Failures (vBT or Relapse)		Virologic Failures, Excl. 8-Wk Duration	
Target	Substitution	GT1a (n=75)	GT1b (n=10)	GT1a (n=67)	GT1b (n=7)	GT1a (n=58)	GT1b (n=6)
NS3/4A	V36A	3 (4.0%)		3 (4.5%)		3 (5.2%)	
	V36M	2 (2.7%)		2 (3.0%)		2 (3.4%)	
	V36T	1 (1.3%)		1 (1.5%)		1 (1.7%)	
	V36A/M/T	**4 (5.3%)**		**4 (6.0%)**		**4 (6.9%)**	
	F43L	**2 (2.7%)**		**2 (3.0%)**		**2 (3.4%)**	
	V55I	**4 (5.3%)**		**4 (6.0%)**		**4 (6.9%)**	
	Y56H	**6 (8.0%)**	**3 (30.0%)**	**6 (9.0%)**	**3 (42.9%)**	**6 (10.3%)**	**3 (50.0%)**
	Q80L	**1 (1.3%)**		**1 (1.5%)**		**1 (1.7%)**	
	I132V (1a)	**4 (5.3%)**		**4 (6.0%)**		**4 (6.9%)**	
	R155K	**9 (12.0%)**		**9 (13.4%)**		**9 (15.5%)**	
	A156G	**1 (1.3%)**		**1 (1.5%)**		**1 (1.7%)**	
	D168V	34 (45.3%)	3 (30.0%)	34 (50.7%)	3 (42.9%)	34 (58.6%)	3 (50.0%)
	D168A/F/H/I/L/N/T/Y	13 (17.3%)	1 (10.0%)	13 (19.4%)	1 (14.3%)	13 (22.4%)	1 (16.7%)
	D168A/F/H/I/L/N/T/V/Y	**42 (56.0%)**	**4 (40.0%)**	**42 (62.7%)**	**4 (57.1%)**	**42 (72.4%)**	**4 (66.7%)**
	P334S	**4 (5.3%)**		**4 (6.0%)**		**4 (6.9%)**	
	S342P	**2 (2.7%)**		**2 (3.0%)**		**2 (3.4%)**	
	E357K	**3 (4.0%)**	**1 (10.0%)**	**3 (4.5%)**	**1 (14.3%)**	**3 (5.2%)**	**1 (16.7%)**
	V406A	2 (2.7%)		2 (3.0%)		2 (3.4%)	
	V406I	1 (1.3%)		1 (1.5%)		1 (1.7%)	
	V406A/I	**3 (4.0%)**		**3 (4.5%)**		**3 (5.2%)**	
	T449I	**3 (4.0%)**		**3 (4.5%)**		**3 (5.2%)**	
	P470S	**3 (4.0%)**		**3 (4.5%)**		**3 (5.2%)**	
	NS4A V23A		**1 (10.0%)**		**1 (14.3%)**		**1 (16.7%)**

		All Subjects w/ Available Data		All Virologic Failures (vBT or Relapse)		Virologic Failures, Excl. 8-Wk Duration	
Target	Substitution	GT1a (n=75)	GT1b (n=10)	GT1a (n=67)	GT1b (n=7)	GT1a (n=58)	GT1b (n=6)
	Any Tx-Emergent Substitution (from list above)	51 (68.0%)	4 (40.0%)	51 (76.1%)	4 (57.1%)	51 (87.9%)	4 (66.7%)
	≥2 Tx-Emergent Substitutions (from list above)	25 (33.3%)	3 (30.0%)	25 (37.3%)	3 (42.9%)	25 (43.1%)	3 (50.0%)
	Any Substitution Detected at Failure (from list above)	54 (72.0%)	5 (50.0%)	54 (80.6%)	4 (57.1%)	53 (91.4%)	4 (66.7%)

The most commonly observed treatment-emergent substitutions in NS3/4A were D168x (any substitution, but mostly V) and R155K in HCV genotype 1a subjects, and D168A/V and Y56H in genotype 1b subjects. Overall, 76% of HCV genotype 1a virologic failures and 57% of HCV genotype 1b virologic failures had viral populations with at least 1 treatment-emergent resistance-associated substitution in NS3/4A. Excluding the 8-week treatment duration arm in Phase 2 trial M11-652, treatment-emergent NS3/4A resistance-associated substitutions were detected in 88% and 67% of HCV genotype 1a and 1b virologic failure subjects' viruses, respectively.

The Y56H substitution, which by itself does not confer a major reduction in the susceptibility to HCV genotype 1a replicons to PTV, emerged in the viruses from 6 genotype 1a virologic failures. All 6 subjects' viruses had treatment-emergent substitutions or pre-existing polymorphisms at key resistance-associated positions including R155K, A156G and/or D168x. Similarly, all 3 HCV genotype 1b subjects' viruses with treatment-emergent Y56H also had treatment-emergent D168A/V.

The substitutions V36T, Q80L and A156G each emerged in the viruses from a single subject. Although their emergence was uncommon, these substitutions emerged at positions known to play a role in resistance to NS3/4A protease inhibitors. The T36 variant was detected only at a single timepoint in a mixture with A, M and V variants; no phenotype data are available regarding the impact of V36T on PTV anti-HCV activity. The Q80L substitution conferred a 2-fold decrease in HCV genotype 1a replicon susceptibility to PTV. No data are available on the impact of an A156G substitution on PTV anti-HCV activity, but an A156T substitution conferred a 17-fold decrease in PTV anti-HCV activity.

5.3 NS5A (OMB, ABT-267) Treatment-Emergent Substitutions

Based on paired Baseline/Post-Baseline analyses, the following NS5A substitutions emerged in the viruses from non-SVR subjects who received the 3-DAA +/- RBV regimen in Phase 2 and Phase 3 trials:

- Genotype 1a subjects (n=75): K24R, M28A/T/V, Q30E/K/R, H58D/P/R, E62D, Y93C/N
- Genotype 1b subjects (n=10): H54Y, Y93H

(b) (4)

The K24R substitution emerged in 3 subjects' viral populations. Although it was no longer detected after virologic failure in the virus from 1 subject with it at Baseline, substitutions at position K24 have been previously described as potential

NS5A inhibitor resistance-associated substitutions (Wong et al., 2013; Bilello et al., 2014). The H54Y substitution emerged in only a single genotype 1b subject's virus, and did not emerge in the viruses from subjects who did not achieve SVR in Phase 2 trials evaluating non-3-DAA regimens. However, position 54 in the NS5A protein interacts with NS5A inhibitors and has been described as a resistance-associated position (Lemm et al., 2010; Ascher et al., 2013), and given the few genotype 1b failures for analysis this reviewer recommends H54Y is described as a treatment-emergent substitution in the prescribing information. Viral populations with E62D emerged in only a single genotype 1a subject, but E62D was also enriched in viruses from two other subjects who had a mixture detected at Baseline, and is a known NS5A inhibitor resistance-associated substitution (Sun et al., 2012).

Of note, no treatment-emergent, resistance-associated substitutions were observed at position L31, which is a key position involved in resistance to other NS5A inhibitors. One genotype 1a subject (M11652-19986-5195) had virus with an M31 polymorphism detected at Baseline that was no longer detected after virologic failure. One genotype 1b subject (M11646-47163-561210) had M31 detected at Baseline and after virologic failure.

There were many highly polymorphic positions in NS5A, particularly beyond the first 100 amino acids, making it difficult to identify patterns of genotypic changes attributable to treatment failure at these sites; in general there were no consistent or obvious treatment-emergent patterns at these polymorphic positions. Other treatment-emergent substitutions in NS5A at positions not known to be associated with resistance were observed in a small number of subjects' viruses and are noted here for possible future analyses of NS5A inhibitor resistance pathways; no phenotype data are available regarding the impact of these substitutions on OMB anti-HCV activity. Because of their low frequency of detection and lack of emergence in other Phase 2 trials (i.e., testing non-3-DAA regimens) they were not considered treatment-emergent, resistance-associated substitutions in these analyses. These NS5A substitutions include:

- V37I/M: Each emerged in a single genotype 1a subject, did not emerge with non-3-DAA regimens
- A61S/V: Each emerged in a single genotype 1a subject, did not emerge with non-3-DAA regimens
- F161Y: Emerged in only a single genotype 1a subject but was enriched from F/Y mixture in two others, relatively common polymorphism in dataset (~10%), did not emerge with non-3-DAA regimens
- I209V: Emerged as I/V mixture in two subjects, did not emerge with non-3-DAA regimens

Table 23 summarizes the numbers of non-SVR subjects who received the 3-DAA +/- RBV regimen in Phase 2/3 trials with viral populations harboring treatment-emergent NS5A substitutions, considering the list indicated above. The most commonly observed treatment-emergent substitutions in NS5A were Q30E/K/R and M28A/T/V in viruses from HCV genotype 1a subjects, and Y93H in viruses from HCV genotype 1b subjects. Overall, 72% of HCV genotype 1a virologic failures and 29% of HCV genotype 1b virologic failures had viral populations with at least 1 treatment-emergent resistance-associated substitution in NS5A. Excluding virologic failure subjects from the 8-week treatment duration arm in Phase 2 trial M11-652, treatment-emergent NS5A resistance-associated substitutions emerged in the viral populations from 78% and 33% of HCV genotype 1a and 1b virologic failure subjects, respectively.

Table 23. Treatment-emergent NS5A substitutions in the viruses from subjects who did not achieve SVR in Phase 2/3 trials of the 3-DAA +/- RBV regimen. Bolded positions summarize all data for the indicated position (b) (4) .

		All Subjects w/ Available Data		All Virologic Failures (vBT or Relapse)		Virologic Failures, Excl. 8-Wk Duration	
Target	Substitution	GT1a (n=75)	GT1b (n=10)	GT1a (n=67)	GT1b (n=7)	GT1a (n=58)	GT1b (n=6)
NS5A	**K24R**	**3 (4.0%)**		**3 (4.5%)**		**3 (5.2%)**	
	M28A	1 (1.3%)		1 (1.5%)		1 (1.7%)	
	M28T	14 (18.7%)		14 (20.9%)		13 (22.4%)	
	M28V	7 (9.3%)		6 (9.0%)		6 (10.3%)	
	M28A/T/V	**21 (28.0%)**		**20 (29.9%)**		**19 (32.8%)**	
	Q30E (1a)	1 (1.3%)		1 (1.5%)		1 (1.7%)	
	Q30K (1a)	1 (1.3%)		1 (1.5%)		1 (1.7%)	
	Q30R (1a)	30 (40.0%)		27 (40.3%)		25 (43.1%)	
	Q30E/K/R (1a)	**32 (42.7%)**		**29 (43.3%)**		**27 (46.6%)**	
	H54Y		**1 (10.0%)**		**1 (14.3%)**		**1 (16.7%)**
	H58D (1a)	2 (2.7%)		2 (3.0%)		2 (3.4%)	
	H58P (1a)	2 (2.7%)		2 (3.0%)		1 (1.7%)	
	H58R (1a)	1 (1.3%)		1 (1.5%)		1 (1.7%)	
	H58D/P/R (1a)	**5 (6.7%)**		**5 (7.5%)**		**4 (6.9%)**	
	E62D	**1 (1.3%)**		**1 (1.5%)**		**0 (0.0%)**	
	Y93C	1 (1.3%)	0 (0.0%)	1 (1.5%)	0 (0.0%)	1 (1.7%)	0 (0.0%)
	Y93H	0 (0.0%)	4 (40.0%)	0 (0.0%)	2 (28.6%)	0 (0.0%)	2 (33.3%)
	Y93N	2 (2.7%)	0 (0.0%)	2 (3.0%)	0 (0.0%)	2 (3.4%)	0 (0.0%)
	Y93C/H/N	**3 (4.0%)**	**4 (40.0%)**	**3 (4.5%)**	**2 (28.6%)**	**3 (5.2%)**	**2 (33.3%)**
	Any Tx-Emergent Substitution (from list above)	**51 (68.0%)**	**4 (40.0%)**	**48 (71.6%)**	**2 (28.6%)**	**45 (77.6%)**	**2 (33.3%)**
	≥2 Tx-Emergent Substitutions (from list above)	**12 (16.0%)**	**1 (10.0%)**	**11 (16.4%)**	**1 (14.3%)**	**10 (17.2%)**	**1 (16.7%)**
	Any Substitution Detected at Failure (from list above)	**65 (86.7%)**	**7 (70.0%)**	**60 (89.6%)**	**5 (71.4%)**	**56 (96.6%)**	**5 (83.3%)**

Several subjects without treatment-emergent NS5A resistance-associated substitutions had viral populations with pre-existing resistance-associated polymorphisms, which may explain the lack of emergence of new substitutions. Among 13 HCV genotype 1a virologic failure subjects in the 8-week-duration-excluded subgroup without treatment-emergent NS5A resistance-associated substitutions, 11 (85%) already had NS5A resistance-associated polymorphisms (relative to standard genotype 1a or 1b strains) detected at Baseline, including Y93C/N (n=4), M28T/V (n=2), M28V + H58P (n=1), H58P + E62D (n=1), Q30E/R (n=1), H58D (n=1) and E62D (n=1). Similarly, 3 of the 4 HCV genotype 1b virologic failure subjects in this subgroup without treatment-emergent NS5A resistance-associated substitutions already had Y93H detected at Baseline. Therefore, 56/58 (97%) HCV genotype 1a subjects and 5/6 (83%) HCV genotype 1b subjects who received 12 or 24 weeks of the 3-DAA +/- RBV regimen and experienced virologic failure had viruses with at least 1 NS5A resistance-associated substitution at the time of virologic failure (Table 23).

5.4 NS5B (DBV, ABT-333) Treatment-Emergent Substitutions

Based on paired Baseline/Post-Baseline analyses, the following NS5B substitutions emerged in the viruses from non-SVR subjects who received the 3-DAA +/- RBV regimen in Phase 2 and Phase 3 trials:

- Genotype 1a subjects (n=74): G307R, C316Y, M414T, E446K/Q, A450V, A553T, G554S, S556G/R, G558R, D559G/I/N/V, Y561H, L588F
- Genotype 1b subjects (n=10): C316Y, M414I, S556G

[redacted] (b) (4) To this reviewer's knowledge some of these substitutions have not been shown to be associated with non-nucleoside NS5B-palm inhibitors, including G307R, A450V and L588F. G307R emerged in 2 genotype 1a subjects' viruses and was enriched in a third subject (M11652-42420-8192, M13099-42808-105111 and M14002-48586-116405, respectively). Position A450 appears to be highly conserved (no Baseline polymorphisms in 3-DAA +/- RBV failure dataset). A450V emerged in the viruses from two genotype 1a subjects (M13099-42808-105111, M13099-47437-320104), and A450T emerged in the virus from one subject who did not achieve SVR in Phase 2 trial M12-746 (non-3-DAA regimen). Position L588 also appears to be highly conserved (no Baseline polymorphisms in 3-DAA +/- RBV failure dataset), and the same variant, L588F, emerged in two subjects' viruses (M11652-42368-5174, M13098-46996-700307). One of the subjects also had viral populations with histidine (H) and tyrosine (Y) detected in a mixture with phenylalanine (F); L588Y requires two nucleotide changes possibly including L588F as an intermediate step. It is not possible to determine if G307R and A450V were selected primarily by RBV or ABT-333, as both substitutions were observed only in the viruses from subjects who received the 3-DAA + RBV regimen. However, viral populations with L588F emerged with the 3-DAA regimen with RBV in 1 subject and without RBV in 1 subject, indicating that if it was in fact selected by the treatment regimen it is likely attributed to DBV. An L588P substitution also emerged in DBV-selected HCV genotype 1b replicons (see Table 4 in Section 2.4).

In addition, E446K/Q, [redacted] (b) (4) was observed in 2 genotype 1a subjects' viruses (1 subject each). Although emergent substitutions were observed in only a small number of subjects, this position is considered by the sponsor as a "signature" DBV resistance-associated position, and is located near other key positions (e.g., C445, Y448) associated with resistance to this class of drugs. Furthermore, E446K and E446Q were shown by the sponsor to confer 54- and 18-fold reductions, respectively, in DBV activity against an HCV genotype 1a replicon (see Appendix C).

Other treatment-emergent substitutions in NS5B at positions not known to be associated with resistance were observed in a small number of subjects and are noted here for possible future analyses of NS5B inhibitor resistance pathways. However, because of their low frequency of detection and lack of emergence in other Phase 2 trials they were not considered confirmed treatment-emergent, resistance-associated substitutions in these analyses; no phenotype data are available regarding the impact of these substitutions on DBV anti-HCV activity. These NS5B substitutions include:

- I413V: Emerged in virus from a single genotype 1b subject, position located near known resistance-associated position (M414), did not emerge with non-3-DAA regimens
- R465G/T: Each substitution emerged in a single genotype 1a subject's virus, did not emerge with non-3-DAA regimens

- A486V: Emerged in a single genotype 1b subject's virus, did not emerge with non-3-DAA regimens (also discussed below for cross-resistance assessment)

A few genotype 1a subjects also had viral populations with changes at position F415; an F415Y substitution is associated with prior treatment failure with RBV-containing regimens (Young et al., 2003; Ward et al., 2008; Bartels et al., 2011). However, the patterns of F415Y changes were not necessarily consistent with selective pressure by RBV. Three subjects, two of whom received RBV with the 3-DAA regimen, had viruses with F415Y detected at Baseline but not after virologic failure. Conversely, two subjects with F/Y detected in viruses at Baseline had enrichment of the Y variant after virologic failure, but only one of the subjects received RBV.

Table 24 summarizes the numbers of non-SVR subjects who received the 3-DAA +/- RBV regimen in Phase 2/3 trials and had viral populations with treatment-emergent NS5B substitutions, considering the list indicated above. The predominant treatment-emergent substitution in NS5B was S556G in HCV genotype 1a subjects. Clear DBV treatment-emergent resistance substitutions were detected in viruses from only two HCV genotype 1b subjects. Excluding the 8-week treatment duration arm in Phase 2 trial M11-652, treatment-emergent NS5B resistance-associated substitutions emerged in viruses from 67% and 33% of HCV genotype 1a and 1b virologic failure subjects, respectively.

Table 24. Treatment-emergent NS5B substitutions in viruses from subjects who did not achieve SVR in Phase 2/3 trials of the 3-DAA +/- RBV regimen. Bolded positions summarize all data for the position (b) (4)

*1 genotype 1a subject did not have Post-Baseline NS5B sequence data available.

		All Subjects w/ Available Data		All Virologic Failures (vBT or Relapse)		Virologic Failures, Excl. 8-Wk Duration	
Target	Substitution	GT1a (n=74*)	GT1b (n=10)	GT1a (n=66*)	GT1b (n=7)	GT1a (n=57*)	GT1b (n=6)
NS5B	**G307R**	**2 (2.7%)**		**2 (3.0%)**		**2 (3.5%)**	
	C316Y	**2 (2.7%)**	**1 (10.0%)**	**2 (3.0%)**	**1 (14.3%)**	**2 (3.5%)**	**1 (16.7%)**
	M414I	0 (0.0%)	1 (10.0%)	0 (0.0%)	1 (14.3%)	0 (0.0%)	1 (16.7%)
	M414T	3 (4.1%)	0 (0.0%)	3 (4.5%)	0 (0.0%)	3 (5.3%)	0 (0.0%)
	M414I/T	**3 (4.1%)**	**1 (10.0%)**	**3 (4.5%)**	**1 (14.3%)**	**3 (5.3%)**	**1 (16.7%)**
	E446K	1 (1.4%)		1 (1.5%)		1 (1.8%)	
	E446Q	1 (1.4%)		1 (1.5%)		1 (1.8%)	
	E446K/Q	**2 (2.7%)**		**2 (3.0%)**		**2 (3.5%)**	
	A450V	**2 (2.7%)**		**2 (3.0%)**		**2 (3.5%)**	
	A553T	**4 (5.4%)**		**4 (6.1%)**		**4 (7.0%)**	
	G554S	**2 (2.7%)**		**2 (3.0%)**		**2 (3.5%)**	
	S556G	22 (29.7%)	1 (10.0%)	22 (33.3%)	1 (14.3%)	20 (35.1%)	1 (16.7%)
	S556R	3 (4.1%)	0 (0.0%)	3 (4.5%)	0 (0.0%)	3 (5.3%)	0 (0.0%)
	S556G/R	**24 (32.4%)**	**1 (10.0%)**	**24 (36.4%)**	**1 (14.3%)**	**22 (38.6%)**	**1 (16.7%)**
	G558R	**2 (2.7%)**		**2 (3.0%)**		**2 (3.5%)**	
	D559G	2 (2.7%)		2 (3.0%)		2 (3.5%)	
	D559I	1 (1.4%)		1 (1.5%)		1 (1.8%)	
	D559N	2 (2.7%)		2 (3.0%)		2 (3.5%)	
	D559V	1 (1.4%)		1 (1.5%)		1 (1.8%)	
	D559G/I/N/V	**4 (5.4%)**		**4 (6.1%)**		**4 (7.0%)**	
	Y561H	**3 (4.1%)**		**3 (4.5%)**		**3 (5.3%)**	
	L588F	**2 (2.7%)**		**2 (3.0%)**		**2 (3.5%)**	

		All Subjects w/ Available Data		All Virologic Failures (vBT or Relapse)		Virologic Failures, Excl. 8-Wk Duration	
Target	Substitution	GT1a (n=74*)	GT1b (n=10)	GT1a (n=66*)	GT1b (n=7)	GT1a (n=57*)	GT1b (n=6)
	Any Tx-Emergent Substitution (from list above)	40 (54.1%)	2 (20.0%)	40 (60.6%)	2 (28.6%)	38 (66.7%)	2 (33.3%)
	≥2 Tx-Emergent Substitutions (from list above)	10 (13.5%)	1 (10.0%)	10 (15.2%)	1 (14.3%)	10 (17.5%)	1 (16.7%)
	Any Substitution Detected at Failure (from list above)	43 (58.1%)	5 (50.0%)	42 (63.6%)	4 (57.1%)	40 (70.2%)	4 (66.7%)

Cross-Resistance Analysis (see also Section 2.4)
Because several other NS5B polymerase inhibitors across multiple different classes are either FDA-approved (sofosbuvir-nucleotide analogue NS5B polymerase inhibitor) or in development, analyses were conducted to determine if NS5B substitutions emerged at any positions associated with resistance to other classes of NS5B inhibitors to assess the potential for cross-resistance. Positions specifically evaluated included the following:

- Nucleot(s)ide analogue NS5B polymerase inhibitors: L159, S282, C316, L320, and V321 (also D61, A112 and E237 recently identified as possible sofosbuvir resistance-association positions)
- Non-nucleoside NS5B-thumb1 polymerase inhibitors: L392, A421, P495, P496 and V499
- Non-nucleoside NS5B-thumb2 polymerase inhibitors: L419, R422, M423, M426, I482, A486 and V494

As shown in Table 25, the emergence of substitutions at NS5B positions associated with resistance to other NS5B polymerase inhibitors was rare. No subjects had detectable viral populations with treatment-emergent substitutions known to be associated with resistance to nucleot(s)ide analogue NS5B polymerase inhibitors. A C316N substitution/polymorphism in HCV genotype 1b has been shown to be potentially associated with reduced sofosbuvir efficacy (Sovaldi™ label, Donaldson et al., 2014; also see Clinical Virology reviews of NDA 204671 by Eric Donaldson, Ph.D., and Lisa Naeger, Ph.D.). Viral populations with the C316N substitution did not emerge in any subjects treated with the AbbVie 3-DAA +/- RBV regimen, although a C316Y substitution emerged in 3 subjects (2 HCV genotype 1a, 1 HCV genotype 1b) and causes a >1,000-fold reduction in DBV anti-HCV activity in cell culture (Appendix C). As discussed in Section 2.4, this reviewer is not aware of any cell culture phenotype data generated by the sponsor on the impact of C316Y on sofosbuvir anti-HCV activity, (b) (4)

A single subject had virus with a treatment-emergent A421V substitution, which is associated with resistance to certain non-nucleoside NS5B-thumb1 polymerase inhibitors. This substitution was also frequently detected as a natural Baseline polymorphism, and in one subject the polymorphism was no longer detected at the time of virologic failure, indicating that A421V is unlikely selected by DBV.

Three different subjects each had emergent viral populations with single substitutions that are associated with resistance to certain non-nucleoside NS5B-thumb2 polymerase inhibitors, including M423I (GT1a), I482T (GT1a) and A486V (GT1b). This reviewer is not aware of any phenotypic resistance data available for these substitutions, although as noted in Section 2.4 an M423T substitution did not reduce DBV anti-HCV activity in a GT1b replicon.

Table 25. Treatment-emergent resistance analysis focusing on positions associated with resistance to other classes of NS5B polymerase inhibitors. *Recently identified sofosbuvir resistance-associated positions.

NS5B position	# Subjects with Tx-Emergent Substitutions		Comments (e.g., Detected at BL?)
	GT1a (n=74)	GT1b (n=10)	
Nucleot(s)ide analogue NS5B polymerase inhibitors			
D61*	0	0	
A112*	0	0	A112T detected in 1 GT1a subject as A/T mixture at BL and T at Failure
L159	0	0	L159F detected in 3 GT1b subjects at BL and Failure
E237*	0	0	
S282	0	0	
C316	2 (C316Y)	1 (C316Y)	No Tx-emergent C316N; C316N (3-GT1b), C316H (1-GT1b) and C316Y (1-GT1a) detected at BL and Failure
L320	0	0	
V321	0	0	V321I detected in 1 GT1b subject at BL and Failure
Non-nucleoside NS5B-thumb1 polymerase inhibitors			
L392	0	0	L392F detected in 3 GT1a subjects at BL and Failure
A421	1 (A421V)	0	A421V is a common polymorphism, detected in 20 subjects (18 GT1a, 2 GT1b) at BL, lost at failure in 1 GT1a subject
P495	0	0	
P496	0	0	
V499	0	0	V499A or V499T detected at BL and Failure in 6 subjects (5 GT1b, 1 GT1a)
Non-nucleoside NS5B-thumb2 polymerase inhibitors			
L419	0	0	
R422	0	0	
M423	1 (M423I)	0	Not detected at BL in any subjects. Subject had no known DBV treatment-emergent resistance substitutions.
M426	0	0	M426L detected at BL in 12 subjects (11 GT1a, 1 GT 1b), detection at BL lost at failure in 1 GT1a subject
I482	1 (I482T)	0	Not detected at BL in any subjects. Subject also had tx-emergent S556G.
A486		1 (A486V)	Not detected at BL in any subjects. Subject had no known DBV treatment-emergent resistance substitutions.
V494	0	0	V494I detected in 1 GT1a subject at BL and Failure.

5.5 Overall Summary of Treatment-Emergent Resistance

Table 26 summarizes the numbers of subjects with viral populations that have treatment-emergent resistance-associated substitutions across each of the 3 drug targets.

Overall, excluding the 8-week treatment duration arm in Phase 2 trial M11-652, NS3/4A, NS5A and NS5B resistance-associated substitutions emerged in viruses from 51 (87.9%), 45 (77.6%) and 38 (66.7%) genotype 1a virologic failure subjects, respectively, and in 4 (66.7%), 2 (33.3%) and 2 (33.3%) genotype 1b virologic failure subjects, respectively.

Among genotype 1a virologic failure subjects (8-week duration excluded), viral populations with treatment-emergent substitutions in ≥1, ≥2 or all 3 drug targets were detected in 54 (93.1%), 50

(86.2%), and 30 (52.6%) subjects, respectively. Among genotype 1b virologic failure subjects (8-week duration excluded), viral populations with treatment-emergent substitutions in ≥1, ≥2 or all 3 drug targets were detected in 5 (83.3%), 2 (33.3%), and 1 (16.7%) subjects, respectively.

Consistent with the tables shown above for the individual drug targets, excluding subjects who were non-virologic failures or who received only 8 weeks of treatment resulted in a higher rate of detection of resistance-associated substitutions across all 3 drug targets. This observation indicates that true virologic failure with the 3-DAA +/- RBV regimen is more likely to be associated with the emergence of resistance-associated substitutions than failure due to non-virologic reasons or a sub-optimal treatment duration.

Table 26. Summary of treatment-emergent resistance-associated substitutions across all 3 drug targets. *1 genotype 1a subject did not have Post-Baseline NS5B sequence data available; denominator was adjusted appropriately.

Target	Substitution	All Subjects w/ Available Data		All Virologic Failures (vBT or Relapse)		Virologic Failures, Excl. 8-WK Duration	
		GT1a (n=75*)	GT1b (n=10)	GT1a (n=67*)	GT1b (n=7)	GT1a (n=58*)	GT1b (n=6)
NS3/4A	Any Tx-Emergent Substitution	51 (68.0%)	4 (40.0%)	51 (76.1%)	4 (57.1%)	51 (87.9%)	4 (66.7%)
	Any Substitution Detected at Failure	54 (72.0%)	5 (50.0%)	54 (80.6%)	4 (57.1%)	53 (91.4%)	4 (66.7%)
NS5A	Any Tx-Emergent Substitution	51 (68.0%)	4 (40.0%)	48 (71.6%)	2 (28.6%)	45 (77.6%)	2 (33.3%)
	Any Substitution Detected at Failure	65 (86.7%)	7 (70.0%)	60 (89.6%)	5 (71.4%)	56 (96.6%)	5 (83.3%)
NS5B	Any Tx-Emergent Substitution	40 (54.1%)*	2 (20.0%)	40 (60.6%)*	2 (28.6%)	38 (66.7%)*	2 (33.3%)
	Any Substitution Detected at Failure	43 (58.1%)*	5 (50.0%)	42 (63.6%)*	4 (57.1%)	40 (70.2%)*	4 (66.7%)
≥1 Tx-Emergent Substitution in NS3/4A, NS5A or NS5B		60 (80.0%)	7 (70.0%)	57 (85.1%)	5 (71.4%)	54 (93.1%)	5 (83.3%)
≥1 Tx-Emergent Substitution in ≥2 Drug Targets		52 (69.3%)	2 (20.0%)	52 (77.6%)	2 (28.6%)	50 (86.2%)	2 (33.3%)
≥1 Tx-Emergent Substitution in All 3 Drug Targets*		30 (40.5%)	1 (10.0%)	30 (45.5%)	1 (14.3%)	30 (52.6%)	1 (16.7%)
≥1 Substitution in All 3 Drug Targets at Failure (emergent or pre-existing)*		39 (52.7%)	4 (40.0%)	39 (59.1%)	4 (57.1%)	38 (66.7%)	4 (66.7%)

5.6 Persistence of Treatment-Emergent Resistance Substitutions

Overview of analyses conducted

Analyses of Phase 2 trials of 2- or 3-DAA +/- RBV combination regimens were conducted to evaluate the persistence of resistance-associated substitutions in NS3, NS5A and NS5B, as limited long-term

follow-up data are available from Phase 3 trials. Analyses were conducted only for subjects with available data through Post-Treatment Week 24 or later. Because of the low virologic failure rate in HCV genotype 1b subjects these analyses were conducted only for HCV genotype 1a subjects. All treatment-emergent resistance-associated positions/substitutions identified in the analyses of the 3-DAA +/- RBV regimens described above were monitored for persistence after treatment, specifically:

- **NS3** V36A/M/T, F43L, V55I, Y56H, Q80L, I132V, R155K, A156G, D168x, P334S, S342P, E357K, V406A/I, T449I and P470S
- **NS5A** K24R, M28A/T/V, Q30E/K/R, H58D/P/R, E62D and Y93C/N
- **NS5B** G307R, C316Y, M414T, E446K/Q, A450V, A553T, G554S, S556G/R, G558R, D559G/I/N/V, Y561H and L588F

Both population and clonal nucleotide sequencing data were considered for the NS3 and NS5B persistence analyses. For clonal analyses, the sponsor only considered substitutions that were detected in at least 2 clones, with a sensitivity to detect minority variants present at approximately 5-10% of the population (see Appendix D for more detailed methods and the numbers of clones analyzed per sample). In general, most substitutions that were detected by clonal nucleotide sequencing were also detected by population nucleotide sequencing when both types of data were available. For analyses of NS5A, only population nucleotide sequencing data were analyzed because limited clonal data were provided and persistence of resistance-associated substitutions was clearly observed based on the population nucleotide sequencing assay.

Of note for the descriptive summaries associated with the following tables, when a subject had no sequence data at Post-Treatment Week 24, HCV treatment-emergent substitutions that were detected at Post-Treatment Week ≥48 were considered detectable at Post-Treatment Week 24 and imputed accordingly. Conversely, when a subject had no data at Post-Treatment Week ≥48, if no treatment-emergent substitutions were detected through Post-Treatment Week 24 it was assumed that no substitutions would have been detected at Post-Treatment Week ≥48. Also, if no data were available for Post-Treatment Week 24 and no emergent substitutions were still detected at Post-Treatment Week ≥48, the subject was considered “missing” from the Post-Treatment Week 24 analysis and therefore not included in the denominator. Also note that the lack of detection of a given substitution does not necessarily mean it does not exist or does not remain enriched in the viral population relative to pre-treatment levels.

Persistence of NS3 resistance-associated substitutions

Table 27 summarizes the persistence of PTV treatment-emergent substitutions for 32 subjects with ≥1 treatment-emergent substitution in NS3 (from list indicated above) and at least 24 weeks of post-treatment follow-up. The following conclusions can be drawn from these data:

- 17/29 (59%) subjects still had detectable viruses with at least 1 treatment-emergent NS3 substitution through at least Post-Treatment Week 24, and 5/22 (23%) through at least Post-Treatment Week 48.
- Treatment-emergent R155K remained detected in viruses from 5/8 (63%) subjects through Post-Treatment Week 24, and in 1/5 (20%) subjects through Post-Treatment Week 48.
- Treatment-emergent D168 substitutions, which are most commonly observed in PTV-based treatment failure subjects, remained detected in viruses from 6/22 (27%) subjects through Post-Treatment Week 24, and in 0/22 (0%) subjects through Post-Treatment Week 48.

Table 27. Persistence of viral populations with treatment-emergent substitutions in NS3. Both population and clonal nucleotide sequence analysis data were considered for these analyses; clonal analyses only covered NS3 amino acids 1-181. ND, no data; PTW, Post-Treatment Week; red cells indicate "positive" detection of substitutions and yellow cells indicate "negative" detection of substitutions (imputed or observed).

USUBJID	Last Visit w/Data	Tx-emergent Substitutions (Pop. Seq., except where noted*)	Detected @PTW24	Detected @PTW48+	Additional Notes about Analyses
M11652-12538-5377	PTW 24	R155K, D168V	none	ND	R155G detected only by clonal seq. (6% of clones) at PTW24 but not earlier, not considered in analyses
M11652-12649-5316	PTW 24	R155K, P334S	R155K	ND	
M11652-22271-5181	PTW 24	V36A, Y56H, D168A/V, P334S	Y56H, D168A	ND	
M11652-36975-5308	PTW 48	D168V, S342P	D168V, S342P	none	
M11652-37388-5593	PTW 48	V406A	ND	V406A	
M11652-37868-5413	PTW 48	D168V	D168V	none	D168V at PTW24 by clonal seq.
M11652-37868-8028	PTW 48	Y56H, D168V, E357K	ND	none	
M11652-37869-8071	PTW 24	D168V	none	ND	
M11652-38624-5256	PTW 24	D168V	none	ND	
M11652-38624-8108	PTW 24	D168F/V/Y, S342P, T449I	S342P	ND	
M11652-38627-5687	PTW 59	D168V	ND	none	
M11652-38646-5751	PTW 24	D168A	none	ND	
M11652-38853-5309	PTW 24	D168V	none	ND	
M11652-40076-5424	PTW 24	Y56H, D168V, E357K	E357K	ND	
M11652-40526-5297	PTW 48	R155K, A156G	R155K	R155K	
M11652-40784-8118	PTW 24	A156V, D168V	none	ND	
M11652-42362-5144	PTW 24	Y56H, D168Y	none	ND	
M11652-42364-8215	PTW 24	V36M, I132V, R155K	I132V, R155K	ND	
M11652-42368-5174	PTW 24	D168V, T449I	T449I	ND	
M11652-42371-5525	PTW 24	D168V	none	ND	
M11652-42420-8192	PTW 24	I132V, D168Y, P334S, P470S	I132V, P334S, P470S	ND	
M11652-42808-5435	PTW 48	D168V	ND	none	V36A detected only by clonal seq. (3% of clones) at PTW48 and not earlier, not considered in analyses.
M11652-42808-8035	PTW 24	V36A/M/T, R155K	R155K	ND	
M11652-44319-8246	PTW 48	V55A, D168V	V55A, D168V	V55A	V55A considered tx-emergent
M11652-44367-8224	PTW 24	D168V, E357K	none	ND	
M11652-44372-5610	PTW 24	D168V*	D168V	ND	D168V detected only by clonal seq. (2% of clones) only at PTW24
M12746-22854-2146	PTW 48	V36M, Y56H, R155K*, D168V	V36M, R155K	V36M	R155K detected only by clonal seq. (3% of clones) at PTW24, V36M detected at PTW48 only by clonal seq.
M12746-37869-2095	PTW 48	Q80L	Q80L	Q80L	Q80L detected at Baseline only by clonal analyses in 7% of clones, considered treatment-emergent (enriched to 100% of clones)
M12746-37869-2096	PTW 48	V55A, I132V, D168V	none	none	V55A considered tx-emergent
M12746-38934-2187	PTW 48	R155K*	none	none	R155K detected only by clonal seq. (2% of clones) at two different timepoints
M12746-40537-2507	PTW 48	V36M, Y56H, R155K, A156G, D168A	none	none	
M12998-37869-3094	PTW 24	Y56H, D168A	Y56H, D168A	ND	R155S detected only by clonal seq. (4% of clones) at PTW24 and was not considered in this analysis

Persistence of NS5A resistance-associated substitutions

Table 28 summarizes the persistence of OMB treatment-emergent substitutions for 24 subjects with ≥1 treatment-emergent substitution in NS5A (from list indicated above) and at least 24 weeks of post-treatment follow-up. All 24 subjects (100%) had treatment-emergent NS5A substitutions detected in their viral populations through at least Post-Treatment Week 24, and 18/18 (100%) subjects with available data had treatment-emergent substitutions detected in their viral populations through at least Post-Treatment Week 48, clearly indicating the long-term persistence of resistance-associated substitutions in NS5A.

Table 28. Persistence of viral populations with treatment-emergent substitutions in NS5A.
Only population sequence analysis data were considered for these analyses. ND, no data; PTW, Post-Treatment Week; red cells indicate "positive" detection of substitutions (imputed or observed).

USUBJID	Last Visit w/Data	Tx-emergent Substitutions	Detected @PTW24	Detected @PTW48+
M11652-02965-5213	PTW 48	M28T, H58P, E62D	M28T, H58P, E62D	M28T, H58P, E62D
M11652-12649-5316	PTW 24	M28A/T, Q30R	M28A/T, Q30R	ND
M11652-36975-5308	PTW 48	K24R, Q30R	Q30R	K24R, Q30R
M11652-37868-5413	PTW 48	Q30R	Q30R	Q30R
M11652-37869-8071	PTW 48	Q30R	Q30R	Q30R
M11652-38624-8108	PTW 48	M28A/T/V, Q30R	Q30R	M28A/T/V, Q30R
M11652-38627-5687	PTW 48	Q30R	ND	Q30R
M11652-38646-5751	PTW 48	M28T, Q30R	M28T	M28T
M11652-38853-5309	PTW 24	Q30R	Q30R	ND
M11652-40784-8118	PTW 24	Q24R, M28T	Q24R, M28T	ND
M11652-42361-5765	PTW 48	M28V	ND	M28V
M11652-42362-5144	PTW 48	M28V, Q30K	M28V, Q30K	M28V, Q30K
M11652-42364-8215	PTW 24	M28T	M28T	ND
M11652-42368-5174	PTW 48	Y93N	Y93N	Y93N
M11652-42371-5525	PTW 48	M28V	M28V	M28V
M11652-42420-8192	PTW 48	Q30R	Q30R	Q30R
M11652-42808-5435	PTW 48	M28T	ND	M28T
M11652-42808-8035	PTW 24	Q30R	Q30R	ND
M11652-43114-5554	PTW 48	H58D	H58D	H58D
M11652-44319-8246	PTW 48	Q30R	Q30R	Q30R
M11652-44367-8224	PTW 59	Q30R	Q30R	Q30R
M11652-44372-5610	PTW 48	Q30R	Q30R	Q30R
M12998-31542-3049	PTW 48	Q30R	ND	Q30R
M12998-37869-3094	PTW 24	Q30R	Q30R	ND

Persistence of NS5B resistance-associated substitutions

Table 29 summarizes the persistence of DBV (or possibly RBV) treatment-emergent substitutions for 16 subjects with ≥1 treatment-emergent substitution in NS5B (from list indicated above) and at least 24 weeks of post-treatment follow-up. The following conclusions can be drawn from these data:

- 11/16 (69%) subjects still had virus with at least 1 treatment-emergent NS5B substitution detected through at least Post-Treatment Week 24, and 8/15 (53%) through at least Post-Treatment Week 48.

- S556G, which is the most commonly observed DBV resistance-associated substitution, remained detected in 8/9 (89%) subjects' viruses through at least Post-Treatment Week 24, 6/9 (67%) through at least Post-Treatment Week 48.

In this reviewer's opinion, the persistence of viral populations with S556G or other key DBV resistance-associated substitutions, while clearly occurring in many virologic failure subjects, is not as concerning as the persistence of substitutions in other drug targets, particularly NS5A. The non-nucleoside NS5B-palm class is not a critical HCV DAA class, and no other drugs in the class are currently approved or in late stage clinical development.

Table 29. Persistence of viral populations with treatment-emergent substitutions in NS5B. Note that both population and clonal nucleotide sequence analysis data were considered for these analyses. ND, no data; PTW, Post-Treatment Week; red cells indicate "positive" detection of substitutions and yellow cells indicate "negative" detection of substitutions (imputed or observed).

USUBJID	Last Visit w/Data	Tx-emergent Substitutions (Pop. Seq.,except where noted*)	Detected @PTW24	Detected @PTW48+	Additional Notes about Analyses
M11652-12649-5316	PTW 24	G554S, S556G	none	ND	
M11652-36975-5308	PTW 48	S556G	S556G	none	S556G detected in 5% of clones at BL, considered tx-emergent (enriched to 100% of clones)
M11652-38624-5256	PTW 48	M414T	M414T	M414T	
M11652-38627-5687	PTW 59	S556G	ND	S556G	
M11652-40526-5297	PTW 24	G307R*, D559N	G307R	ND	G307R detected only by clonal seq. (5% of clones) only at PTW24
M11652-42362-5144	PTW 48	S556G	S556G	S556G	
M11652-42364-8215	PTW 24	D559G	none	ND	
M11652-42368-5174	PTW 48	M414T, S556G	S556G	S556G	L588F/H/Y detected only at PTW48 and not at earlier follow-up timepoints, and was not considered in this analysis
M11652-42420-8192	PTW 48	G307R, S556G	G307R, S556G	G307R, S556G	
M11652-42808-8035	PTW 24	G307R*, G558R	none	ND	G307R detected only by clonal seq. (2% of clones) during treatment
M11652-44367-8224	PTW 24	G307R*, A553T	none	ND	G307R detected only by clonal seq. (5% of clones in 2 samples)
M11652-44372-5610	PTW 48	S556G	S556G	S556G	
M12746-22854-2146	PTW 48	S556G	S556G	S556G	
M12746-37869-2095	PTW 48	A450T	A450T	A450T	A450T was not a treatment-emergent substitution in pooled analyses of the 3-DAA +/- RBV regimens, but A450V was; A450T considered tx-emergent.
M12746-37869-2096	PTW 48	C316Y, A553D, G554S*, S556G, G558R, D559G/N*	S556G	none	A553D was not a treatment-emergent substitution in pooled analyses of the 3-DAA +/- RBV regimens, but A553T was; A553D considered tx-emergent. G554S and D559G/N detected only by clonal sequencing on-treatment (2-7% of clones)
M12746-40537-2507	PTW 48	M414T*, G554S, G558R*	none	none	M414T and G558R detected only by clonal seq. on-treatment (3% of clones)

5.7 Baseline Resistance Analysis (Genotype 1a)

Two sets of analyses were conducted to determine if Baseline polymorphisms in NS3, NS5A or NS5B impacted the efficacy of the 3-DAA +/- RBV regimen in HCV genotype 1a subjects. These analyses were dictated in part by the analyses conducted by the sponsor. The first set of analyses evaluated the proportion Baseline polymorphisms in virologic failure subjects compared to a subset of SVR subjects from the pooled Phase 3 trials. The second set of analyses compared the SVR rates for subjects with or without specific Baseline polymorphisms in the large Phase 2b trial M11-652.

Given the extremely low (0.5%, Table 18) virologic failure rate among HCV genotype 1b subjects who received the 3-DAA +/- RBV regimen in Phase 2/3 trials, it is not possible to identify any Baseline polymorphisms that impacted response among HCV genotype 1b subjects. Therefore, all of these analyses focused only HCV genotype 1a subjects.

Pooled Phase 3 Analysis

The first analysis focused on the pooled Phase 3 trials of the 3-DAA +/- RBV regimen. For these trials, the sponsor conducted population nucleotide sequence analyses on Baseline samples from all subjects who experienced virologic failure, and also from a subset of subjects who achieved SVR, with 2 SVR subjects for every 1 virologic failure subject matched by trial, HCV subtype, IL28B genotype, baseline HCV RNA, and sex to the extent possible. Because only a subset of SVR subjects was analyzed, a direct comparison of SVR rates for subjects with or without Baseline resistance-associated polymorphisms cannot be conducted. However, the proportions of virologic failure and SVR subjects with Baseline polymorphisms can be determined and compared to determine if the polymorphisms were more common in virologic failure subjects, which would indicate that the polymorphisms may have impacted treatment efficacy. Furthermore, as described below, based on the known SVR and virologic failure rates in the trials an SVR rate for subjects with Baseline polymorphisms can be estimated.

In total, Baseline samples from 94 SVR subjects and 47 virologic failure subjects were analyzed for the presence of resistance-associated polymorphisms. The specific Baseline polymorphisms evaluated for their association with treatment response included the variants identified in the treatment-emergent resistance analyses (see Sections 5.2-5.4). For NS3 (amino acids 1-360 were analyzed), the analyses also included Q80K, which did not emerge in the Phase 3 trials but has been shown to impact the efficacy of certain other NS3/4A protease inhibitors, most notably simeprevir (Olysio™ label). The polymorphisms detected in the viruses from these subjects include the following (expressed relative to the "wild-type" genotype 1a consensus):

- NS3: V36M, V55I, Q80K, Q80L, I132V, R155K, P334S, S342P
- NS5A: K24R, M28T/V, Q30E/R, H54Y, H58D/P, E62D, Y93C/H/N
- NS5B: G307R, S556G

Note that the list of NS5A polymorphisms evaluated does not include position L31. Although substitutions or polymorphisms at position L31 are associated with resistance to other NS5A inhibitors, (b) (4) no subjects in the pooled Phase 3 trials of the 3-DAA +/- RBV regimen had detectable viral populations with treatment-emergent substitutions at this position. Three of the 141 HCV genotype 1a subjects included in this Phase 3 analysis had viruses with an L31M (or I/M) polymorphism detected at Baseline and all 3 achieved SVR.

As shown in Figure 7, several polymorphisms in NS3 and NS5A were enriched in virologic failure subjects relative to SVR subjects. As expected, NS3 Q80K was a common polymorphism in this group of genotype 1a subjects. Interestingly, Q80K was enriched ~2.4-fold among virologic failure subjects relative to SVR subjects, indicating that it may have impacted treatment response in some subjects.

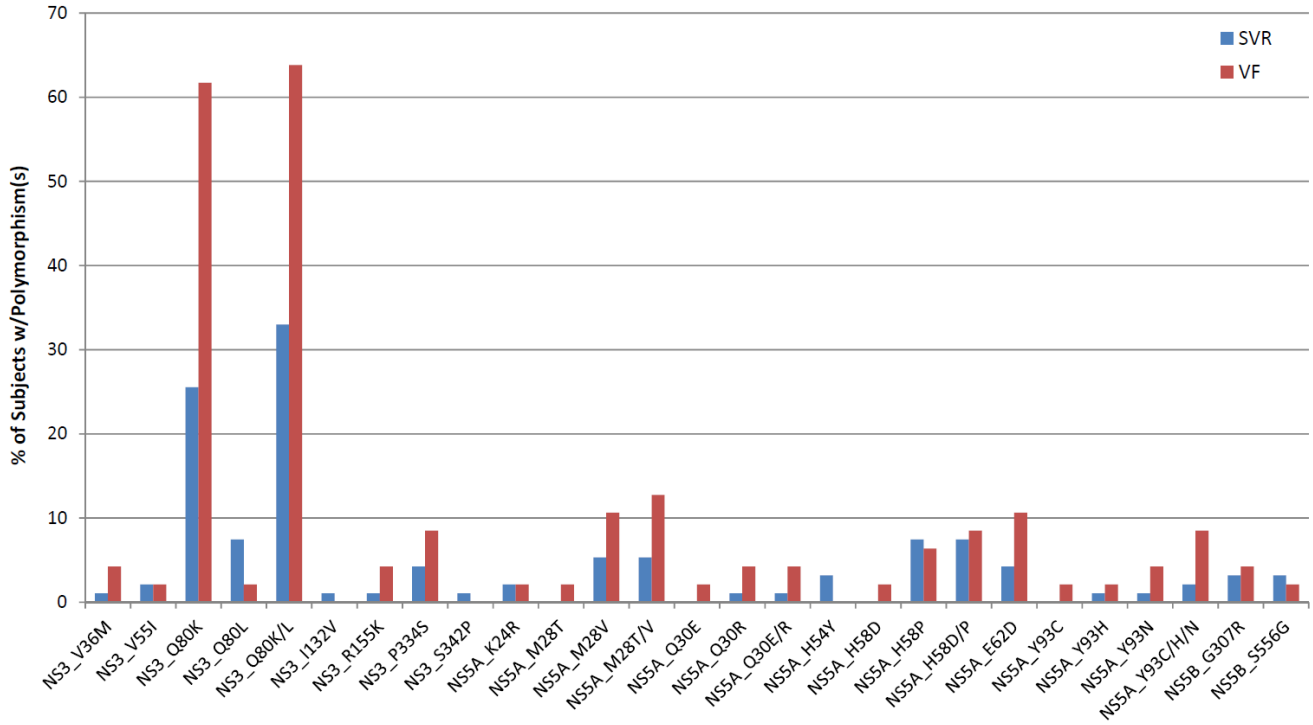


Figure 7. Proportion of virologic failure (VF) and SVR subjects with specific Baseline polymorphisms in NS3, NS5A or NS5B in Pooled Phase 3 trials. Analysis was conducted on Baseline sequences from 94 SVR subjects and 47 VF subjects. The polymorphisms are expressed relative to the "wild-type" genotype 1a consensus.

Considering the detection of any Baseline polymorphism, subjects who experienced virologic failure were more likely than those who achieved SVR to have Baseline viral populations with resistance-associated polymorphisms in NS3 and NS5A (Figure 8). Polymorphisms in NS5B known to potentially impact DBV anti-HCV activity were rare and were not enriched in virologic failure subjects' viruses relative to SVR subjects, indicating that virologic failure was not associated with the detection of resistance-associated polymorphisms in NS5B.

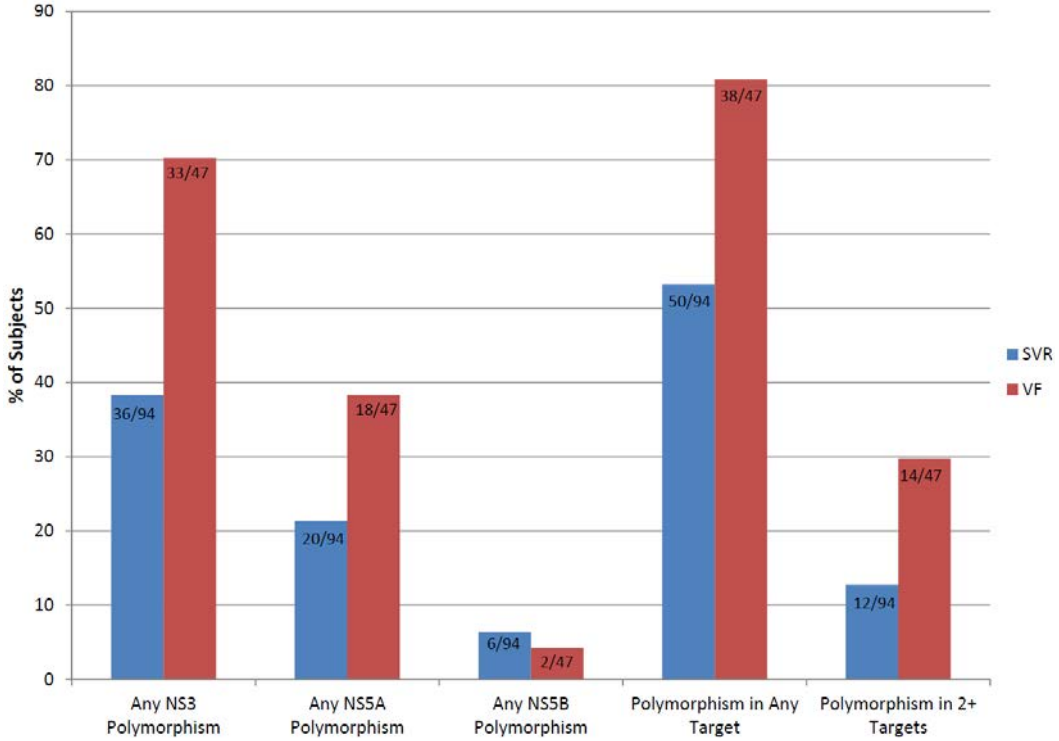
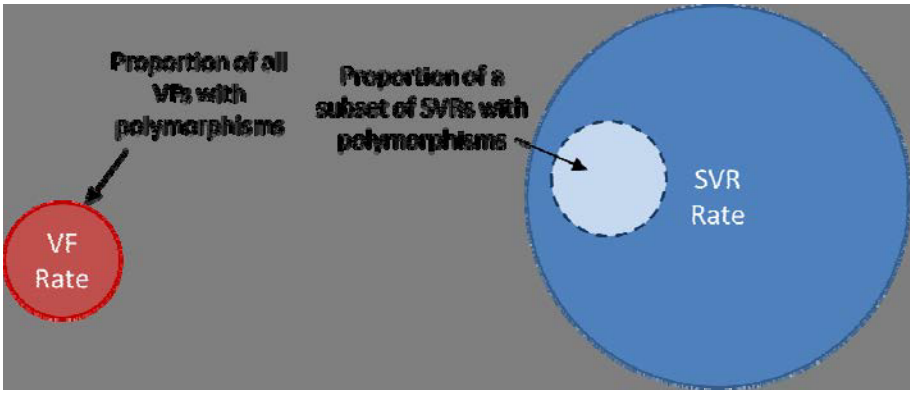


Figure 8. Proportion of virologic failure (VF) and SVR subjects who have Baseline viral populations with polymorphisms in NS3, NS5A or NS5B in Pooled Phase 3 trials.
Polymorphisms detected and considered in this analysis included (expressed relative to "wild-type" genotype 1a consensus) NS3 V36M, V55I, Q80K, Q80L, I132V, R155K, P334S and S342P; NS5A K24R, M28T/V, Q30E/R, H54Y, H58D/P, E62D and Y93C/H/N; and NS5B G307R and S556G.

To better understand the overall impact of Baseline polymorphisms in subjects treated with the 3-DAA +/- RBV regimen, a formula was developed to estimate the SVR rate in subjects with or without Baseline polymorphisms based on the overall SVR rate, overall virologic failure rate, and the proportions of SVR and virologic failure subjects with Baseline polymorphisms (Figure 9). Multiple analyses (not shown) using other available datasets for SVR rates for subjects with or without Baseline polymorphisms were conducted to ensure the formula accurately reproduced the SVR rates from the 4 variables (pSVR, pPM_{svr}, pVF and pPM_{vf}).



$$\text{SVR Rate (PMs)} = \frac{(pSVR)(pPM_{svr})}{(pSVR)(pPM_{svr}) + (pVF)(pPM_{vf})}$$

$$\text{SVR Rate (no PMs)} = \frac{(pSVR)(1 - pPM_{svr})}{(pSVR)(1 - pPM_{svr}) + (pVF)(1 - pPM_{vf})}$$

pSVR = SVR rate for population in question
pPM_{svr} = Proportion of SVRs with polymorphisms
pVF = Virologic failure rate for population in question
pPM_{vf} = Proportion of VFs with polymorphisms

Figure 9. Formula used to estimate SVR rates for subjects with or without Baseline polymorphisms in Phase 3 trials.

Using this formula, it was estimated that the presence of any resistance-associated polymorphism in NS3, NS5A or NS5B was associated with an approximately 5% lower SVR rate compared to subjects without any resistance-associated polymorphisms (Table 30). Based on these calculations it appears that the detection of NS3 K80 had a bigger impact on SVR rate than the detection of NS5A resistance-associated polymorphisms; these results reflect the 2.4-fold enrichment of K80 in virologic failure subjects relative to SVR subjects, versus the 1.8-fold enrichment of NS5A polymorphisms in virologic failure subjects relative to SVR subjects.

Table 30. Estimated SVR rate for subjects with Baseline polymorphisms in Pooled Phase 3 trials. "Any" polymorphisms (PMs) considered in this analysis included (expressed relative to "wild-type" genotype 1a consensus) NS3 V36M, V55I, Q80K, Q80L, I132V, R155K, P334S and S342P; NS5A K24R, M28T/V, Q30E/R, H54Y, H58D/P, E62D and Y93C/H/N; and NS5B G307R and S556G.

Polymorphism	Estimated SVR rate w/PM(s)	Estimated SVR rate w/o PM(s)
NS3, NS5A, NS5B (any)	93%	98%
NS3 Q80K	89%	97%
NS5A (any)	91%	96%
NS3 Q80K or NS5A (any)	92%	98%

There are two important limitations of this approach to estimate SVR rates, and therefore these results should be interpreted with caution. First, these analyses assume that the subset of SVR subjects analyzed to determine the rate of detection of Baseline polymorphisms (pPM_{svr}) accurately

sampled all of the subjects who achieved SVR. It is possible that due to inaccurate sampling the calculated SVR rates over-estimate the impact of Baseline polymorphisms for all subjects in the Phase 3 trials, because: (1) the group of SVR subjects analyzed was demographically matched with virologic failure subjects, (2) virologic failure is often associated with multiple baseline disease factors that reduce treatment efficacy, and (3) Baseline polymorphisms likely have a greater impact on treatment efficacy in patients who already have other negative baseline disease factors. Furthermore, half of the virologic failure subjects included in these analyses were treated with regimens with suboptimal efficacy, including cirrhotic subjects treated with the 3-DAA + RBV regimen for 12 weeks in clinical trial M13-099 (see Section 4.4), and noncirrhotic subjects treated with the 3-DAA + placebo regimen in clinical trial M14-002 (see Section 4.7). Therefore, while the enrichment of Baseline polymorphisms in virologic failure subjects indicates that certain Baseline polymorphisms can impact the efficacy of the 3-DAA +/- RBV regimen in HCV genotype 1a infected patients, it is likely that the impact is minimized in the overall population when optimally effective treatment regimens are used.

A second limitation of this approach is that even with unbiased sampling only approximately 1% of SVR-achieving HCV genotype 1a subjects in Phase 3 trials were analyzed for the detection of Baseline polymorphisms. No additional analyses were conducted to assess variance or determine confidence intervals around the SVR estimates based on the sampling of subjects in these analyses.

Baseline Resistance Analysis of M11-652

Because of the limitations of the Phase 3 Baseline resistance analyses, additional independent analyses of Baseline data from HCV genotype 1a subjects were conducted for Phase 2b clinical trial M11-652, in which Baseline samples from all subjects were analyzed with the exception of missing data for technical reasons. Therefore, SVR rates in M11-652 for subjects with or without Baseline polymorphisms can be calculated directly without data extrapolation. These analyses focused primarily on the NS3 K80 and NS5A polymorphisms, as these were the polymorphisms that were identified as possibly impacting treatment efficacy in Phase 3 trials. Note that available data for NS5A position L31 were included in these analyses. Treatment-naïve and P/R-experienced subjects were pooled according to treatment regimen, and different PTV dose levels were also pooled, as PTV dose level did not appear to impact efficacy in M11-652 (see Section 4.8). Subjects who failed to achieve SVR for non-virologic reasons were censored, and post-SVR12 relapsers were considered treatment failures.

As shown in Table 31, the NS3 Q80K polymorphism was associated with a ~5% overall lower SVR rate for subjects treated with the 3-DAA +/- RBV regimen dosed for 8-24 weeks. However, differences in SVR rates were not necessarily consistent across arms. For example, Q80K was associated with an 8% lower SVR rate for subjects with treated with the 3-DAA + RBV regimen for 12 weeks, but a 5% higher SVR rate for subjects treated with the 3-DAA (no RBV) regimen for 12 weeks. Note that it should not be interpreted that the higher SVR rate for the 3-DAA + RBV 24-Week group compared to the 3-DAA + RBV 12-Week group indicates the 24-week treatment duration improved efficacy; 3 of the 4 virologic failures in the 3-DAA + RBV 12-Week group were on-treatment failures and therefore would not have achieved SVR with a longer treatment duration. In this analysis 90/220 (41%) HCV genotype 1a subjects had viral populations with the K80 polymorphism detected.

Table 31. SVR rates (non-VF-censored) for HCV genotype 1a subjects with or without Baseline NS3 Q80K (expressed relative to "wild-type" genotype 1a consensus) included in M11-652. Only subjects treated with the 3-DAA +/- RBV regimen were included in this analysis.

	3-DAA ± RBV 8-24 Weeks	3-DAA ± RBV 12-24 Weeks	3-DAA+RBV 12 Weeks	3-DAA+RBV 24 Weeks	3-DAA 12 Weeks	3-DAA+RBV 8 Weeks
Q80K	79/90 (88%)	65/71 (92%)	25/28 (89%)	22/23 (96%)	18/20 (90%)	14/19 (74%)
w/o Q80K	121/130 (93%)	95/100 (95%)	38/39 (97%)	40/41 (98%)	17/20 (85%)	26/30 (87%)

Approximately 20% of HCV genotype 1a subjects treated with the 3-DAA +/- RBV regimen for 8-24 weeks had 1 or more NS5A resistance-associated polymorphisms, although there was no clear indication that the polymorphisms impacted treatment efficacy (Table 32).

Table 32. SVR rates (non-VF-censored) in M11-652 for HCV genotype 1a subjects who have viral populations with Baseline NS5A polymorphisms. Polymorphisms shown are expressed relative to "wild-type" genotype 1a consensus.

NS5A Polymorphism	Overall SVR 3-DAA ± RBV 8-24 Weeks	Overall SVR 3-DAA ± RBV 12-24 Weeks
K24R	4/4 (100.0%)	2/2 (100.0%)
M28T	1/1 (100.0%)	1/1 (100.0%)
M28V	11/13 (84.6%)	9/10 (90.0%)
M28T/V	12/14 (85.7%)	10/11 (90.9%)
Q30R	3/3 (100.0%)	3/3 (100.0%)
L31M	0/1 (0.0%)	n/a
L31V	1/1 (100.0%)	n/a
L31M/V	1/2 (50.0%)	n/a
H54Y	9/9 (100.0%)	7/7 (100.0%)
H58P	3/3 (100.0%)	2/2 (100.0%)
H58R	3/3 (100.0%)	3/3 (100.0%)
H58P/R	6/6 (100.0%)	5/5 (100.0%)
E62D	5/5 (100.0%)	4/4 (100.0%)
Y93C	1/1 (100.0%)	1/1 (100.0%)
Y93H	3/3 (100.0%)	n/a
Y93N	0/1 (0.0%)	0/1 (0.0%)
Y93C/H/N	4/5 (80.0%)	1/2 (50.0%)
NS5A any (from above)	39/43 (90.7%)	28/30 (93.3%)
No NS5A Polymorphism	165/181 (91.2%)	140/149 (94.0%)

Overall, the impact of NS3 K80 or NS5A Baseline polymorphisms on 3-DAA +/- RBV treatment efficacy in M11-652 was minimal, and clearly smaller than the estimated impact in the Phase 3 trials overall (Table 33). Among those subjects with Baseline sequence data for both NS3 and NS5A, 55% (112/205) had NS3 K80 or NS5A polymorphisms.

Consistent with the Phase 3 analyses, NS5B resistance-associated polymorphisms were uncommon and not associated with treatment efficacy in M11-652 (Table 33).

Table 33. Summary of SVR rates for 3-DAA +/- RBV-treated subjects (HCV genotype 1a) with Baseline HCV polymorphisms in M11-652 and Phase 3 trials. *Note that pooled Phase 3 subjects include cirrhotic and noncirrhotic subjects, whereas M11-652 included only noncirrhotic subjects.

Baseline Polymorphism	M11-652 3-DAA +/- RBV (8-24W)	M11-652 3-DAA +/- RBV (12-24W)	Estimated Phase 3 3-DAA +/- RBV (12-24W)*
NS3 K80	79/90 (88%)	65/71 (92%)	89%
w/o NS3 K80	121/130 (93%)	95/100 (95%)	97%
NS5A Polymorphism	39/43 (91%)	28/30 (93%)	91%
w/o NS5A Polymorphism	165/181 (91%)	140/149 (94%)	96%
NS3 K80 or NS5A Polymorphism	103/116 (89%)	84/91 (92%)	92%
NS3 K80 and NS5A Polymorphism	15/17 (88%)	9/10 (90%)	Not Determined
No NS3 K80 or NS5A Polymorphism	86/93 (92%)	70/74 (95%)	98%
NS5B Polymorphism	17/18 (94%)	11/12 (92%)	97%
w/o NS5B Polymorphism	210/229 (92%)	172/182 (95%)	95%

A key review question is whether there are certain subgroups of treatment-naïve, noncirrhotic, HCV genotype 1a infected subjects that do not require the use of RBV for optimal efficacy of the 3-DAA regimen. Therefore, an analysis was conducted to determine if RBV was associated with an efficacy benefit in treatment-naïve, noncirrhotic, HCV genotype 1a subjects with or without NS3 K80 or NS5A polymorphisms in M11-652. As shown in Table 34, the use of RBV in these subjects appeared to be consistently associated with an improvement in SVR rates, even in subjects without NS3 K80 or NS5A polymorphisms, although the number of subjects in some subgroups was small.

Table 34. Impact of RBV on SVR rates for treatment-naïve, HCV genotype 1a subjects with or without NS3 Q80K or NS5A polymorphisms in M11-652 (Arm E vs. F/G).

Baseline Polymorphism	3-DAA 12W	3-DAA + RBV 12W
NS3 K80	18/20 (90%)	19/20 (95%)
w/o NS3 K80	17/20 (85%)	23/23 (100%)
NS5A Polym.	7/8 (88%)	8/9 (89%)
w/o NS5A Polym.	32/36 (89%)	40/40 (100%)
NS3 K80 or NS5A Polym.	21/24 (88%)	24/25 (96%)
No NS3 K80 or NS5A Polym.	13/15 (87%)	17/17 (100%)

Reviewer's Perspective on Baseline Resistance Testing to Guide Treatment

The totality of evidence does not seem to support broad use of Baseline resistance testing to guide treatment decision making with the AbbVie 3-DAA +/- RBV regimen for HCV genotype 1a or 1b patients. Although the analyses of Phase 3 trials indicate that certain Baseline resistance-associated polymorphisms may have impacted the efficacy of the 3-DAA +/- RBV regimens in HCV genotype 1a subjects, the impact was likely modest and also was not clearly reproduced in analyses of Phase 2b trial M11-652. Given that a majority of HCV genotype 1a patients likely carry NS3 K80 or NS5A polymorphisms (either or both), yet a very low (~5%) virologic failure rate was observed in genotype 1a subjects in Phase 2/3 trials of the 3-DAA +/- RBV regimens, it is clear that these polymorphisms do not have a major impact on treatment efficacy. The targeting of three different HCV proteins that are essential for viral replication provides coverage to reduce the impact of pre-existing Baseline resistance-associated polymorphisms. Maximizing treatment efficacy for all patient populations, for example including RBV in the regimen for all HCV genotype 1a subjects and treating cirrhotic HCV genotype 1a patients for 24 weeks, would likely further minimize the impact of Baseline resistance-associated polymorphisms. Finally as a practical consideration, although an NS3 sequencing assay may be commercially available for some patients, this reviewer is not aware of an NS5A sequencing assay that is commercially available at this time, although an assay may become available in the future. No NS3 or NS5A sequencing assays are FDA-approved.

6. CONCLUSION

This Original NDA is approvable from a Clinical Virology perspective for the treatment of chronic HCV genotype 1 infected patients who are either naïve to prior anti-HCV therapy or who have failed prior therapy with Peg-IFNα/RBV, including patients with compensated cirrhosis.

7. PACKAGE INSERT

7.1 Proposed Package Insert (with initial Reviewer-recommended changes)

Clinical virology-related sections of the proposed Viekira Pak™ label and suggested edits are shown below. Note that at the time of finalization of this review the review team was still negotiating labeling and comparing analysis results with the sponsor, so the edits shown below should be considered preliminary.

General comment to AbbVie: Specify the non-nucleoside sub-class ("palm") throughout the label.

12.1 Mechanism of Action

VIEKIRA PAK combines three direct-acting hepatitis C virus antiviral agents with distinct mechanisms of action (b) (4)

Ritonavir is not active against HCV. Ritonavir is a (b) (4) potent CYP3A inhibitor that increases peak and trough plasma drug concentrations of paritaprevir and overall drug exposure (i.e., area under the curve).

12.4 Microbiology

7 Page(s) of Draft Labeling has been Withheld in Full as B4 (CCI/TS) immediately following this page

(b) (4)

[redacted]

7.2 Reviewer's Proposed Package Insert (clean)

12.1 Mechanism of Action

VIEKIRA PAK combines three direct-acting hepatitis C virus antiviral agents with distinct mechanisms of action *[see Microbiology (12.4)]*.

Ritonavir is not active against HCV. Ritonavir is a potent CYP3A inhibitor that increases peak and trough plasma drug concentrations of paritaprevir and overall drug exposure (i.e., area under the curve).

12.4 Microbiology

Mechanism of Action

VIEKIRA PAK combines three direct-acting antiviral agents with distinct mechanisms of action and non-overlapping resistance profiles to target HCV at multiple steps in the viral lifecycle.

Ombitasvir

Ombitasvir is an inhibitor of HCV NS5A, which is essential for viral RNA replication and virion assembly. The mechanism of action of ombitasvir has been characterized based on cell culture antiviral activity and drug resistance mapping studies.

Paritaprevir

Paritaprevir is an inhibitor of the HCV NS3/4A protease which is necessary for the proteolytic cleavage of the HCV encoded polyprotein (into mature forms of the NS3, NS4A, NS4B, NS5A, and NS5B proteins) and is essential for viral replication. In a biochemical assay, paritaprevir inhibited the proteolytic activity of recombinant HCV genotype 1a and 1b NS3/4A protease enzymes with IC_{50} values of 0.18 nM and 0.43 nM, respectively. Paritaprevir inhibited the activity of NS3/4A enzymes from single isolates of genotypes 2a, 2b, 3a, and 4a with IC_{50} values of 2.4 nM, 6.3 nM, 14.5 nM, and 0.16 nM, respectively.

Dasabuvir

Dasabuvir is a non-nucleoside inhibitor of the HCV RNA-dependent RNA polymerase encoded by the NS5B gene, which is essential for replication of the viral genome. In a biochemical assay, dasabuvir inhibited the polymerase activity of recombinant HCV genotype 1a and 1b NS5B enzymes with IC_{50} values of 2.8 nM and 10.7 nM, respectively. Dasabuvir inhibited a panel of genotype 1a and 1b NS5B polymerases with median IC_{50} values of 2.8 nM (range 2.4 nM to 4.2 nM; n = 3) and 3.7 nM (range 2.2 nM to 10.7 nM; n = 4), respectively. Based on drug resistance mapping studies of HCV genotypes 1a and 1b, dasabuvir targets the palm domain of the NS5B polymerase, and is therefore referred to as a non-nucleoside NS5B-palm polymerase inhibitor. Dasabuvir had reduced activity in biochemical assays against NS5B polymerases from HCV genotypes 2a, 2b, 3a and 4a (IC_{50} values ranging from 900 nM to >20 μM).

Antiviral Activity

Ombitasvir

The EC_{50} values of ombitasvir against genotype 1a-H77 and 1b-Con1 strains in HCV replicon cell culture assays were 14.1 pM and 5 pM, respectively. The median EC_{50} values of ombitasvir against HCV replicons containing NS5A genes from a panel of genotype 1a and 1b isolates from treatment-naïve subjects were (b) (4) pM (range 0.35 to 0.88 pM; n = 11) and (b) (4) pM (range 0.74 to 1.5 pM; n = 11), respectively. Ombitasvir had EC_{50} values of 12 pM, 4.3 pM, 19 pM, 1.7 pM, 3.2 pM, and 366 pM against chimeric replicons constructed with NS5A from single isolates representing genotypes 2a, 2b, 3a, 4a, 5a, and 6a, respectively. (b) (4)

Paritaprevir

The EC_{50} values of paritaprevir against genotype 1a-H77 and 1b-Con1 strains in the HCV replicon cell culture assay were 1.0 nM and 0.21 nM, respectively. The median EC_{50} values of paritaprevir against HCV replicons containing NS3 genes from a panel of genotype 1a and 1b isolates from treatment-naïve subjects were 0.68 nM (range 0.43 nM to 1.87 nM; n = 11) and 0.06 nM (range 0.03 nM to 0.09 nM; n = 9), respectively. Paritaprevir had an EC_{50} value of 5.3 nM against the HCV genotype 2a-JFH-1 replicon cell line, and EC_{50} values of 19 nM, 0.09 nM, and 0.68 nM against replicon cell lines containing NS3 from a single isolate each of genotype 3a, 4a, and 6a, respectively. (b) (4)

In HCV replicon cell culture assays, ritonavir did not exhibit a direct antiviral effect and the presence of ritonavir did not affect the antiviral activity of paritaprevir.

Dasabuvir

The EC_{50} values of dasabuvir against genotype 1a-H77 and 1b-Con1 strains in HCV replicon cell culture assays were 7.7 nM and 1.8 nM, respectively. The median EC_{50} values of dasabuvir against HCV replicons containing NS5B genes from a panel of genotype 1a and 1b isolates from treatment-naïve subjects were 0.6 nM (range 0.4 nM to 2.1 nM; n = 11) and 0.3 nM (range 0.2 nM to 2 nM; n = 10), respectively (b) (4)

Combination Antiviral Activity

(b) (4)

Resistance

In Cell Culture

(b) (4) of HCV genotype 1a and 1b replicons (b) (4) ombitasvir, paritaprevir or dasabuvir resulted in the emergence of drug resistant replicons carrying amino acid substitutions in NS5A, NS3, or NS5B, respectively. Amino acid substitutions in NS5A, NS3, or NS5B selected in cell culture or identified in Phase 2b and 3 clinical trials were phenotypically characterized in genotype 1a or 1b replicons.

For ombitasvir, in HCV genotype 1a replicons single NS5A substitutions M28T/V, Q30E/R, L31V, H58D, and Y93C/H/L/N (b) (4) reduced ombitasvir antiviral activity by (b) (4)- to 67,000-fold. In genotype 1b replicons, single NS5A substitutions L28T, L31F/V, and Y93H reduced ombitasvir antiviral activity by 8- to 661-fold. A P29 deletion in NS5A reduced HCV genotype 1b replicon susceptibility to ombitasvir by >300,000-fold. In general, combinations of ombitasvir resistance-associated substitutions in HCV genotype 1a or 1b replicons further reduced ombitasvir antiviral activity.

For paritaprevir, in HCV genotype 1a replicons single NS3 substitutions F43L, R155G/K/S/ (b) (4) A156T, and D168A/E/F/H/N/V/Y reduced paritaprevir antiviral activity by (b) (4) to 219-fold. An NS3 Q80K substitution in a genotype 1a replicon reduced paritaprevir antiviral activity by 3-fold. Combinations of V36M, Y56H, or E357K with R155K or D168 substitutions reduced the activity of paritaprevir by an additional 2- to 7-fold relative to the single R155K or D168 substitutions in genotype 1a replicons. In genotype 1b replicons single NS3 substitutions A156T and D168A/(b) (4)/H/ (b) (4) /V/ (b) (4) reduced paritaprevir antiviral activity by (b) (4) to (b) (4)-fold. The combination of Y56H with D168 substitutions reduced the activity of paritaprevir by an additional (b) (4)- to 26-fold relative to the single D168 substitutions in genotype 1b replicons.

For dasabuvir, in HCV genotype 1a replicons single NS5B substitutions C316Y, M414I/T/ (b) (4) E446K/Q, Y448C/H, A553T, G554S, S556G/R, and Y561H reduced dasabuvir antiviral activity by (b) (4) to 1,472-fold. In genotype 1b replicons, single NS5B substitutions C316H/N/Y, S368T, N411S, M414I/T/ (b) (4) Y448C/H, A553V, S556G and D559G reduced dasabuvir antiviral activity by 5- to 1,569-fold.

In Clinical Studies

Treatment-emergent substitutions observed in the viral populations of subjects (b) (4) are shown in Table 8. Treatment-emergent substitutions were detected in all 3 HCV drug targets in 30/57 (53%) HCV genotype 1a infected subjects, and 1/6 (17%) HCV genotype 1b infected subjects.

Table 8. Treatment-Emergent Amino Acid Substitutions in the Pooled Analysis of VIEKIRA PAK with and without Ribavirin Regimens (12- or 24-week durations) in Phase 2b and Phase 3 Clinical Trials (N = 2418)

Target	Emergent Amino Acid Substitutions	Genotype 1a N = 58[a] % (n)	Genotype 1b N = 6 % (n)
(b) (4)			

Target	Emergent Amino Acid Substitutions	Genotype 1a N = 58[a] % (n)	Genotype 1b N = 6 % (n)
b. Substitutions were observed in combination with other emergent substitutions at NS3 position R155 or D168. c. Position located in NS3 helicase domain. d. (b) (4)			

Persistence of Resistance-Associated Substitutions

The persistence of ombitasvir, paritaprevir, and dasabuvir resistance-associated amino acid substitutions in NS5A, NS3, and NS5B, respectively, was assessed in HCV genotype 1a-infected subjects in Phase 2 trials whose virus had at least 1 treatment-emergent resistance-associated substitution in the drug target, and with available data through at least 24 weeks post-treatment. Population and clonal nucleotide sequence analyses (assay sensitivity approximately 5-10%) were conducted to detect the persistence of viral populations with resistance-associated substitutions.

(b) (4) treatment-emergent substitutions in NS5A persisted at detectable levels through at least Post-Treatment Week 24 in 24/24 (100%) subjects, and through Post-Treatment Week 48 in 18/18 (100%) subjects with available data.

For paritaprevir, viral populations with 1 or more treatment-emergent substitutions in NS3 persisted at detectable levels through at least Post-Treatment Week 24 in 17/29 (59%) subjects, and through Post-Treatment Week 48 in 5/22 (23%) subjects with available data. (b) (4)

For dasabuvir, viral populations with 1 or more treatment-emergent substitutions in NS5B persisted at detectable levels through at least Post-Treatment Week 24 in 11/16 (69%) subjects, and through Post-Treatment Week 48 in 8/ (b) (4) subjects with available data. Treatment-emergent S556G persisted through Post-Treatment Week 48 in 6/9 (67%) subjects.

Due to (b) (4) subjects infected with HCV genotype 1b, trends in persistence of treatment-emergent substitutions in this genotype could not be established.

The lack of detection of virus containing a resistance-associated substitution does not indicate that the resistant virus is no longer present at clinically significant levels. The long-term clinical impact of the emergence or persistence of virus containing VIEKIRA PAK-resistance-associated substitutions is unknown.

Effect of Baseline HCV Polymorphisms on Treatment Response

A pooled analysis of subjects in the Phase 3 clinical trials of ombitasvir, paritaprevir, and dasabuvir with or without ribavirin was conducted to explore the association between baseline HCV NS5A, NS3 (b) (4) or NS5B resistance-associated polymorphisms and treatment outcome. Baseline samples

from HCV genotype 1a infected subjects who experienced virologic failure (n=47), as well as samples from a subset of demographically matched subjects who achieved SVR (n=94), were analyzed to compare the frequencies of resistance-associated polymorphisms in these two populations. The NS3 Q80K polymorphism was detected in approximately 38% of subjects in this analysis and was enriched approximately 2-fold in virologic failure subjects compared to SVR-achieving subjects. Ombitasvir resistance-associated polymorphisms in NS5A (pooling data from all resistance-associated amino acid positions) were detected in approximately (b) (4)% of subjects in this analysis and similarly enriched approximately 2-fold in virologic failure subjects. Dasabuvir resistance-associated polymorphisms in NS5B were detected in approximately 5% of subjects in this analysis and were not enriched in virologic failure subjects. (b) (4)

(b) (4)

Cross-resistance

Cross-resistance is expected among NS5A inhibitors, NS3/4A protease inhibitors, and non-nucleoside NS5B-palm inhibitors by class. Dasabuvir retained full activity against HCV replicons containing (b) (4) single NS5B (b) (4) S282T associated with resistance to nucleos(t)ide analogue NS5B polymerase inhibitor (b) (4) In clinical trials of VIEKIRA PAK, no subjects who experienced virologic failure had treatment-emergent substitutions potentially associated with resistance to nucleot(s)ide analogue NS5B polymerase inhibitors.

The impact of prior ombitasvir, paritaprevir, or dasabuvir treatment experience on the efficacy of other NS5A inhibitors, NS3/4A protease inhibitors, or NS5B inhibitors has not been studied.

7.3 Sponsor's Responses to Initial Label Recommendations (SDN 032)

This reviewer's summary of the sponsor's responses and any further action items are indicated in italicized font.

12.1 Mechanism of Action

Reviewer's comments: The sponsor accepted our recommended changes to this section. ***No further action is indicated for this section.***

12.4 Microbiology
Mechanism of Action

Reviewer's comments: the sponsor accepted our recommended changes to this section for the descriptions related to ombitasvir and paritaprevir. For dasabuvir, the sponsor removed one point (see below), arguing that it was redundant with the following sentence. The sponsor also confirmed our calculations of median IC_{50} values. This reviewer accepts the sponsor's change. ***No further action is indicated for this section.***

Dasabuvir is a non-nucleoside inhibitor of the HCV RNA-dependent RNA polymerase encoded by the NS5B gene, which is essential for replication of the viral genome. In a biochemical assay, dasabuvir (b) (4) -inhibited a panel of genotype 1a and 1b NS5B polymerases with median IC_{50} values of 2.8 nM (range 2.4 nM to 4.2 nM; n = 3) and 3.7 nM (range 2.2 nM to 10.7 nM; n = 4), respectively. Based on drug resistance mapping studies of HCV genotypes 1a and 1b, dasabuvir targets the palm domain of the NS5B polymerase, and is therefore referred to as a non-nucleoside NS5B-palm polymerase inhibitor. Dasabuvir had reduced activity in biochemical assays against NS5B polymerases from HCV genotypes 2a, 2b, 3a and 4a (IC_{50} values ranging from 900 nM to >20 µM).

Antiviral Activity

Reviewer's comments: The sponsor added/confirmed median EC_{50} values as requested. All other changes were accepted. ***No further action is indicated for this section.***

Combination Antiviral Activity

Reviewer's comments: The sponsor proposed the following change for this section, (b) (4) *. These types of assays are not standardized and their primary purpose is to detect overt antagonism.*

(b) (4)

Reviewer's counter-proposal:

Evaluation of combinations of ombitasvir, paritaprevir, dasabuvir and ribavirin in HCV genotype 1 replicon cell culture assays showed no evidence of antagonism in antiviral activity.

Resistance
In Cell Culture

Reviewer's comments: The sponsor generally agreed with our recommended changes, (b) (4) *and adjusted the fold-resistance values accordingly.* (b) (4) ***No further action is indicated for this section.***

For ombitasvir, in HCV genotype 1a replicons single NS5A substitutions M28T/V, Q30E/R, L31V, H58D, and Y93C/H/L/N (b) (4) reduced ombitasvir antiviral activity by 58 (b) (4) to 67,000-fold. In genotype 1b replicons, single NS5A substitutions L28T, L31F/V, and Y93H reduced ombitasvir antiviral activity by 8- to 661-fold. (b) (4)

(b) (4) In general, combinations of ombitasvir resistance-associated substitutions in HCV genotype 1a or 1b replicons further reduced ombitasvir antiviral activity.

For paritaprevir, in HCV genotype 1a replicons single NS3 substitutions F43L, R155G/K/S (b) (4) A156T, and D168A/E/F/H/N/V/Y reduced paritaprevir antiviral activity by 7 (b) (4) to 219-fold. An NS3 Q80K substitution in a genotype 1a replicon reduced paritaprevir antiviral activity by 3-fold. Combinations of V36M, Y56H, or E357K with R155K or D168 substitutions reduced the activity of paritaprevir by an additional 2- to 7-fold relative to the single R155K or D168 substitutions in genotype 1a replicons. In genotype 1b replicons single NS3 substitutions A156T and D168A (b) (4) /H (b) (4) V (b) (4) reduced paritaprevir antiviral activity by 7 (b) (4) to 159 (b) (4) -fold. The combination of Y56H with D168 substitutions reduced the activity of paritaprevir by an additional 16 (b) (4) to 26-fold relative to the single D168 substitutions in genotype 1b replicons.

For dasabuvir, in HCV genotype 1a replicons single NS5B substitutions C316Y, M414I/T (b) (4) E446K/Q, Y448C/H, A553T, G554S, S556G/R, and Y561H reduced dasabuvir antiviral activity by 8 (b) (4) to 1,472-fold. In genotype 1b replicons, single NS5B substitutions C316H/N/Y, S368T, N411S, M414I/T (b) (4) Y448C/H, A553V, S556G and D559G reduced dasabuvir antiviral activity by 5- to 1,569-fold.

Resistance cont.
In Clinical Studies

Reviewer's comments: The sponsor agreed with most of our recommended changes. The first statement proposed by the sponsor summarizing the virologic failure rate in the opening paragraph (see below) is potentially misleading as it does not include any post-SVR12 relapses from the Phase 3 trials (n=9 known to date). This reviewer recommends either removal of the statement or inclusion of the 9 additional late relapsers in the numerator. This reviewer prefers that a pooled virologic failure rate is not reported in this section, as the data come from different trials/regimens/populations in which the failure rates differed to some extent. Furthermore, 18 subjects from Phase 2b/3 trials (20 if including 2 additional open-label rollover treatment subjects) experienced on-treatment virologic failure, so the phrase "primarily post-treatment relapse" should either be deleted or changed to "including on-treatment virologic failure and post-treatment relapse."

(b) (4)

Treatment-emergent substitutions observed in the viral populations of these subjects (b) (4) are shown in Table 8. Treatment-emergent substitutions were detected in all 3 HCV drug targets in 30/57 (53%) HCV genotype 1a infected subjects, and 1/6 (17%) HCV genotype 1b infected subjects.

Resistance cont.
(Table 8-treatment-emergent substitutions)

Reviewer's comments: The sponsor confirmed our analyses that were incorporated into the revised Table 8. In general, the sponsor accepted most of the substitutions that were added to the table, with some minor formatting changes for clarity that for the most part are acceptable to this reviewer. (b) (4)

(b) (4)

Reviewer's responses:

- (b) (4)
- *NS5B C316Y: Please include this substitution also for genotype 1a, as it emerged in 2 HCV genotype 1a infected subjects.*
- (b) (4)

Table 8. Treatment-Emergent Amino Acid Substitutions in the Pooled Analysis of VIEKIRA PAK with and without Ribavirin Regimens (12- or 24-week durations) in Phase 2b and Phase 3 Clinical Trials (N = 2418)

Target	Emergent Amino Acid Substitutions	Genotype 1a N = 58[a] % (n)	Genotype 1b N = 6 % (n)
(b) (4)			

(b) (4)

a. N = 57 for the NS5B target.
b. Substitutions were observed in combination with other emergent substitutions at NS3 position R155 or D168.
c. Position located in NS3 helicase domain.
d. (b) (4) -D168A/F/H/I/L/N/T/V/Y.

Persistence of Resistance-Associated Substitutions

Effect of Baseline HCV Polymorphisms on Treatment Response

Cross-resistance

Reviewer's comments: The sponsor has not yet responded to our recommended changes for these sections. For the cross-resistance section, we plan to recommend removal of the statements referring to other classes of non-nucleoside NS5B polymerase inhibitors, as no drugs in these classes are currently FDA-approved.

7.4 Final Approved Package Insert

Due to the timing of NDA milestones and PDUFA goal deadlines, the final approved package insert was not available at the time of finalization of this review.

8. RECOMMENDATIONS

1) Please conduct site-directed mutant phenotype analyses of sofosbuvir against HCV replicons carrying the following NS5B substitutions associated with dasabuvir resistance: C316Y (1a and 1b), S556G (1a).

2) Please conduct site-directed mutant phenotype analyses of dasabuvir against HCV genotype 1a and 1b replicons carrying the following sofosbuvir treatment-emergent substitutions: L159F and V321A.

3) Please conduct site-directed mutant phenotype analyses of dasabuvir against HCV replicons carrying the following substitutions that are associated with resistance to certain non-nucleoside NS5B-thumb2 polymerase inhibitors: M423I (GT1a), I482T (GT1a) and A486V (GT1b).

4) Please conduct paritaprevir phenotypic analyses of substitutions in the NS3 helicase (e.g., P334S, S342P, V406A/I, T449I, P470S) that emerged in virologic failure subjects treated with the 3-DAA +/- RBV regimen. Evaluate the impact of these substitutions alone and in combination with other key resistance-associated substitutions (e.g., R155K or D168x) that were often detected in combination.

5) Please submit the complete study reports from Phase 3 trials, including complete data from later follow-up timepoints for HCV RNA levels and persistence of resistance-associated substitutions.

9. APPENDICES

APPENDIX A: Site-directed mutant phenotype data for paritaprevir

GT	NS3 Substitution	nM EC_{50}	FC EC_{50}	nM EC_{90}	FC EC_{90}	% Replication Capacity	Comment
1a	**WT (H77)**	**1.4**	**1**	**3.0**	**1**	**100**	
1a	V36A	4.4	3	8.8	3	130	
1a	V36L	2.5	2	5.2	2	82	
1a	V36M	3.0	2	6.1	2	81	
1a	F43L	27	19	35	12	17	
1a	T54S	0.5	0.4	1.2	0.4	6.2	
1a	V55I	1.4	1	2.5	1	81	
1a	Y56H	4.1	3	11	4	3.5	
1a	Q80K	3.9	3	6.4	2	91	
1a	Q80L	2.2	2	3.1	1	38	
1a	Q80R	3.3	2	6.6	2	44	
1a	R155G	19	14	107	36	2.0	
1a	R155K	51	36	96	32	31	
1a	R155M	1.2	1	2.6	1	5.6	
1a	R155S	10	7	37	12	2.1	
1a	R155T	10	7	43	14	5.1	
1a	R155V	6.3	5	13	4	20	
1a	R155W	16	11	66	22	5.3	
1a	A156T	24	17	43	14	5.2	
1a	D168A	70	50	365	122	35	
1a	D168E	20	14	25	8	34	
1a	D168F	289	206	322	107	4	
1a	D168H	87	62	151	50	24	
1a	D168N	19	14	42	14	28	
1a	D168V	135	96	274	91	1.5	
1a	D168Y	307	219	658	219	3.5	
1a	E357K	2.4	2	2.7	1	131	
1a	V23A (NS4A)	2.6	2	6.7	2	70	
1a	V36L + R155K	96	69	294	98	51	
1a	V36M + R155K	111	79	352	117	29	
1a	F43L + R155K	139	99	1,000	333	7.2	EC90 >
1a	F43L + D168V	246	176	1,699	566	23	
1a	Y56H + D168A	493	352	1,424	475	46	
1a	Y56H + D168Y	632	451	1,705	568	1.1	
1a	Y56H + D168V	785	561	1,487	496	15	
1a	Q80K + R155K	27	19	135	45	77	
1a	Q80K + D168A	108	77	467	156	28	
1a	Q80K + D168V	340	243	821	274	5	
1a	Q80K + D168Y	468	334	800	267	2	
1a	R155T + D168N	88	63	127	42	52	
1a	E357K + D168V	574	410	630	210	24	
1a	E357K + D168Y	487	348	599	200	8.2	
1a	V23A (NS4A) + D168V	ND	ND	ND	ND	<0.5	
1a	V23A (NS4A) + D168Y	376	269	735	245	18	
GT	**NS3 Substitution**	**nM EC_{50}**	**FC EC_{50}**	**nM EC_{90}**	**FC EC_{90}**	**% Replication Capacity**	**Comment**
1b	**WT (Con1)**	**0.1**	**1**	**0.3**	**1**	**100**	
1b	T54A	0.09	1	0.41	2	59	
1b	T54S	ND	ND	ND	ND	<0.5	
1b	V55A	0.07	1	0.31	1	14	

1b	Y56H	ND	ND	ND	ND	<0.5	
1b	R155K	4.4	40	17	65	73	
1b	R155Q	ND	ND	ND	ND	<0.5	
1b	A156S	0.06	1	0.3	1	61	
1b	A156T	0.81	7	3.4	13	19	
1b	D168A	3	27	11	42	69	
1b	D168E	0.48	4	1.1	4	80	
1b	D168H	8.3	75	16	62	108	
1b	D168K	96	873	203	781	50	
1b	D168T	5.4	49	15	58	129	
1b	D168V	17	155	40	154	157	
1b	D168Y	37	336	57	219	70	
1b	V170A	0.09	1	0.29	1	64	
1b	Y56H + D168A	77	700	219	842	6	
1b	Y56H + D168V	272	2,473	519	1,996	22	
1b	Y56H + D168Y	453	4,118	591	2,273	8	

FC, fold-change; ND, no data

APPENDIX B: Site-directed mutant phenotype data for OMB (ABT-267)

GT	NS5A Substitution	pM EC_{50}	FC EC_{50}	pM EC_{90}	FC EC_{90}	% Replication Capacity	Comment
1a	**WT (H77)**	**2.7**	**1**	**7.6**	**1**	**100**	
1a	K24R	1	1	9	1	172	
1a	M28T	24,475	9,065	71,764	9,443	100	
1a	M28V	159	59	627	83	87	
1a	Q30E	3,619	1,340	10,000	1,316	70	EC90 >
1a	Q30H	8	3	34	4	64	
1a	Q30R	2,184	809	22,570	2,970	60	
1a	L31M	5	2	20	3	141	
1a	L31V	422	156	2,166	285	241	
1a	H58D	664	246	4,046	532	66	
1a	H58P	1	1	4	1	129	
1a	Y93C	4,573	1,694	31,740	4,176	24	
1a	Y93H	112,975	41,843	200,000	26,316	18	EC90 >
1a	Y93L	8,116	3,006	39,622	5,213	42	
1a	Y93N	182,200	67,481	358,450	47,164	25	
1a	Y93S	2,765	1,024	12,425	1,635	ND	
1a	I269V	1	1	4	1	60	
1a	K24R + M28V	120	44	844	111	155	
1a	K24R + M28T	8,684	3,216	45,327	5,964	107	
1a	K24R + Q30R	2,566	950	26,940	3,545	83	
1a	M28V + Q30R	115,567	42,803	252,267	33,193	17	
1a	M28V + Y93L	286,867	106,247	368,700	48,513	31	
1a	M28V + Y93C	30,455	11,280	84,120	11,068	ND	
1a	M28T + Q30R	9,656,500	3,576,481	10,000,000	1,315,789	32	EC90 >
1a	M28T + Y93C	1,725,850	639,204	2,000,000	263,158	22	EC90 >
1a	Q30L + Y93S	596	221	4,299	566	1	
1a	Q30L + Y93H	1,137	421	6,133	807	30	
1a	Q30R + L31M	1,377	510	7,735	1,018	49	
1a	Q30R + H58D	875,650	324,315	978,800	128,789	50	
1a	Q30R + Y93C	118,350	43,833	206,300	27,145	6	
1a	Q30R + Y93H	958,450	354,981	1,000,000	131,579	21	EC90 >
1a	L31M + Y93C	5,386	1,995	23,280	3,063	32	
1a	L31V + Y93H	88,710	32,856	220,600	29,026	20	
1a	M28T + Q30R + L31M	1,176,800	435,852	1,000,000	131,579	45	EC90 >

1a	Q30R + L31M + Y93C	52,905	19,594	115,635	15,215	7	
1a	Q30R + I269V	2,259	837	23,290	3,064	52	
1a	Y93N + I269V	168,950	62,574	341,250	44,901	27	
GT	**NS5A Substitution**	**pM EC_{50}**	**FC EC_{50}**	**pM EC_{90}**	**FC EC_{90}**	**% Replication Capacity**	**Comment**
1b	**WT (Con1)**	**0.8**	**1**	**1.6**	**1**	**100**	
1b	L28M	1	2	4	3	114	
1b	L28T	522	661	34,234	21,396	17	
1b	P29 deletion	285,300	361,139	440,666	275,416	6	
1b	R30Q	0.3	0.4	1	1	ND	
1b	L31F	8	10	23	14	127	
1b	L31M	1	1	1	1	119	
1b	L31V	7	8	29	18	86	
1b	P58S	1	1	2	1	80	
1b	P58T	0.3	0.4	1	1	ND	
1b	Y93H	60	76	254	159	73	
1b	L28M + Y93H	328	415	1,213	758	104	
1b	R30Q + Y93H	224	284	737	461	60	
1b	L31F + Y93H	8,115	10,272	63,695	39,809	35	
1b	L31M + Y93H	112	142	572	358	11	
1b	L31V + Y93H	9,739	12,328	46,766	29,229	24	
1b	P58S + Y93H	1,107	1,401	5,451	3,407	34	

FC, fold-change; ND, no data

APPENDIX C: Site-directed mutant phenotype data for DBV (ABT-333)

GT	NS5B Substitution	nM EC_{50}	FC EC_{50}	nM EC_{90}	FC EC_{90}	% Replication Capacity	Comment
1a	**WT (H77)**	**1.0**	**1**	**5.1**	**1**	**100**	
1a	S282T	1.0	1	7.2	1	12.7	
1a	G307R	0.9	1	4.9	1	45	
1a	C316Y	1,413	1,472	5,024	985	82	
1a	S368T	2.0	2	13	3	70	
1a	M414I	8.0	8	94	18	75	
1a	M414T	31	32	207	41	110	
1a	M414V	5.1	5	83	16	75	
1a	A421T	0.7	1	3.3	1	46	
1a	A421V	0.8	1	4.3	1	54	
1a	E446K	52	54	147	29	30	
1a	E446Q	17	18	99	19	55	
1a	Y448C	902	940	2,675	525	19	
1a	Y448H	936	975	2,996	587	41	
1a	A450V	2.9	3	18	4	75	
1a	C451R	ND	ND	ND	ND	<0.5	
1a	A553T	146	152	285	56	66	
1a	G554D	2.5	3	18	4	51	
1a	G554S	190	198	321	63	22	
1a	Y555H	ND	ND	ND	ND	<0.5	
1a	S556G	29	30	161	32	59	
1a	S556R	251	261	1,213	238	74	
1a	G557R	ND	ND	ND	ND	<0.5	
1a	G558R	ND	ND	ND	ND	<0.5	
1a	D559G	ND	ND	ND	ND	<0.5	
1a	D559N	ND	ND	ND	ND	<0.5	
1a	Y561H	20	21	46	9	31	
1a	Y561N	ND	ND	ND	ND	<0.5	

1a	C316Y + S556G	2,788	2,904	6,747	1,323	64	
1a	M414I + S556G	225	234	942	185	53	
1a	M414I + S556R	980	1,021	2,643	518	39	
1a	M414T + S556G	1,042	1,085	2,764	542	58	
1a	M414T + S556R	4,583	4,774	6,057	1,188	17	
1a	A450V + M414T	29	30	210	41	75	
GT	**NS5B Substitution**	**nM EC_{50}**	**FC EC_{50}**	**nM EC_{90}**	**FC EC_{90}**	**% Replication Capacity**	**Comment**
1b	**WT (Con1)**	**0.6**	**1**	**3.5**	**1**	**100**	
1b	Con1 wt	0.6	1	3.5	1	100	
1b	C316H	136	231	786	225	6.7	
1b	C316N	3.2	5	14	4	154	
1b	C316W	ND	ND	ND	ND	<0.5	
1b	C316Y	926	1,569	2,639	754	96	
1b	S368T	82	139	141	40	11	
1b	N411S	50	85	105	30	13	
1b	M414I	10	17	56	16	54	
1b	M414T	28	47	61	17	31	
1b	M414V	11	19	40	11	51	
1b	C445Y	ND	ND	ND	ND	<0.5	
1b	Y448C	244	414	567	162	7.2	
1b	Y448H	27	46	117	33	58	
1b	A553V	71	120	119	34	14	
1b	S556G	6.5	11	27	8	62	
1b	D559G	65	110	129	37	4.9	
1b	C316H + V321I	90	153	652	186	52	
1b	C316N + S556G	23	39	112	32	125	
1b	C316N + C451N + S556G	104	176	318	91	105	
1b	C316Y + M414I	1,544	2,617	3,387	968	43	
1b	C316Y + M414I + Y448H	2,223	3,768	4,440	1,269	4.5	

FC, fold-change; ND, no data

Additional phenotype data from Report R&D/08/212				
Report R&D/08/212				
GT	**NS5B Substitution**	**nM EC_{50}**	**FC EC_{50}**	**% Replication Capacity**
1b	**WT (N)**	**0.7**	**1**	**100**
1b	S96T	1.4	2	24
1b	S282T	1.3	2	4
1b	M414T	190	270	ND
1b	M423T	1.0	1	4
1b	Y448H	70	100	ND
1b	P495S	1.1	2	1
1b	P495A	0.6	1	19
1b	P496S	0.6	1	10
1b	V499A	1.1	2	21
Biochemical Assays				
GT	**NS5B Substitution**	**nM IC_{50}**	**FC IC_{50}**	**% Replication Capacity**
1a	**WT (H77)**	**2.8**	**1**	**n/a**
1a	C316H	210	75	n/a
1a	C316Y	1100	390	n/a
1a	M414T	9.3	3	n/a
1a	P495L	2.6	1	n/a
GT	**NS5B Substitution**	**nM IC_{50}**	**FC IC_{50}**	**% Replication Capacity**
1b	**WT (BK)**	**3.1**	**1**	**n/a**
1b	V201A	18,200	5870	n/a

1b	N316H	200	65	n/a
1b	N316Y	1860	600	n/a
1b	S365L	85	27	n/a
1b	S368T	13	4	n/a
1b	M414T	15.9	5	n/a
1b	M423T	6.8	2	n/a
1b	Y448H	34	11	n/a
1b	P495A	5.1	2	n/a
1b	A553T	14	4	n/a
1b	G554D	10.9	4	n/a
1b	S556N	8.7	3	n/a
1b	D559G	8.4	3	n/a

FC, fold-change; ND, no data

APPENDIX D: Communications with sponsor during NDA review

Appendix D1. Clinical Virology request communicated on 5/12/2014 (in bold type)

For the integrated resistance analysis and summary in the prescribing information, it is unclear to DAVP how you calculated a total of (b) (4) HCV genotype 1 infected subjects in Phase 2b/3 trials who were treated with the 3-DAA +/- RBV regimen for 8, 12 or 24 weeks. Our calculations are illustrated below. Please provide a similar table that shows the breakdown of trials and regimens that make up your total of (b) (4) subjects.

Trial	Phase of trial	N (3-DAA +/- RBV)	N (3-DAA)	N (3-DAA + RBV)	N placebo
M11-646	Phase 3	473	0	473	158
M13-098	Phase 3	297	0	297	97
M13-099	Phase 3	380	0	380	
M13-961	Phase 3	419	209	210	
M13-389	Phase 3	186	95	91	
M14-002	Phase 3	305	205	100	
M11-652	Phase 2b	406	79	327	
M14-103	Phase 2b	38	0	38	
Total		2504	588	1916	
Total according to AbbVie				(b) (4)	

Sponsor's response in SDN 008 (in normal type)

(b) (4)

For Study M13-389, the 179 subjects in the GT1b Efficacy subset were included (see Table 2.6__4 of the ISE), along with 1 subject with unknown genotype. This subject (Subject 10171) was not included in the ISE as his genotype/subgenotype could not be provided by the central lab; however, was confirmed to be genotype 1b by BLAST and phylogenetic analysis and used as such in the resistance datasets. The 6 who were not included in the GT1b efficacy subset for Study M13-389 (and thus not considered ITT for the ISE) were Subjects 10003, 10005, and 10007 with HCV GT1a infection and Subjects 10010, 10014, 10015 with GT1b infection who were enrolled before Protocol Amendment No. 5; all 6 were not administered ABT-450/r/ABT-267 coformulated drug. AbbVie's calculations are illustrated below.

Trial	Phase of trial	N (3-DAA +/- RBV)	N (3-DAA)	N (3-DAA + RBV)	N placebo
M11-646	Phase 3	473	0	473	158
M13-098	Phase 3	297	0	297	97
M13-099	Phase 3	380	0	380	
M13-961	Phase 3	419	209	210	
M13-389	Phase 3	~~186~~ 180	~~95~~ 92	~~91~~ 88	
M14-002	Phase 3	305	205	100	
M11-652	Phase 2b	406	79	327	
M14-103	Phase 2b	38	0	38	
(b) (4)					
Total		**2504**	**588**	**1916**	
Total according to AbbVie		(b) (4)			

Reviewer's Comments (to be communicated with label edits)
This reviewer disagrees (b) (4)

This reviewer agrees with the sponsor's calculations for M13-389 excluding the 6 subjects noted above, although arguably the 3 genotype 1b subjects (or even all 6 subjects) enrolled in the "Phase 2" stage of the trial could be included in the totals. Subject 10171, who did not have a genotype/subtype result determined by the central laboratory, received the 3-DAA regimen without RBV. Below is an updated table:

Trial	Type of trial	N (3-DAA +/- RBV)	N (3-DAA)	N (3-DAA + RBV)	N placebo	Comments
M11-646	Phase 3	473	0	473	158	
M13-098	Phase 3	297	0	297	97	
M13-099	Phase 3	380	0	380		
M13-961	Phase 3	419	209	210		
M13-389	Phase 3	180	92	88		Total excludes 6 subjects who enrolled during "Phase 2" version, but includes 1 subject who did not have a subtype (all achieved SVR)
M14-002	Phase 3	305	205	100		Total includes 1 subject who was GT1b and censored from my efficacy analysis
M11-652	Phase 2b	406	79	327		
M14-103	Phase 2b	38	0	38		
Total		2498	585	1913		
Total from AbbVie		(b) (4)				
(b) (4)						

Appendix D2. Clinical Virology requests communicated on 07/17/2014 (in bold type), and sponsor's responses in SDN 021 (in normal type)

1. **Please provide additional information about the clonal sequencing methods for the Phase 2 trials that were used for analyses of persistence of resistance-associated substitutions. What were the median and range for the numbers of clones analyzed per sample for each drug target for subjects with available follow-up data? Also, your analyses of clonal sequencing data considered only substitutions that were detected in at least 2 clones from a given sample. For the clonal sequencing datasets, were substitution results from single clones reported, or was detection in at least 2 clones required to report a substitution for a given sample?**

   Report R&D/13/948, submitted in Module 5, Section 5.3.5.4 of the NDA submission, provides a full description the clonal sequencing assay methods and characteristics in detecting variants in NS3, NS5A and NS5B.

   The Phase 2 studies used for analyses of persistence of resistance-associated substitutions were Studies M12-998, M12-746 and M11-652. In the Phase 2 Study M13-386, all subjects achieved SVR so there were no follow-up data. For Study M11-652, clonal sequencing was performed only if variants at resistance-associated amino acid positions were not detected by population sequencing, whereas clonal sequencing was performed on follow-up samples from each subject in Studies M12-998 and M12-746. The attached Table 1_1 indicates the median and range of the numbers of clones analyzed by target for the set of samples in each time window (baseline, time of failure if before Post-Treatment Week 24, Post-Treatment Week 24 and Post-Treatment Week 48), integrated across these 3 Phase 2 studies.

TABLE 1__1

MEDIAN AND RANGE FOR THE NUMBERS OF CLONES ANALYZED PER SAMPLE FOR EACH DRUG TARGET FOR SUBJECTS WITH AVAILABLE DATA

TARGET	VISIT WINDOW	NUMBER OF SAMPLES	MEDIAN	MIN	MAX	RANGE
NS3						
	BASELINE	59	87	72	94	22
	PRIOR TO POST-TREATMENT WEEK 24	52	87.5	60	94	34
	POST-TREATMENT WEEK 24	25	84	68	92	24
	POST-TREATMENT WEEK 48	13	92	79	95	16
NS5A						
	BASELINE	60	77	54	94	40
	PRIOR TO POST-TREATMENT WEEK 24	39	80	52	94	42
	POST-TREATMENT WEEK 24	5	87	81	89	8
	POST-TREATMENT WEEK 48	4	90	88	91	3
NS5B						
	BASELINE	39	86	62	95	33
	PRIOR TO POST-TREATMENT WEEK 24	35	86	66	94	28
	POST-TREATMENT WEEK 24	15	88	54	93	39
	POST-TREATMENT WEEK 48	6	83.5	63	94	31

   The clonal sequencing datasets from the Phase 2 studies noted above include substitutions present in at least 2 clones. The details are included in Section 5.2 of the Analysis Datasets – Reviewers Guide (Module 1, Section 1.2). A list of the tables that include all variants relative to baseline seen in ≥ 1 clone can be found in the CSR for each of these individual Phase 2 studies.

   Reviewer's comments:
   In the referenced study report the sponsor claimed the following regarding assay sensitivity: "Based on an assumption of uniform amplification of each RNA species and subsequent sequencing of 75 clones per sample, there is a 95% statistical probability that an individual variant present at a prevalence of 6.5% will be detected in at least 2 clones in a single determination." For the purposes of this review, because a range of clones was analyzed per sample, this reviewer considers the assay to be capable of detecting minority variants at a prevalence of approximately 5-10%, assuming uniform sampling and amplification of each RNA species.

2. **Please confirm that the FDA-approved Roche COBAS® TaqMan® HCV v2.0 test was used for HCV RNA assessments in Phase 3 trials.**

AbbVie confirms the FDA-approved Roche COBAS® TaqMan® HCV v2.0 test was used for HCV RNA assessments in Phase 3 trials.

Appendix D3. AbbVie 8/22/2014 labeling-related questions, and DAVP Clinical Virology Responses communicated 8/26/2014

AbbVie Question 1:
(A) Could the agency provide further clarification on the amino acid positions within NS5A that were included in the analyses in section "*Effect of baseline HCV polymorphisms on treatment response*"?

(b) (4)

(B) Could the agency provide their numerical analysis, if different from the above table, leading to the statement below in the USPI?

"*Ombitasvir resistance-associated polymorphisms in NS5A (pooling data from all resistance-associated amino acid positions) were detected in approximately* (b) (4) *% of subjects in this analysis and similarly enriched approximately 2-fold in virologic failure subjects.*"

DAVP RESPONSE TO QUESTION 1

We believe the discrepancies are based on the specific NS5A polymorphisms considered in these analyses. The specific polymorphisms considered by DAVP were based on the substitutions that emerged in Phase 2/3 trials of the 3-DAA +/- RBV regimens: K24R, M28A/T/V, Q30E/K/R, H54Y, H58D/P, E62D, Y93C/H/N. This list is based on all available treatment-emergent resistance data from all subjects who did not achieve SVR, including genotype 1a and 1b subjects. From this list, the following NS5A polymorphisms were detected in the 141 subjects included in the analysis (censoring subjects who failed for non-virologic reasons): K24R (n=3), M28T/V (n=11), Q30E/R (n=3), H54Y (n=3), H58D/P (n=11), E62D (n=9), Y93C/H/N (n=6). A total of 38/141 (27%) subjects in the Phase 3

analysis population had one or more of these baseline polymorphisms; we apologize that the "approximately (b)(4)%" quoted in the label was a rounding error.

This "FDA List" of NS5A resistance-associated polymorphisms/substitutions does not include NS5A position L31. Although L31 is a key resistance-associated position for other NS5A inhibitors, there was no evidence from our treatment-emergent resistance analyses that this position plays a role in ombitasvir resistance, as no L31 substitutions appeared to emerge in any subjects. Three HCV genotype 1a subjects with Baseline L31M achieved SVR.

The H54Y polymorphism was included in our list as this position has been described as being associated with NS5A inhibitor resistance, and H54Y emerged in a single HCV genotype 1b subject who experienced virologic failure. Nevertheless, DAVP acknowledges that H54Y is unlikely to play a major role in ombitasvir resistance, particularly in HCV genotype 1a, and all 3 subjects with Baseline H54Y achieved SVR in this analysis.

The E62D polymorphism was included in our list as this position has also been described as being associated with NS5A inhibitor resistance, and E62D emerged in a single HCV genotype 1a subject who experienced virologic failure; this subject failed treatment with the 8-week regimen in M11-652. (b) (4)

Table 1 below summarizes our re-analysis considering the "FDA List," "AbbVie List," and the "FDA List without H54Y/E62D." As shown in the table, we were able to reproduce your analysis. Furthermore, even if excluding H54Y and E62D there is still a 1.6-fold enrichment of NS5A polymorphisms in the virologic failure population.

Table 1. FDA re-analysis of baseline polymorphism prevalence in subjects who experienced virologic failure versus those who achieved SVR in Phase 3 trials of 3-DAA +/- RBV regimen.

	Any NS5A PM (FDA List)		Any NS5A PM (AbbVie List)		Any NS5A PM (FDA List w/o 54/62)	
EFFICFL	**#**	**%**	**#**	**%**	**#**	**%**
N	18	38.3%	(b) (4)		14	29.8%
Y	20	21.3%			17	18.1%
Fold-enrichment in VFs		1.8				1.6

Despite the small number of subjects with any single specific NS5A polymorphism, DAVP believes that the overall trend observed across each position further strengthens the observation that NS5A resistance-associated polymorphisms were enriched in virologic failures. Figure 1 illustrates the prevalence of specific NS5A polymorphisms in the SVR and virologic failure groups. Polymorphisms at NS5A positions 28, 30 and 93 were clearly enriched in the virologic failure group, which is consistent with their strong association with ombitasvir resistance based on treatment-emergent resistance patterns and site-directed mutant phenotype analyses. It is our understanding that the impact of E62D on ombitasvir anti-HCV activity (alone or in combination with other NS5A substitutions) has not been evaluated in a phenotype assay.

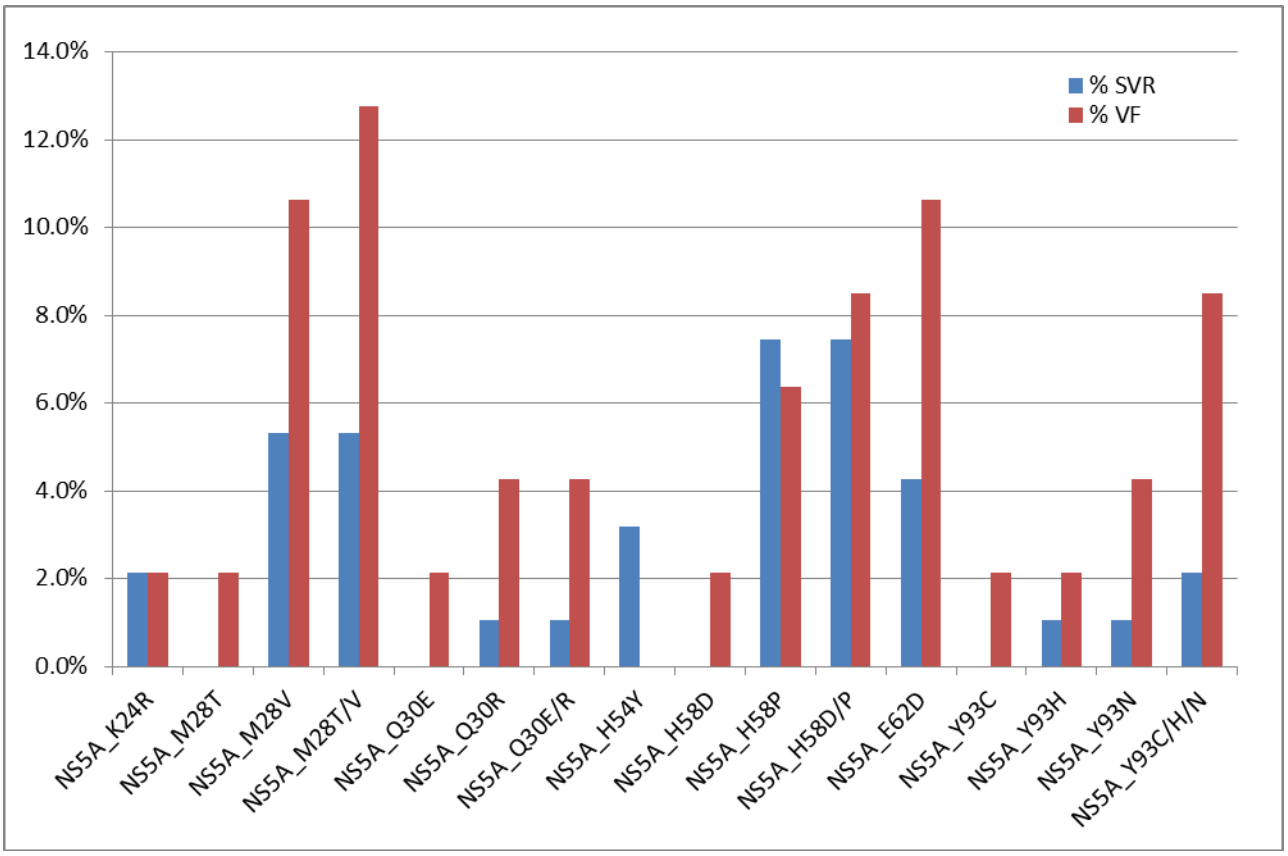


Figure 1. Proportion of SVR and virologic failure (VF) subjects with specific NS5A polymorphisms.

The statement about the lack of an association in the Phase 2b trial comes from an analysis of SVR rates for subjects in M11-652 with or without any NS5A polymorphisms of interest (Table 2). In this analysis L31 polymorphisms are also included for completeness, although only 2 subjects had L31 polymorphisms, both from the 8-week duration arm in M11-652.

Table 2. SVR rates (non-VF-censored) for HCV genotype 1a subjects with or without Baseline NS5A polymorphisms in M11-652.

NS5A Polymorphism	Overall SVR 3-DAA ± RBV 8-24 Weeks	Overall SVR 3-DAA ± RBV 12-24 Weeks
K24R	4/4 (100.0%)	2/2 (100.0%)
M28T	1/1 (100.0%)	1/1 (100.0%)
M28V	11/13 (84.6%)	9/10 (90.0%)
M28T/V	12/14 (85.7%)	10/11 (90.9%)
Q30R	3/3 (100.0%)	3/3 (100.0%)
L31M	0/1 (0.0%)	n/a
L31V	1/1 (100.0%)	n/a
L31M/V	1/2 (50.0%)	n/a
H54Y	9/9 (100.0%)	7/7 (100.0%)
H58P	3/3 (100.0%)	2/2 (100.0%)
H58R	3/3 (100.0%)	3/3 (100.0%)

H58P/R	6/6 (100.0%)	5/5 (100.0%)
E62D	5/5 (100.0%)	4/4 (100.0%)
Y93C	1/1 (100.0%)	1/1 (100.0%)
Y93H	3/3 (100.0%)	n/a
Y93N	0/1 (0.0%)	0/1 (0.0%)
Y93C/H/N	4/5 (80.0%)	1/2 (50.0%)
NS5A any (from above)	39/43 (90.7%)	28/30 (93.3%)
No NS5A Polymorphism	165/181 (91.2%)	140/149 (94.0%)

For clarity in labeling, DAVP proposes that the specific polymorphisms considered in the Phase 3 analyses be listed: "Ombitasvir resistance-associated polymorphisms in NS5A (~~pooling data from all resistance associated amino acid positions~~ (b) (4) were detected in approximately (b) (4) %…"

AbbVie Question 2:
We are in general agreement with the agency's edits to the section "*Persistence of resistance-associated substitutions*", but request additional clarification on how the analysis time windows and the total number of subjects at the follow-up time points were calculated to aid in our validation process.

(b) (4)

(b) (4)

[redacted]

DAVP RESPONSE TO QUESTION 2

Analyses of Phase 2 trials of 2- or 3-DAA +/- RBV combination regimens were conducted to evaluate the persistence of resistance-associated substitutions in NS3, NS5A and NS5B, as limited long-term follow-up data are available from Phase 3 trials. Analyses were conducted only for subjects with available data through Post-Treatment Week 24 or later. Because of the low virologic failure rate in HCV genotype 1b subjects these analyses were conducted only for HCV genotype 1a subjects. All treatment-emergent resistance-associated positions/substitutions identified in the analyses of the 3-DAA +/- RBV regimens were monitored for persistence after treatment, specifically:

- **NS3** V36A/M/T, F43L, V55I, Y56H, Q80L, I132V, R155K, A156G, D168x, P334S, S342P, E357K, V406A/I, T449I and P470S (note that V55A was also considered as it emerged in 2 subjects in this analysis population)
- **NS5A** K24R, M28A/T/V, Q30E/K/R, H58D/P/R, E62D and Y93C/N
- **NS5B** G307R, C316Y, M414T, E446K/Q, A450V, A553T, G554S, S556G/R, G558R, D559G/I/N/V, Y561H and L588F (note that A450T was also considered as it emerged in 1 subject in this analysis population)

Both population and clonal nucleotide sequencing data were considered for the NS3 and NS5B persistence analyses. For analyses of NS5A, only population nucleotide sequencing data were analyzed because limited clonal data were provided and persistence of resistance-associated substitutions was clearly observed based on the population nucleotide sequencing assay. The timepoints analyzed were based on the visits indicated in the resistance datasets; visit windows were

not adjusted except that the visits after Post-Treatment Week 48 were considered in the analyses of persistence through Post-Treatment Week ≥48.

Tables 3-5 below include the data for all subjects considered in these analyses. Each subject was analyzed individually, and in some cases the clonal sequence data were not considered subjectively based on the timing and frequency of detection (see 'Additional Notes' in tables).

For the descriptive summaries associated with the tables (which were included in the recommended label edits), when a subject had no sequence data at Post-Treatment Week 24, substitutions that were detected at Post-Treatment Week ≥48 were considered detectable at Post-Treatment Week 24 and imputed accordingly. Conversely, when a subject had no data at Post-Treatment Week ≥48, if no treatment-emergent substitutions were detected through Post-Treatment Week 24 it was assumed that no substitutions would have been detected at Post-Treatment Week ≥48. Also, if no data were available for Post-Treatment Week 24 and the subject had no emergent substitutions detected at Post-Treatment Week ≥48, the subject was considered "missing" from the Post-Treatment Week 24 analysis and therefore not included in the denominator.

Persistence of NS3 resistance-associated substitutions

Table 3 summarizes the persistence of ABT-450 treatment-emergent substitutions for 32 subjects with ≥1 treatment-emergent substitution in NS3 (from list indicated above) and at least 24 weeks of post-treatment follow-up. The following conclusions can be drawn from these data:

- 17/29 (59%) subjects still had at least 1 treatment-emergent NS3 substitution detected through at least Post-Treatment Week 24, and 5/22 (23%) through at least Post-Treatment Week 48.
- Treatment-emergent R155K remained detected in 5/8 (63%) subjects through Post-Treatment Week 24, and in 1/5 (20%) subjects through Post-Treatment Week 48.
- Treatment-emergent D168 substitutions, which are most commonly observed in ABT-450-based treatment failure subjects, remained detected in 6/22 (27%) subjects through Post-Treatment Week 24, and in 0/22 (0%) subjects through Post-Treatment Week 48.

Table 3. Persistence of treatment-emergent substitutions in NS3. Note that both population and clonal nucleotide sequence analysis data were considered for these analyses, although clonal analyses only covered NS3 amino acids 1-181. ND, no data; PTW, Post-Treatment Week. Red cells indicate "positive" detection of substitutions and yellow cells indicate "negative" detection of substitutions (imputed or observed).

USUBJID	Last Visit w/Data	Tx-emergent Substitutions (Pop. Seq., except where noted*)	Detected @PTW24	Detected @PTW48+	Additional Notes about Analyses
M11652-12538-5377	PTW 24	R155K, D168V	none	ND	R155G detected only by clonal seq. (6% of clones) at PTW24 but not earlier, not considered in analyses
M11652-12649-5316	PTW 24	R155K, P334S	R155K	ND	
M11652-22271-5181	PTW 24	V36A, Y56H, D168A/V, P334S	Y56H, D168A	ND	
M11652-36975-5308	PTW 48	D168V, S342P	D168V, S342P	none	
M11652-37388-5593	PTW 48	V406A	ND	V406A	
M11652-37868-5413	PTW 48	D168V	D168V	none	D168V at PTW24 by clonal seq.
M11652-37868-8028	PTW 48	Y56H, D168V, E357K	ND	none	
M11652-37869-8071	PTW 24	D168V	none	ND	
M11652-38624-5256	PTW 24	D168V	none	ND	
M11652-38624-8108	PTW 24	D168F/V/Y, S342P, T449I	S342P	ND	
M11652-38627-5687	PTW 59	D168V	ND	none	
M11652-38646-5751	PTW 24	D168A	none	ND	
M11652-38853-5309	PTW 24	D168V	none	ND	
M11652-40076-5424	PTW 24	Y56H, D168V, E357K	E357K	ND	
M11652-40526-5297	PTW 48	R155K, A156G	R155K	R155K	
M11652-40784-8118	PTW 24	A156V, D168V	none	ND	
M11652-42362-5144	PTW 24	Y56H, D168Y	none	ND	
M11652-42364-8215	PTW 24	V36M, I132V, R155K	I132V, R155K	ND	
M11652-42368-5174	PTW 24	D168V, T449I	T449I	ND	
M11652-42371-5525	PTW 24	D168V	none	ND	
M11652-42420-8192	PTW 24	I132V, D168Y, P334S, P470S	I132V, P334S, P470S	ND	
M11652-42808-5435	PTW 48	D168V	ND	none	V36A detected only by clonal seq. (3% of clones) at PTW48 and not earlier, not considered in analyses.
M11652-42808-8035	PTW 24	V36A/M/T, R155K	R155K	ND	
M11652-44319-8246	PTW 48	V55A, D168V	V55A, D168V	V55A	V55A considered tx-emergent
M11652-44367-8224	PTW 24	D168V, E357K	none	ND	
M11652-44372-5610	PTW 24	D168V*	D168V	ND	D168V detected only by clonal seq. (2% of clones) only at PTW24
M12746-22854-2146	PTW 48	V36M, Y56H, R155K*, D168V	V36M, R155K	V36M	R155K detected only by clonal seq. (3% of clones) at PTW24, V36M detected at PTW48 only by clonal seq.
M12746-37869-2095	PTW 48	Q80L	Q80L	Q80L	Q80L detected at Baseline only by clonal analyses in 7% of clones, considered treatment-emergent (enriched to 100% of clones)
M12746-37869-2096	PTW 48	V55A, I132V, D168V	none	none	V55A considered tx-emergent
M12746-38934-2187	PTW 48	R155K*	none	none	R155K detected only by clonal seq. (2% of clones) at two different timepoints
M12746-40537-2507	PTW 48	V36M, Y56H, R155K, A156G, D168A	none	none	
M12998-37869-3094	PTW 24	Y56H, D168A	Y56H, D168A	ND	R155S detected only by clonal seq. (4% of clones) at PTW24 and was not considered in this analysis

Persistence of NS5A resistance-associated substitutions

Table 4 summarizes the persistence of ombitasvir treatment-emergent substitutions for 24 subjects with ≥1 treatment-emergent substitution in NS5A (from list indicated above) and at least 24 weeks of post-treatment follow-up. All 24 subjects (100%) had treatment-emergent NS5A substitutions detected through at least Post-Treatment Week 24, and 18/18 (100%) subjects with available data had treatment-emergent substitutions detected through at least Post-Treatment Week 48, clearly indicating the long-term persistence of resistance-associated substitutions in NS5A.

Table 4. Persistence of treatment-emergent substitutions in NS5A. Only population sequence analysis data were considered for these analyses. ND, no data; PTW, Post-Treatment Week. Red cells indicate "positive" detection of substitutions (imputed or observed).

USUBJID	Last Visit w/Data	Tx-emergent Substitutions	Detected @PTW24	Detected @PTW48+
M11652-02965-5213	PTW 48	M28T, H58P, E62D	M28T, H58P, E62D	M28T, H58P, E62D
M11652-12649-5316	PTW 24	M28A/T, Q30R	M28A/T, Q30R	ND
M11652-36975-5308	PTW 48	K24R, Q30R	Q30R	K24R, Q30R
M11652-37868-5413	PTW 48	Q30R	Q30R	Q30R
M11652-37869-8071	PTW 48	Q30R	Q30R	Q30R
M11652-38624-8108	PTW 48	M28A/T/V, Q30R	Q30R	M28A/T/V, Q30R
M11652-38627-5687	PTW 48	Q30R	ND	Q30R
M11652-38646-5751	PTW 48	M28T, Q30R	M28T	M28T
M11652-38853-5309	PTW 24	Q30R	Q30R	ND
M11652-40784-8118	PTW 24	Q24R, M28T	Q24R, M28T	ND
M11652-42361-5765	PTW 48	M28V	ND	M28V
M11652-42362-5144	PTW 48	M28V, Q30K	M28V, Q30K	M28V, Q30K
M11652-42364-8215	PTW 24	M28T	M28T	ND
M11652-42368-5174	PTW 48	Y93N	Y93N	Y93N
M11652-42371-5525	PTW 48	M28V	M28V	M28V
M11652-42420-8192	PTW 48	Q30R	Q30R	Q30R
M11652-42808-5435	PTW 48	M28T	ND	M28T
M11652-42808-8035	PTW 24	Q30R	Q30R	ND
M11652-43114-5554	PTW 48	H58D	H58D	H58D
M11652-44319-8246	PTW 48	Q30R	Q30R	Q30R
M11652-44367-8224	PTW 59	Q30R	Q30R	Q30R
M11652-44372-5610	PTW 48	Q30R	Q30R	Q30R
M12998-31542-3049	PTW 48	Q30R	ND	Q30R
M12998-37869-3094	PTW 24	Q30R	Q30R	ND

Persistence of NS5B resistance-associated substitutions

Table 5 summarizes the persistence of dasabuvir (or possibly RBV) treatment-emergent substitutions for 16 subjects with ≥1 treatment-emergent substitution in NS5B (from list indicated above) and at least 24 weeks of post-treatment follow-up. The following conclusions can be drawn from these data:

- 11/16 (69%) subjects still had at least 1 treatment-emergent NS5B substitution detected through at least Post-Treatment Week 24, and 8/15 (53%) [Note: in our label edits we stated (b) (4) in error, please correct when you resubmit the updated label] through at least Post-Treatment Week 48.
- S556G, which was the most commonly observed dasabuvir resistance-associated substitution, remained detected in 8/9 (89%) subjects through at least Post-Treatment Week 24, 6/9 (67%) through at least Post-Treatment Week 48.

Table 5. Persistence of treatment-emergent substitutions in NS5B. Note that both population and clonal nucleotide sequence analysis data were considered for these analyses. ND, no data; PTW, Post-Treatment Week. Red cells indicate "positive" detection of substitutions and yellow cells indicate "negative" detection of substitutions (imputed or observed).

USUBJID	Last Visit w/Data	Tx-emergent Substitutions (Pop. Seq.,except where noted*)	Detected @PTW24	Detected @PTW48+	Additional Notes about Analyses
M11652-12649-5316	PTW 24	G554S, S556G	none	ND	
M11652-36975-5308	PTW 48	S556G	S556G	none	S556G detected in 5% of clones at BL, considered tx-emergent (enriched to 100% of clones)
M11652-38624-5256	PTW 48	M414T	M414T	M414T	
M11652-38627-5687	PTW 59	S556G	ND	S556G	
M11652-40526-5297	PTW 24	G307R*, D559N	G307R	ND	G307R detected only by clonal seq. (5% of clones) only at PTW24
M11652-42362-5144	PTW 48	S556G	S556G	S556G	
M11652-42364-8215	PTW 24	D559G	none	ND	
M11652-42368-5174	PTW 48	M414T, S556G	S556G	S556G	L588F/H/Y detected only at PTW48 and not at earlier follow-up timepoints, and was not considered in this analysis
M11652-42420-8192	PTW 48	G307R, S556G	G307R, S556G	G307R, S556G	
M11652-42808-8035	PTW 24	G307R*, G558R	none	ND	G307R detected only by clonal seq. (2% of clones) during treatment
M11652-44367-8224	PTW 24	G307R*, A553T	none	ND	G307R detected only by clonal seq. (5% of clones in 2 samples)
M11652-44372-5610	PTW 48	S556G	S556G	S556G	
M12746-22854-2146	PTW 48	S556G	S556G	S556G	
M12746-37869-2095	PTW 48	A450T	A450T	A450T	A450T was not a treatment-emergent substitution in pooled analyses of the 3-DAA +/- RBV regimens, but A450V was; A450T considered tx-emergent.
M12746-37869-2096	PTW 48	C316Y, A553D, G554S*, S556G, G558R, D559G/N*	S556G	none	A553D was not a treatment-emergent substitution in pooled analyses of the 3-DAA +/- RBV regimens, but A553T was; A553D considered tx-emergent. G554S and D559G/N detected only by clonal sequencing on-treatment (2-7% of clones)
M12746-40537-2507	PTW 48	M414T*, G554S, G558R*	none	none	M414T and G558R detected only by clonal seq. on-treatment (3% of clones)

Appendix D4. Clinical Virology request communicated on 8/27/2014 (in bold type), and sponsor's response in SDN 033 (in normal type)

Please confirm [redacted] (b) (4)
[redacted] **Also, please indicate** [redacted] (b) (4)

AbbVie confirms [redacted] (b) (4) For reference, the information is located in Appendix 16.2_5.3 of the clinical study reports. [redacted] (b) (4)

Appendix D5. Clinical Virology request communicated on 9/11/2014 (in bold type), and sponsor's response in SDN 034 (in normal type)

The 4-Month Safety Update Report included a summary of post-SVR12 relapse rates, with relapse defined as confirmed HCV RNA ≥LLOQ. Please comment if there were any additional subjects in this analysis who were not counted as relapsers but had HCV RNA ≥LLOQ during follow-up that had not yet been confirmed (e.g., lost to follow-up or pending confirmation). Please provide USUBJIDs for these subjects.

Relapse was defined using confirmed HCV RNA ≥ LLOQ post treatment. However, if the last available post-treatment value is ≥ LLOQ, then the subject will be considered a relapse (i.e., will not require confirmation). This is described in the ISE SAP Section 9.1 as follows: "If the last available post-treatment value is ≥ LLOQ, then the subject will be considered a relapse (i.e., will not require confirmation)."

If any subject had an unconfirmed increase in HCV RNA as their final value, they were counted as a relapse. Therefore, there were no subjects who were not counted as relapsers but had HCV RNA ≥ LLOQ during follow-up that had not yet been confirmed (e.g., lost to follow-up or pending confirmation) in the 4-Month Safety Update.

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/s/

PATRICK R HARRINGTON
09/18/2014

JULIAN J O REAR
09/18/2014

VIROLOGY FILING CHECKLIST FOR NDA or Supplement

NDA Number: 206619 **Applicant:** AbbVie **Stamp Date:** 04/21/2014

Drug Names: ABT-450/ritonavir/ABT-267/ABT-333 **NDA Type:** Original

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

	Content Parameter	Yes	No	Comments
1	Is the virology information (nonclinical and clinical) provided and described in different sections of the NDA organized in a manner to allow substantive review to begin?	X		
2	Is the virology information (nonclinical and clinical) indexed, paginated and/or linked in a manner to allow substantive review to begin?	X		
3	Is the virology information (nonclinical and clinical) legible so that substantive review can begin?	X		
4	On its face, has the applicant submitted cell culture data in necessary quantity, using necessary clinical and non-clinical strains/isolates, and using necessary numbers of approved current divisional standard of approvability of the submitted draft labeling?	X		
5	Has the applicant submitted any required animal model studies necessary for approvability of the product based on the submitted draft labeling?		X	Not applicable
6	Has the applicant submitted all special/critical studies/data requested by the Division during pre-submission discussions?	X		
7	Has the applicant submitted the clinical virology datasets in the appropriate format as described in the relevant guidance documents and are the datasets complete?	X		
8	Has the applicant used standardized or nonstandardized methods for virologic outcome measures? If nonstandardized methods were used, has the applicant included complete details of the method, the name of the laboratory where actual testing was done and performance characteristics of the assay in the laboratory where the actual testing was done?	X		Following published HCV resistance data draft guidance
9	Has the applicant submitted draft labeling consistent with current regulation, divisional and Center policy, and the design of the development package?	X		
10	Has the applicant submitted annotated microbiology draft labeling consistent with current divisional policy, and the design of the development package?	X		
11	Have all the study reports, published articles, and other	X		

VIROLOGY FILING CHECKLIST FOR NDA or Supplement

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

IS THE MICROBIOLOGY SECTION OF THE APPLICATION FILEABLE? __yes___

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

N/A

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

None at this time.

Reviewing Microbiologist Date

Microbiology Team Leader Date

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/s/

PATRICK R HARRINGTON
05/22/2014

JULIAN J O REAR
05/22/2014

M E M O R A N D U M

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: 06 May 2014

TO: NDA 206619

FROM: Erika Pfeiler, Ph.D.
Microbiologist
CDER/OPS/NDMS

THROUGH: Stephen Langille, Ph.D.
Senior Review Microbiologist
CDER/OPS/NDMS

cc: Katherine Schumann
Senior Regulatory Health Project Manager

SUBJECT: Product Quality Microbiology assessment of Microbial Limits for Ombitasvir/ABT-450/Ritonavir and Dasabuvir Film Coated Tablets [Submission Date: 17 March 2014]

NDA 206619 does not include a microbial limits release specification for drug product release; however, the applicant provides a suitable rationale for the exclusion of this testing. Therefore, this submission is recommended for approval from the standpoint of product quality microbiology.

The drug product consists of two copackaged film-coated tablets, an Ombitasvir/ABT450/Ritonavir tablet and a Dasabuvir tablet. The product is intended for oral administration.

The Ombitasvir/ABT450/Ritonavir tablet is produced [redacted] (b) (4) This tablet has demonstrated a [redacted] (b) (4) total aerobic microbial counts and total yeast and mold counts that are consistent with acceptance criteria listed in USP <1111> (Microbiological Examination of Non-sterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use). (Tests for specified microorganisms were not performed as a part of these studies.)

The Dasabuvir tablet is produced [redacted] (b) (4) This tablet has demonstrated a [redacted] (b) (4) total aerobic microbial counts and total yeast and mold counts

that are consistent with acceptance criteria listed in USP <1111>. (Tests for specified microorganisms were not performed as a part of these studies.)

The applicant does not propose to perform microbial enumeration testing for product release; however, to support the microbiological quality of the drug product, the applicant proposes performing microbial enumeration tests/tests for *Escherichia coli* on 20 batches of drug product in order to establish historic data. After 20 batches, this testing will be discontinued, but will be performed as a part of the stability program with testing performed at the initial and final time points.

The Microbial Limits test methods were verified to be appropriate for use with both tablets following procedures consistent with those in USP Chapter <61> and <62>.

ADEQUATE

Reviewer Comments – The microbiological quality of the drug product is controlled via a suitable testing protocol.
The manufacturing process and microbiological data submitted would be suitable to support an outright waiver of microbial limits testing; however the applicant wishes to gather more data from batches manufactured for commercial use. Since the applicant's proposal to perform microbial limits testing on the first 20 commercial batches is independent of the release specification, a post-approval manufacturing supplement is not necessary for this change.

END

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/s/

ERIKA A PFEILER
05/06/2014

STEPHEN E LANGILLE
05/06/2014