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APPLICATION NUMBER:

206843Orig1s000

MICROBIOLOGY / VIROLOGY REVIEW(S)

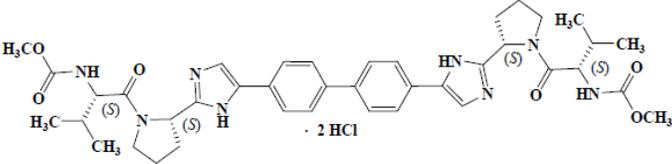
**DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
CLINICAL VIROLOGY REVIEW**

NDA: 206843 SDN: 036 (Original NDA resubmission) REVIEW COMPLETED: 06/26/2015
Clinical Virology Reviewers: Patrick R. Harrington, Ph.D. and Eric Donaldson, Ph.D.

NDA#: 206843 **SDN:** 036 (NDA resubmission)
Reviewer's Names: Patrick R. Harrington, Ph.D.
Eric Donaldson, Ph.D.

Sponsor's Name and Address: Bristol-Myers Squibb Company
5 Research Parkway
Wallingford, CT 06492
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Director, Global Regulatory, Safety and Biometrics-US

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Correspondence Date: 2/13/2015
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Assigned Date: 2/18/2015
Review Complete Date: 06/26/2015
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NDA #	<u>206843</u>
Proprietary Name	DAKLINZA™
Drug Name	daclatasvir (DCV, BMS-790052)
Drug Class	NS5A inhibitor
Associated IND #s	<u>79599</u> , (b) (4)
Chemical Name	Methyl((1S)-1-(((2S)-2-(5-(4'-(2-((2S)-1-((2S)-2-((methoxycarbonyl) amino)-3-methylbutanoyl)-2-pyrrolidinyl)-1H-imidazol-5-yl)-4-biphenyl)-1H-imidazol-2-yl)-1-pyrrolidinyl)carbonyl)-2-methylpropyl)carbamate dihydrochloride
Structure	 <p align="center">daclatasvir</p>
Molecular Formula	C ₄₀ H ₅₀ N ₈ O ₆ · 2HCl
Molecular Weight	811.80 (dihydrochloride), 738.88 (free base)

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Amendments: none

Related/Supporting Documents:

- Clinical Virology Reviews of Original NDAs 206843 and 206844
- Additional NDA 206843 resubmission SDNs/eCTDs (thru 06/26/2015): 37/0035 (correspondence and additional resistance data), 38/0036 (antiviral activity data), 39/0037 (updated resistance datasets), 40/0038 (antiviral activity data), 45/0043 (cell culture resistance data), 46/0044 (correspondence regarding PMR), 48/0046 (updated labeling); note that there were multiple other submissions referring to the availability of observational cohort data that were not reviewed here

Dosage Form/Route of Administration: 30 and 60 mg tablets/oral

Dispensed: Rx X OTC__

Proposed Indication/Usage: DAKLINZA is a hepatitis C virus (HCV) NS5A inhibitor indicated for the treatment of chronic HCV infection as a component of a combination antiviral treatment regimen in adults. (1)

- DAKLINZA efficacy has been established in combination with sofosbuvir in treatment-naive and -experienced adults with HCV genotype 3 infection and compensated liver disease (including cirrhosis). (1, 14)
- DAKLINZA monotherapy is not recommended.

Abbreviations: ASV, asunaprevir; DAA, direct acting antiviral agent; DCV, daclatasvir; EC, effective concentration; FC, fold-change; GT, genotype; HCV, hepatitis C virus; IFN(α), interferon (alfa); LiPA, line-probe assay; LLOQ, lower limit of quantification; NDA, new drug application; NGS, next generation sequencing; Peg-IFN α , pegylated interferon alpha; PM, polymorphism; P/R, pegylated interferon alpha plus ribavirin; RBV, ribavirin; RT-PCR, reverse transcription polymerase chain reaction; SOF, sofosbuvir; SVR, sustained virologic response; VF, virologic failure; WT, wild-type

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EXECUTIVE SUMMARY

1. RECOMMENDATIONS

1.1 Recommendation and Conclusion on Approvability

NDA 206843 is approvable from a Clinical Virology perspective for daclatasvir in combination with sofosbuvir for the treatment of adults with chronic HCV genotype 3 infection.

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

- Conduct a trial to determine if a longer duration of treatment or addition of ribavirin improves the efficacy (i.e., sustained virologic response rate) of daclatasvir plus sofosbuvir for hepatitis C virus genotype 3 infected subjects with cirrhosis.
- Characterize the long-term persistence of treatment-emergent, daclatasvir resistance-associated substitutions in HCV genotype 3 infected subjects.

2. SUMMARY OF OND VIROLOGY ASSESSMENTS

This Clinical Virology review covers the resubmitted NDA application for daclatasvir (DCV) for the treatment of chronic HCV genotype 3 (GT3) infection. This review specifically focuses on efficacy and drug resistance data from clinical trial A1444218 (“ALLY-3”) conducted in subjects with chronic HCV GT3 infection. See the Clinical Virology review of NDA 206843 by Lalji Mishra, Ph.D., for additional review of DCV nonclinical virology. Also see the archived virology reviews by Patrick Harrington, Ph.D. and Lalji Mishra, Ph.D., of the original NDAs submitted on 3/31/2014 for 206843 and 206844 (for the NS3/4A protease inhibitor, asunaprevir) for additional details regarding the efficacy and drug resistance characteristics of DCV and asunaprevir in the context of HCV GT1a, GT1b and GT4.

2.1 Nonclinical Virology

Daclatasvir (DCV) is an inhibitor of the HCV NS5A protein. DCV had a median EC₅₀ value of 0.4 nM (range 0.006 nM to >5,000 nM) against a panel of 25 HCV replicons carrying GT3 NS5A genes from 21 subject-derived samples and 4 commercial samples. At least some of the variability in DCV activity against the different replicon constructs could be explained by the detection of polymorphisms at known resistance-associated positions in NS5A. By phylogenetic analysis, 21 of the 25 GT3 NS5A sequences aligned with subtype 3a. All of the non-GT3a isolates, including 3 GT3b isolates and 1 GT3i isolate, harbored resistance-associated changes (relative to a GT3a reference) at NS5A positions 30 and/or 31, and had reduced susceptibility to DCV with EC₅₀ values ≥3,620 nM. As previously noted for HCV GT1a and GT1b, DCV has a low resistance barrier in HCV GT3 and activity can be substantially reduced by the presence of 1 or more resistance-associated substitutions in NS5A. The key resistance-associated polymorphism/substitution Y93H alone confers a >3,000-fold reduction in DCV anti-HCV activity in the HCV GT3a replicon system.

Please see the review of NDA 206843 by Lalji Mishra, Ph.D., for a more detailed review of DCV nonclinical virology.

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2.2 Clinical Virology

The efficacy of DCV for HCV GT3 was demonstrated in clinical trial AI444218 (ALLY-3), which evaluated 12 weeks of combination therapy with DCV 60 mg QD and sofosbuvir 400 mg QD in subjects with chronic HCV GT3 infection. Sofosbuvir (SOF) is an FDA-approved uridine nucleotide analogue prodrug NS5B polymerase inhibitor. Approximately 100 treatment-naïve and 50 treatment-experienced subjects were to be enrolled. Previous treatment experience could have included interferon alfa with or without ribavirin (IFN α \pm RBV), SOF/RBV (except for failure due to intolerance other than anemia), or other anti-HCV agents, but previous exposure to NS5A inhibitors was prohibited. Up to 50% of subjects in each group could have compensated cirrhosis. The primary efficacy assessment was the proportion of subjects with a sustained virologic response at Follow-up Week 12 (SVR12), defined as HCV RNA <LLOQ (target detected or target not detected).

A total of 152 subjects were enrolled and treated in AI444218, including 101 treatment-naïve subjects and 51 treatment-experienced subjects, and 32 subjects with cirrhosis. Phylogenetic analyses of 147 available baseline NS5A sequences demonstrated that 100% of subject-derived NS5A sequences (amino acid positions 1-125) aligned with published HCV GT3a sequences.

Overall, 135/152 (89%) of treated subjects achieved SVR12, with similar SVR12 rates in treatment-naïve and treatment-experienced subjects. SVR12 rates were 63% and 96% in subjects with and without cirrhosis, respectively. Among 7 subjects who previously failed treatment with SOF either with RBV (n=5) or with P/R (n=2), 5 (71%) achieved SVR12 with DCV + SOF in AI444218. Of the 17 subjects who did not achieve SVR12, 16 (94%) subjects experienced virologic relapse post-treatment, and 1 (6%) subject had low, quantifiable HCV RNA (53 IU/mL) at the end of treatment; thus, all non-SVR12 results in AI444218 were attributed to virologic failure.

Independent analyses of population nucleotide sequence analysis data from subjects who experienced virologic failure identified NS5A Y93H as the primary DCV resistance-associated substitution, consistent with the sponsor's conclusions. This substitution is known to reduce the anti-HCV activity of DCV as well as other NS5A inhibitors. Of the 17 subjects who experienced virologic failure, 15 (88%) had the Y93H substitution detected at the time of virologic failure. Six of the subjects had Y93H detected as a baseline polymorphism, and the other 9 subjects had treatment-emergent Y93H. One subject without Y93H detected at failure had treatment-emergent L31I, which is another known NS5A resistance-associated substitution. Overall, 16/17 (94%) subjects had L31I or Y93H detected at virologic failure.

Among 16 virologic failure subjects with available NS5B sequence analysis data, one subject had the S282T SOF resistance-associated substitution detected at the time of failure. The subject did not have baseline sequence data available to assess treatment-emergence of S282T; however, the S282T substitution is rarely, if ever, detected as a natural baseline polymorphism, and therefore the detection of S282T at the time of failure in this subject is almost certainly attributed to DCV + SOF treatment failure. The other 15 virologic failure subjects did not have treatment-emergent substitutions detected at any known SOF resistance-associated NS5B positions, including L159, S282, C316, L320 or V321.

Analyses of pre-treatment NS5A sequences demonstrated the key DCV resistance-associated substitution, Y93H, was detected as a natural baseline polymorphism in 13/148 (9%) subjects with available data and was associated with reduced efficacy of DCV + SOF in both noncirrhotic and cirrhotic subjects. Overall, 7/13 (54%) subjects with the Y93H baseline polymorphism achieved SVR12 compared to 124/135 (92%) subjects without the Y93H baseline polymorphism. Reflecting the

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clear efficacy impact of both the Y93H polymorphism and cirrhosis, of the 17 subjects who experienced virologic failure in AI444218, 15 (88%) had either cirrhosis or the NS5A Y93H baseline polymorphism.

In contrast to data previously observed in HCV GT1b infected subjects with DCV resistance-associated polymorphisms, clear emergence of additional major DCV resistance-associated substitutions was not observed among HCV GT3 infected subjects with the Y93H polymorphism who experienced virologic failure in AI444218. This difference likely reflects that in HCV GT3a, the Y93H polymorphism by itself confers a clinically significant reduction in DCV susceptibility (>3,000-fold) in the absence of any other DCV resistance-associated polymorphisms or substitutions.

Baseline sofosbuvir resistance analyses focused on known resistance-associated positions in NS5B. Of 150 subjects with available data, no subjects had polymorphisms at positions L159, S282, C316, L320 or V321, including the 7 subjects who previously were treated with a SOF-containing regimen. Next generation sequencing (NGS) analyses conducted for the 7 SOF treatment-experienced subjects also did not detect any SOF resistance-associated substitutions at baseline. Furthermore, post-treatment NGS analyses for 2 prior SOF-experienced subjects who experienced virologic failure with DCV + SOF did not identify any known SOF resistance-associated substitutions. The NGS data were independently confirmed by Dr. Eric Donaldson, Ph.D.

No long-term follow-up data are available from AI444218 to assess the durability of SVR12. Previously reviewed data demonstrated durability of SVR12 for HCV GT1 infected subjects treated with DCV-containing regimens.

Similarly, no long-term follow-up data are available from AI444218 to assess the long term persistence of daclatasvir resistance-associated substitutions for HCV GT1 infected subjects who experienced virologic failure. Previous analyses have demonstrated persistence of DCV resistance-associated substitutions for >1 year in most subjects.

3. ADMINISTRATIVE

3.1 Reviewers' Signatures

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

Eric F. Donaldson, Ph.D.
Clinical Virology Reviewer, Division of Antiviral Products

3.2 Concurrence

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

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OND CLINICAL VIROLOGY REVIEW

1. INTRODUCTION AND BACKGROUND

1.1 Introduction and scope of this review

This Clinical Virology review covers the resubmission of New Drug Application (NDA) 206843 for daclatasvir (DCV, DAKLINZA™), a hepatitis C virus (HCV) NS5A inhibitor. The original complete NDA for DCV was submitted on 3/31/2014 and was supported by pivotal clinical trials in which DCV was administered in combination with asunaprevir (ASV, NS3/4A protease inhibitor), which was concurrently submitted for approval under NDA 206844. The proposed regimens covered by original NDAs 206843 and 206844 were ASV plus DCV for 24 weeks for HCV genotype 1b (GT1b) infected patients, and ASV plus DCV plus pegylated interferon alpha (Peg-IFN α) and ribavirin (RBV) for 24 weeks for HCV GT1 or GT4 infected patients. On 10/6/2014 the sponsor withdrew NDA 206844 for ASV, and as a result the DCV NDA could not be approved because it did not contain sufficient safety and efficacy data to support its use in drug combinations without ASV, particularly with other HCV direct acting antivirals (DAAs).

The resubmission of NDA 206843 includes data from clinical trial A1444218 (ALLY-3), which studied DCV dosed in combination with sofosbuvir (SOF, SOVALDI™), an FDA-approved uridine nucleotide analogue NS5B polymerase inhibitor, in subjects with chronic HCV GT3 infection. These data are intended to provide additional safety and efficacy data to support the use of DCV in combination with SOF in HCV GT3 infected patients.

HCV GT3 is responsible for approximately 20-30% of HCV infections worldwide, and approximately 10% of HCV infections in North America ([Gower et al., 2014](#); [Messina et al., 2015](#)). HCV GT3a appears to be the most prevalent GT3 subtype in the U.S. and possibly also worldwide, particularly in HCV-infected intravenous drug users ([Clement et al., 2010](#); [Zein 2000](#); [Morice et al., 2006](#)).

The Clinical Virology review of the DCV NDA 206843 resubmission included independent analyses of HCV RNA and drug resistance data associated with clinical trial A1444218. Next generation sequencing analyses were conducted by the sponsor for a small subset of subjects in clinical trial A1444218; these data were reviewed by Eric Donaldson, Ph.D. and Dr. Donaldson's key conclusions are included in this review. Please also see the review by Lalji Mishra, Ph.D., for additional review of DCV focusing on the nonclinical (i.e., cell culture) virology and resistance characteristics of DCV for HCV GT3. Previously archived virology reviews by Patrick Harrington, Ph.D. and Lalji Mishra, Ph.D., of the original NDAs for 206843 and 206844 include additional details regarding the efficacy and drug resistance profiles of DCV and ASV in the context of HCV GT1a, GT1b and GT4.

1.2 Methodology

HCV genotype/subtype determination

In clinical trial A1444218 HCV genotype determination for inclusion was based on the Abbott RealTime HCV Genotype II assay, which is an FDA-approved, real-time RT-PCR-based HCV genotyping assay. For samples with results that were unavailable or inconclusive, the Siemens Versant HCV genotype 2.0 line-probe assay (LiPA) was used. These analyses were conducted by (b) (4)

Also, retrospective phylogenetic analyses of NS5A sequences were conducted to confirm HCV genotype 3 infection, and identify genotype 3 subtypes.

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HCV viral load assessments

In clinical trial AI444218 HCV RNA levels were determined using the FDA-approved Roche COBAS® TaqMan® HCV v2.0 test, which has a lower limit of quantification (LLOQ) of 25 IU/mL. These analyses were conducted by [REDACTED] (b) (4).

Resistance-related Assessments

In clinical trial AI444218, drug resistance testing on plasma samples was performed by population nucleotide sequence analysis of baseline samples from all subjects, as well as samples collected near the time of virologic failure for subjects who did not achieve a sustained virologic response (SVR). Results were reported for NS5A amino acid positions 1-125 as well as full-length NS5B (amino acids 1-591), although incomplete/unresolved sequences were reported for some subjects across certain stretches of NS5B. These analyses were conducted by [REDACTED] (b) (4). Additional population nucleotide sequence analyses were conducted “in-house” by the sponsor for a few samples without available data generated by the contract laboratory. Next generation nucleotide sequence analyses were conducted by [REDACTED] (b) (4) for 7 subjects who previously failed treatment with SOF + RBV or SOF + Peg-IFN α /RBV (P/R). Phenotypic analyses of NS5A substitutions of interest were conducted by inserting substitutions of interest into a hybrid HCV JFH-1 (GT2a) replicon carrying an HCV GT3 NS5A cassette (amino acids 3-430), and HCV replicon susceptibility to DCV was evaluated based on luciferase reporter expression.

2. NONCLINICAL VIROLOGY

This review section includes a brief summary of key DCV nonclinical virology characteristics to support the review of clinical trial AI444218. Please see the Clinical Virology review of NDA 206843 by Lalji Mishra, Ph.D., for a more detailed review of DCV nonclinical virology data.

2.1 Mechanism of action and antiviral activity in cell culture

DCV is an NS5A inhibitor. The mechanism of action of DCV has been characterized in HCV replicon resistance selection studies, [REDACTED] (b) (4) biochemical assays evaluating phosphorylation of NS5A, and NS5A binding 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 DCV appear to target primarily the N-terminus of the protein. Inhibition of HCV replicons with picomolar EC₅₀ values indicates that DCV targeting of NS5A inhibits HCV RNA replication. Based on viral RNA kinetic modeling in treated subjects and HCV cell culture studies, NS5A inhibitors also impair HCV assembly or release ([Guedj et al., 2013](#); [McGivern et al., 2014](#)).

DCV had a median EC₅₀ value of 0.4 nM (range 0.006 nM to >5,000 nM) against a panel of 25 HCV replicons carrying GT3 NS5A genes from 21 subject-derived samples and 4 commercial samples (Table 1; Report [930086934 v1.0](#) pg. 16). At least some of the variability in DCV activity against the different replicon constructs could be explained by the detection of polymorphisms at known resistance-associated positions in NS5A. By phylogenetic analysis, 21/25 GT3 NS5A sequences aligned with subtype 3a, whereas the other 4 aligned with subtypes 3b (n=3) or 3i (Figure 1; Report [930086934 v1.0](#) pg. 19). Importantly, the sponsor noted that all of the non-GT3a isolates harbored resistance-associated polymorphisms (presumably relative to a GT3a reference) at positions 30 and/or 31, and had reduced susceptibility to DCV with EC₅₀ values $\geq 3,620$ nM. Therefore, since all efficacy data from clinical trial AI444218 were derived from HCV GT3a infected subjects (see efficacy analysis in Section 3.2), the DCV prescribing information should note that all subjects in AI444218 were infected with HCV GT3a, and Section 12.4 should note that DCV had reduced activity against HCV replicons derived from other GT3 subtypes.

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Table 1. DCV activity against HCV replicons carrying NS5A sequences from different HCV genotype/subtype isolates.

GT	Total			Without NS5A Polymorphisms ^a			With NS5A Polymorphisms ^a		
	No of NS5A sequences	Median EC50 (nM)	Range of EC50 values (nM)	No of NS5A sequences	Median EC50 (nM)	Range of EC50 values (nM)	No of NS5A sequences	Median EC50 (nM)	Range of EC50 values (nM)
1a	40	0.008	0.002 - 2409	35 ^b	0.008	0.002 - 0.03	5	76	4.6 - 2409
1b	42	0.002	0.0007 - 10	30	0.002	0.0007 - 0.006	12 ^c	0.05	0.002 - 10
2	21	16	0.005 - 60	5	0.008	0.005 - 0.02	16	17.5	0.3 - 60
3	25	0.4	0.006 - >5000	17	0.2	0.006 - 3.2	8	1835	1.3 - >5000
4	14	0.025	0.001 - 158	4	0.003	0.001 - 0.007	10	0.035	0.007 - 158
5	3	0.004	0.003 - 0.019	0	NA	NA	3	0.004	0.003 - 0.019
6	1	NA	0.054	0	NA	NA	1	NA	0.054

DCV - daclatasvir, GT - genotype, HCV - hepatitis c virus, No - number, NS5A - nonstructural protein 5A

^a GT-1a NS5A polymorphisms at M28 (excluded M28V), Q30, L31 and Y93; GT-1b at L31 and Y93H; GT-2 at F28 (excluded F28L), K30, L31 and Y93; GT-3 at M28 (excluded M28V), A30, L31 and Y93; GT-4 at L28, L30, M31 (excluded M31L) and Y93.

^b 1 subjects had an NS5A sequence that more closely aligned with GT-1a H77c (80% sequence identity) than GT-1b Con1 (78% sequence identity); in this current analysis, the NS5A sequence was shown to more closely segregate with a GT-1c NS5A sequence (94% sequence identity with NS5A accession number D14853) than GT-1a (Figure 1)

^c 2 subjects who were determined as GT-1b by the line probe genotyping assay had NS5A sequences that aligned with GT-1c and GT-1l (Figure 2)

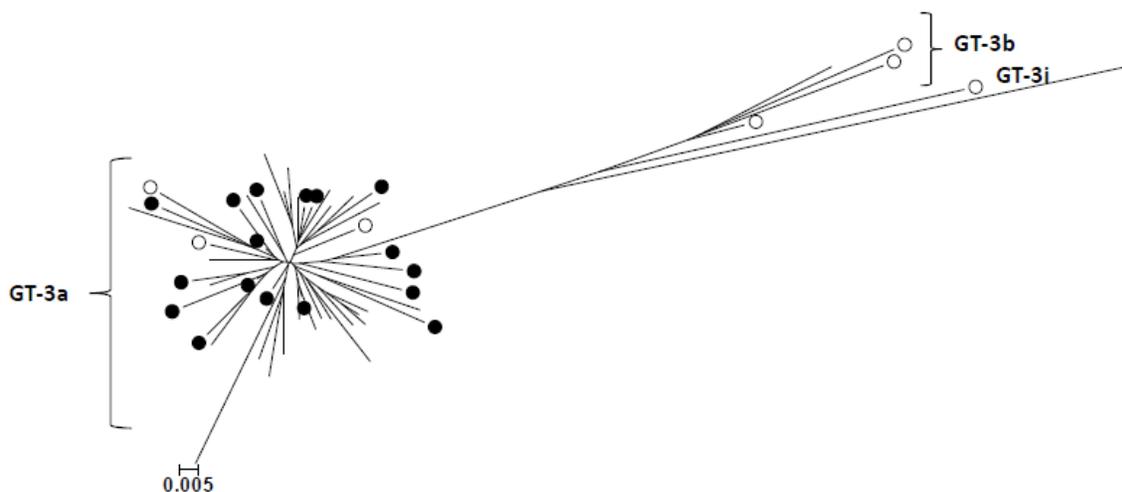


Figure 1. Phylogenetic analysis of GT3 NS5A sequences (amino acid positions 1-213) evaluated for susceptibility to DCV, aligned with 30 GT3 sequences with confirmed subtypes from European HCV database. One sequence with data for amino acid positions 1-100 that was excluded from the analysis also aligned with GT3a. Open circles indicate subject-derived NS5A sequences with DCV resistance-associated polymorphisms at positions M28 (excluding M28V), A30, L31 and Y93. Analysis is based on amino acid; the scale represents the genetic distance at the amino acid level.

2.2 Effect of individual amino acid substitutions on DCV anti-HCV activity

As previously noted for HCV GT1a and GT1b, DCV has a low resistance barrier and activity can be substantially reduced by the presence of 1 or more resistance-associated substitutions in NS5A. Table 2 (adapted from [930086934 v1.0](#), pgs. 28-29) provides a listing of all available site-directed mutant phenotype results for HCV GT3a using a chimeric HCV JFH-1/GT3a replicon construct. The

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key resistance-associated polymorphism/substitution Y93H alone confers a >3,000-fold reduction in DCV anti-HCV activity in the HCV GT3a replicon system. See the referenced report and the Clinical Virology review of Original NDA 206843/206844 submissions for site-directed mutant phenotype data for other HCV genotypes and subtypes.

Table 2. Site-directed mutagenesis DCV phenotype analysis of individual NS5A substitutions.

GT	NS5A Substitution	EC ₅₀ (nM)	FC EC ₅₀	EC ₉₀ (nM)	FC EC ₉₀	Rep. Capacity (%)
3a	WT (AI444031-30-154)	0.3	1	0.5	1	100
3a	M28V	0.006	<0.1	0.019	<0.1	125
3a	A30E	335	1117	501	1002	5
3a	A30K	35	117	53	106	98
3a	A30S	0.6	2	1.3	3	34
3a	A30T	0.2	1	0.44	1	40
3a	A30V	0.023	0.1	0.074	0.1	155
3a	L31I	72	240	101	202	84
3a	L31M	209	697	330	660	138
3a	S54T	0.4	1	0.9	2	60
3a	S54W	0.3	1	0.5	1	130
3a	S54Y	ND	ND	ND	ND	ND
3a	P58A	0.6	2	1	2	33
3a	P58S	0.2	1	0.5	1	16
3a	S62A	0.4	1	0.8	2	88
3a	S62D	0.6	2	1.1	2	139
3a	S62I	0.6	2	0.9	2	198
3a	S62L	0.3	1	0.7	1	125
3a	S62N	1.2	4	2	4	75
3a	S62P	ND	ND	ND	ND	ND
3a	S62Q	0.9	3	1.7	3	82
3a	S62T	0.7	2	1.3	3	70
3a	S62V	ND	ND	ND	ND	ND
3a	E92A	0.005	0	0.02	0	95
3a	E92D	4.6	15	7.2	14	NA
3a	Y93H	1120	3733	1369	2738	35
3a	A30E,S62T	375	1250	606	1212	6
3a	A30K,L31M	3640	12133	4306	8612	31
3a	A30K,P58A	68	227	114	228	17
3a	A30K,S62A	39	130	60	120	20
3a	A30K,S62I	105	350	162	324	22
3a	A30K,S62L	111	370	161	322	27
3a	A30K,S62P	158	527	219	438	10
3a	A30K,S62T	143	477	189	378	14
3a	A30K,S62V	125	417	225	450	47
3a	A30K,E92D	347	1157	1002	2004	ND
3a	A30S,S62T	1.8	6	3.4	7	42
3a	A30T,S62P	1.1	4	2.2	4	23
3a	A30T,S62T	0.4	1	0.6	1	115
3a	A30V,Y93H	4.6	15	9.3	19	52
3a	S54A,Y93H	1308	4360	2115	4230	14
3a	P58R,S62V	ND	ND	ND	ND	ND
3a	P58S,Y93H	1784	5947	2833	5666	9
3a	S62A,Y93H	1447	4823	1662	3324	43
3a	S62I,Y93H	1412	4707	3172	6344	35
3a	S62L,Y93H	3083	10277	4342	8684	18
3a	S62R,Y93H	304	1013	494	988	14
3a	S62T,Y93H	1355	4517	1688	3376	23
3a	E92A,Y93H	135	450	263	526	108
3a	M28V,S62P,Y93H	545	1817	746	1492	7
3a	A30S,S62A,Y93H	359	1197	569	1138	19
3a	A30V,S62P,Y93H	27	90	54	108	20

FC, fold-change; ND, not determined

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3. CLINICAL VIROLOGY REVIEW OF EFFICACY AND DRUG RESISTANCE

3.1 AI444218 (ALLY-3) study design

Clinical trial AI444218 (ALLY-3) evaluated 12 weeks of combination therapy with DCV (60 mg QD) and SOF (400 mg QD) in subjects with chronic HCV genotype 3 infection. Approximately 100 treatment-naïve and 50 treatment-experienced subjects were planned for enrollment. Previous treatment experience could have included IFN α \pm RBV, SOF/RBV (except for failure due to intolerance other than exacerbations of anemia), or other anti-HCV agents (e.g., cyclophilin inhibitors and inhibitors of microRNA). Previous exposure to NS5A inhibitors was prohibited. Up to 50% of subjects in each group could have compensated cirrhosis. Coinfection with human immunodeficiency virus or hepatitis B virus was exclusionary. The trial was conducted at 30 sites in the U.S. and 1 site in Puerto Rico. The primary efficacy assessment was the proportion of subjects with SVR12, defined as HCV RNA <LLOQ (target detected or target not detected) at Follow-up Week 12.

3.2 AI444218 (ALLY-3) HCV genotype/subtype analyses

As noted above, HCV genotype determination for inclusion was based on the Abbott RealTime HCV Genotype II assay, and for any samples with results that were unavailable or inconclusive, the Siemens Versant HCV genotype 2.0 line-probe assay (LiPA) was used. Also, retrospective phylogenetic analyses of NS5A sequences were conducted to confirm HCV genotype 3 infection, and identify genotype 3 subtypes.

Among the 152 subjects who were enrolled and treated in AI444218, 143 (94%) had their HCV GT3 infection identified by the Abbott RealTime assay, while 9 (6%) other subjects with inconclusive or ambiguous results had their HCV GT3 infection identified by the Siemens Versant HCV genotype 2.0 assay. All but one of the 9 subjects with initially inconclusive HCV GT results from the Abbott assay achieved SVR12; the single non-SVR12 subject (AI444218-24-71) had a Baseline NS5A Y93H polymorphism that may have impacted treatment efficacy (see resistance analysis described below).

Phylogenetic analyses of 147 available baseline NS5A sequences demonstrated that 100% of subject-derived NS5A sequences (amino acid positions 1-125) aligned with published **HCV GT3a** sequences (Figure 2; adapted from [Supplementary Resistance Report](#) pg. 21).

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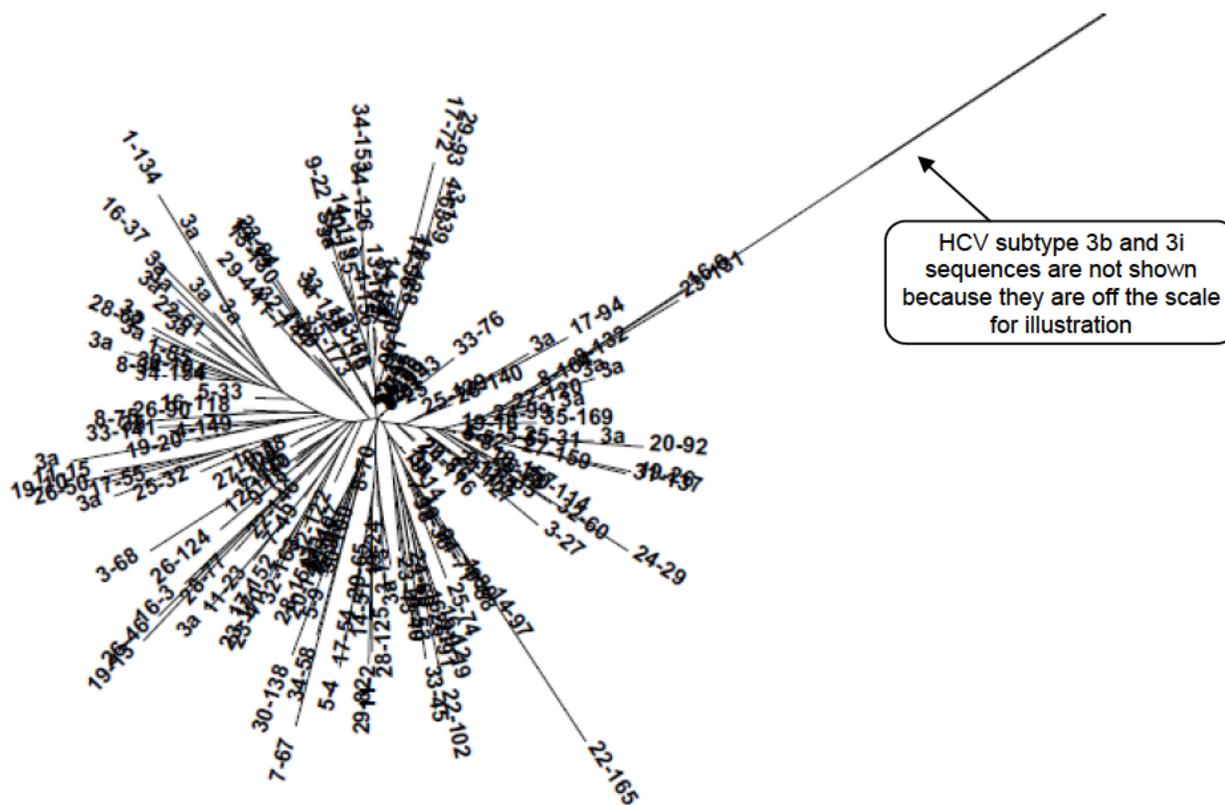


Figure 2. Phylogenetic analysis of 147 baseline NS5A sequences (amino acid positions 1-125) demonstrating clustering with HCV GT3a sequences. Subject IDs are shown, and European HCV sequence database GT3a sequences are noted as “3a”. Analysis is based on amino acid, and the scale represents the genetic distance at the amino acid level.

The sponsor conducted an additional phylogenetic analysis for 138 subjects with complete NS5A domain 1 sequences (amino acid positions 1-213) to investigate whether phylogenetic clustering was associated with treatment outcome. As shown in Figure 3 (adapted from [Supplementary Resistance Report](#) pg. 22), there was no apparent clustering of virologic failure subjects based on NS5A sequences. Furthermore, there was no apparent clustering of NS5A sequences for subjects who initially had inconclusive Abbott genotype assay results.

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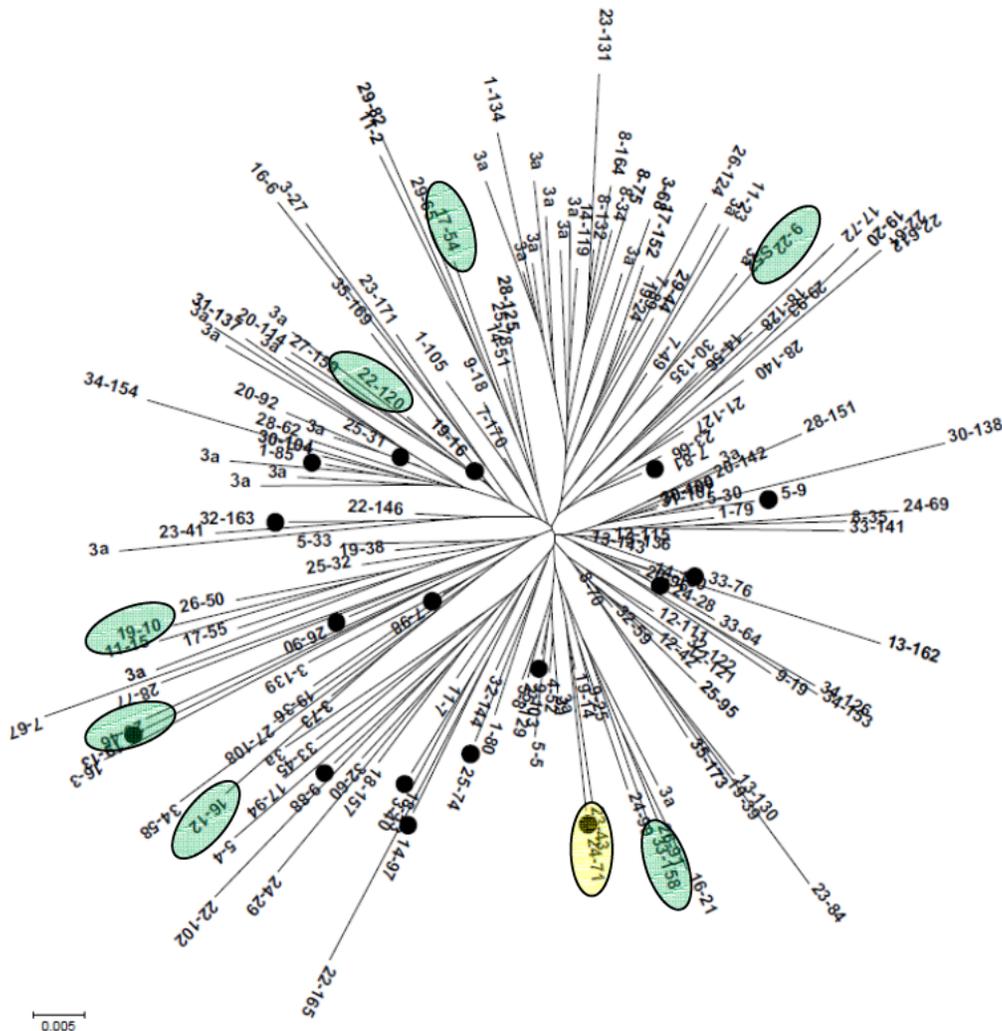


Figure 3. Phylogenetic analysis of 138 baseline NS5A sequences (amino acid positions 1-213) and treatment outcome. Subject IDs are shown, and European HCV sequence database GT3a sequences are noted as “3a”. Closed black circles represent NS5A sequences from subjects who did not achieve SVR12. The highlighted oval annotations added by this reviewer indicate NS5A sequences from 8 subjects (sequence from 1 other subject not available) who initially had inconclusive genotype results by the Abbott assay; the yellow oval indicates Subject AI444218-24-71, who did not achieve SVR12. Analysis is based on amino acid, and the scale represents the genetic distance at the amino acid level.

3.3 AI444218 (ALLY-3) efficacy analysis

SVR rates for all subjects and key subgroups based on independent analyses of HCV RNA data are summarized in Table 3 (FDA analysis). Overall, 135/152 (89%) subjects achieved SVR12. Consistent with the sponsor’s analyses, SVR12 rates were ~30% lower among subjects with cirrhosis compared to subjects without cirrhosis. Of the 17 subjects who did not achieve SVR12, 16 (94%) subjects experienced virologic relapse post-treatment, and 1 (6%) subject had low, quantifiable HCV RNA (53 IU/mL) at the end of treatment; thus, all non-SVR12 results were attributed to virologic failure. The subject with quantifiable HCV RNA at the end of treatment (AI444218-32-163, cirrhotic) had HCV RNA results of >LLOQ/detected at two prior visits, indicating a relatively slow HCV RNA decline; the 53 IU/mL level at the end of treatment likely represented an early indication of virologic

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breakthrough which was subsequently followed by HCV RNA levels of 4.7-6.8 log₁₀ IU/mL post-treatment.

Table 3. AI444218 (ALLY-3) efficacy summary.

	SVR12 Rate
All Subjects	135/152 (89%)
Tx-Naïve Cohort	91/101 (90%)
Tx-Experienced Cohort	44/51 (86%)
Cirrhosis=No	105/109 (96%)
Cirrhosis=Yes	20/32 (63%)
Cirrhosis Unknown	10/11 (91%)
IL28B CC	55/60 (92%)
IL28B CT	57/68 (84%)
IL28B TT	23/24 (96%)
U.S. Sites	129/146 (88%)
Puerto Rico	6/6 (100%)
Non-SVR12 Subjects	17/152 (11%)
On Treatment VF	1/152 (1%)
Relapse	16/152 (11%)
-Among EOT responders	16/151 (11%)

Seven subjects in AI444218 previously received SOF either with RBV (n=5) or with P/R (n=2), of whom 5 (71%) achieved SVR12 (Table 4, FDA analysis). One of the virologic failure subjects had the baseline Y93H polymorphism and the other had baseline cirrhosis. As noted below in Section 3.5, none of the 7 subjects had NS5B substitutions or polymorphisms detected at key SOF resistance-associated positions, so it is not possible to assess the impact of potential SOF resistance on efficacy of DCV/SOF, although prior SOF experience clearly did not preclude an effective response to DCV/SOF.

Table 4. Efficacy of DCV/SOF in prior SOF failures.

USUBJID	Prior SOF Tx	IL28B	CIRRFLL	Y93H PM	SVR12
AI444218-14-97	SOF + RBV	CT	No	Yes	No
AI444218-17-54*	SOF + P/R	CT	No	No	Yes
AI444218-17-94	SOF + RBV	CT	No	No	Yes
AI444218-23-84	SOF + P/R	TT	No	No	Yes
AI444218-33-64	SOF + RBV	CT	No	No	Yes
AI444218-33-76	SOF + RBV	CT	Yes	No	No
AI444218-5-4	SOF + RBV	CC	Yes	No	Yes

*Subject received SOF two prior times, unclear if administered with P/R both times.

3.4 AI444218 (ALLY-3) treatment-emergent resistance analysis

Independent analyses of the sponsor's population nucleotide sequence analysis data were conducted to identify patterns of treatment-emergent substitutions among subjects who experienced virologic failure in clinical trial AI444218. These analyses included both NS5A for DCV, and NS5B for SOF.

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NS5A (daclatasvir)

Analyses of NS5A sequences were conducted for all 17 subjects who did not achieve SVR12. These analyses considered both known DCV resistance-associated positions, as well as all positions (NS5A 1-125) for which sequence analysis data were obtained.

Consistent with the sponsor's conclusions, NS5A Y93H was the primary DCV resistance-associated substitution (Table 5, FDA analysis), which is also consistent with the ~3,000-fold reduction in DCV anti-HCV activity conferred by Y93H. Of the 17 subjects who experienced virologic failure, 15 (88%) had the Y93H substitution detected at the time of virologic failure. Six of the subjects had Y93H detected as a baseline polymorphism, and the other 9 subjects had treatment-emergent Y93H. One other subject without Y93H detected at failure had treatment-emergent L31I, which conferred a 240-fold reduction in DCV activity (Table 2). Overall, 16/17 (94%) subjects had L31I or Y93H detected at virologic failure.

Cirrhotic subject AI444218-32-163, who had a slow HCV RNA decline with HCV RNA of 53 IU/mL at the end of treatment, did not have L31I or Y93H detected at virologic failure. Therefore, the precise reasons for treatment failure in this subject are not known. This subject did, however, have three other treatment-emergent substitutions at positions not known to be associated with DCV resistance: K68R, L74I and W111L. The subject also had a baseline A30K polymorphism, which confers ~100-fold reduced DCV activity in a GT3 replicon (Table 2). It is tempting to speculate that the A30K polymorphism impacted treatment response in this subject, but 5 other subjects with baseline A30K achieved SVR. Interestingly, a W111L polymorphism was possibly associated with reduced treatment efficacy in AI444218 (see Section 3.5), although a review of data from the Phase 3 DUAL/QUAD trials did not identify any polymorphisms or substitutions at this position among 112 HCV GT1b infected subjects or 21 HCV GT1a infected subjects.

Table 5. AI444218 (ALLY-3) NS5A treatment-emergent resistance analysis summary.

NS5A Position/ Substitution	Known Resist.- Assoc. Position	Number (%) w/Tx- Emergence (n=17)	Number (%) at Failure (n=17)
M28-any	Y	0 (0%)	0 (0%)
P29-any	Y	0 (0%)	0 (0%)
A30K or A30S ^a	Y	0 (0%)	2 (12%)
L31I	Y	1 (6%)	1 (6%)
P32-any	Y	0 (0%)	0 (0%)
P58-any	Y	0 (0%)	0 (0%)
S62A/L/P/R/T ^b	Y	2 (12%)	10 (59%)
E92-any	Y	0 (0%)	0 (0%)
Y93H	Y	9 (53%)	15 (88%)
L31I or Y93H	Y	10 (59%)	16 (94%)
Treatment-emergent substitutions in Subject AI444218-32-163: K68R, L74I, W111L			

^a1 subject each had A30K or A30S detected as a baseline polymorphism, which remained detected at treatment failure. Two other subjects had A30 polymorphisms (T or V) that were no longer detected at treatment failure.

^bPosition S62 was highly polymorphic, and the detection of polymorphisms at this position was not consistently associated with treatment failure (see baseline analysis summary-Table 6). Only S62L emerged, in 2 subjects.

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NS5B (sofosbuvir)

Among the 17 subjects who did not achieve SVR12, paired baseline and failure NS5B population nucleotide sequences were obtained from 15 subjects. In addition, data were obtained from Subject AI444218-19-13 at the time of failure but no baseline data are available from this subject. Treatment-emergent resistance analyses considered known SOF resistance-associated positions (NS5B L159, S282, C316, L320 and V321). In addition, an independent analysis of all NS5B positions was conducted to identify substitutions that emerged in at least two virologic failure subjects, excluding changes towards reference/consensus.

None of the 15 subjects included in the paired baseline-failure analysis had treatment-emergent substitutions at NS5B positions L159, S282, C316, L320 or V321. However, Subject AI444218-19-13, who did not have baseline sequence data, had S282T detected at the time of virologic failure. The S282T substitution is rarely, if ever, detected as a natural baseline polymorphism, and therefore the detection of S282T at the time of failure in this subject almost certainly reflects treatment emergence.

An H571Y substitution emerged in 3 subjects, although the Y571 sequence is a common polymorphism and the reverse change was observed in one subject (note that not all subjects had baseline data for this position). According to Dr. Eric Donaldson, similar changes in both directions at position 571 were observed in prior GT3 SOF trials, indicating that these changes are not likely associated with SOF resistance. Changes at other NS5B positions that are highly polymorphic (e.g., A116, A150, R374) were observed in 1-2 subjects each, but again given the small size of the resistance dataset and the polymorphic nature of these positions it is not possible to conclude these changes were associated with SOF resistance.

3.5 AI444218 (ALLY-3) baseline resistance analysis

NS5A (daclatasvir)

As in the treatment-emergent resistance analysis, independent analyses of the sponsor's population nucleotide sequence analysis data were conducted to identify baseline polymorphisms that were associated with virologic failure in clinical trial AI444218. These analyses considered both known DCV resistance-associated positions, as well as all positions (NS5A 1-125) for which sequence analysis data were obtained. Other positions not known to be associated with DCV resistance were flagged as possibly being associated with treatment response if polymorphisms were detected in ≥ 2 virologic failure subjects and associated with a lower SVR rate. Baseline NS5A sequence data were available from 148/152 (97%) subjects.

Table 6 (FDA analysis) summarizes the independent baseline resistance analyses. In general, only the Y93H polymorphism was detected in a significant number of subjects and clearly associated with treatment failure. Although other polymorphisms were detected at known DCV resistance-associated positions in NS5A, the polymorphisms either were not associated with a lower SVR rate or they were not consistently associated with drug resistance and often confounded by the detection of Y93H in the same samples.

Two other baseline polymorphisms at NS5A positions not known to be associated with DCV resistance, K41R and W111L, were enriched in virologic failure subjects. The K41R polymorphism was detected only in two virologic failure subjects, and both subjects had treatment-emergent Y93H, and given the lack of other evidence no conclusions regarding the impact of K41R on DCV resistance can be made at this time. The W111L variant was observed as a treatment-emergent substitution in 1 subject.

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Table 6. Summary of AI444218 (ALLY-3) baseline resistance analysis. PM, polymorphism

"Signature" DCV resistance-associated positions								
Position	PM(s)	# Failure	# SVR	SVR rate	Failures w/Y93H PM	PM Tx changes	Tx-emergence?	Comments
M28	V	0	1	100%	n/a	n/a	No	Conserved position. M28V detected only in a single subject who achieved SVR.
A30	V/K/S/T	4	10	71%	2	to ref.(2), stable(2)	No	Not considered a resistance-associated PM. PMs at position A30 changed to reference in 2 of 4 subjects at the time of VF. No evidence of tx-emergent substitutions in other VFs. Lower SVR rate confounded by Y93H polymorphism. A30K possibly associated with failure in 1 subject.
P58	R/S	0	3	100%	n/a	n/a	No	Not considered a resistance-associated polymorphism. P58R/S detected in 3 subjects, all of whom achieved SVR.
S62	Several	9	57	86%	4	to ref.(1), stable (8)	Yes (S62L, n=2)	Possible resistance-associated polymorphism (S62L). Highly polymorphic position with treatment response associations going in both directions depending on specific PM. Subjects with the most common PM (S62T) had SVR rate of 37/40 (93%). Only S62L was observed as a tx-emergent subst.; SVR rate for subjects with S62L was 4/7 (57%), with 2 of 3 failures having Y93H PM.
Y93	H	6	7	54%	n/a	stable (6)	Yes (Y93H, n=9)	Resistance-associated PM, and primary tx-emergent resistance-associated substitution.
Other "non-signature" positions/polymorphisms of interest								
Position	PM(s)	# Failure	# SVR	SVR rate	Failures w/Y93H PM	PM Tx changes	Tx-emergence?	Comments
K41	R	2	2	50%	0	stable (2)	No	Possibly associated with reduced SVR rate. Two VFs w/PM (both w/ tx-emergent Y93H), and no changes at K41 emerged in other VFs.
W111	L	3	6	67%	1	stable (all)	Yes (W111L, n=1)	Possibly associated with reduced SVR rate. Tx-emergent substitution in 1 subject. One of the 3 VFs with PM also had Y93H PM.

Considering all "signature" DCV resistance-associated positions, a modestly lower SVR rate was observed in subjects with the NS5A polymorphisms (84%) compared to those without the polymorphisms (93%) (Table 7, FDA analysis). However, most of this difference could be attributed to Y93H, as the SVR rate was comparable between those with or without NS5A polymorphisms excluding Y93H. Because Y93H was clearly associated with a reduced SVR12 rate, consistent with its treatment-emergence and major impact on DCV anti-HCV activity in cell culture, additional analyses were conducted focusing only on this polymorphism.

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Table 7. Summary of AI444218 (ALLY-3) SVR rates according to detection of polymorphisms at DCV resistance-associated positions in NS5A. PM, polymorphism

	n	# Failure	#SVR	SVR rate
Any PM at signature position	77	12	65	84%
No PM at signature position	71	5	66	93%
Any PM at signature position other than Y93	64	6	58	91%

As shown in Table 8 (FDA analysis), the Y93H polymorphism was detected overall in 13/148 (9%) subjects and was associated with a ~30-40% lower SVR12 rate in both cirrhotic and noncirrhotic subjects; note that only 4 subjects with cirrhosis had the Y93H polymorphism, of whom 1 (25%) achieved SVR. Reflecting the clear impact of both baseline Y93H and cirrhosis status, of the 17 subjects who experienced virologic failure, 15 (88%) had either cirrhosis or the NS5A Y93H baseline polymorphism.

Table 8. SVR12 rate according to detection of Y93H polymorphism and cirrhosis status.

	n	# Failure	#SVR	SVR rate
All subjects:				
Y93H	13	6	7	54%
No Y93H	135	11	124	92%
Cirrhotic subjects:				
Y93H	4	3	1	25%
No Y93H	28	9	19	68%
Noncirrhotic subjects:				
Y93H	8	3	5	63%
No Y93H	97	1	96	99%
Cirrhosis not reported:				
Y93H	1	0	1	100%
No Y93H	10	1	9	90%
Pooling noncirrhotic/not reported (as "noncirrhotic" in label):				
Y93H	9	3	6	67%
No Y93H	107	2	105	98%

An additional treatment-emergent resistance analysis was conducted focusing on subjects with the baseline NS5A Y93H polymorphism to determine if 12 weeks of suboptimal DCV-containing treatment selected for any additional emergent substitutions that may impact subsequent re-treatment. Table 9 (FDA analysis) illustrates all treatment-emergent changes observed at any NS5A position (amino acids 1-125) for the 6 virologic failure subjects who had the NS5A Y93H baseline polymorphism. Not surprisingly, in two subjects an H/Y93 mixture was enriched to H93 at the time of virologic failure, consistent with drug selection of H93-containing viral populations.

In contrast to data previously observed in HCV GT1b infected subjects with DCV resistance-associated polymorphisms, clear emergence of additional major DCV resistance-associated substitutions was not observed among HCV GT3 infected subjects with the Y93H polymorphism who

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experienced virologic failure. This difference likely reflects that in HCV GT3a, the Y93H polymorphism by itself confers a major reduction in DCV susceptibility (>3,000-fold) in the absence of any other DCV resistance-associated substitutions. Emergence of an S62L substitution was observed in 2/6 (33%) subjects with the Y93H polymorphism; however, position S62 is highly polymorphic and is not known to be a major NS5A inhibitor resistance-associated position. In GT3 hybrid replicon cell culture assays single substitutions at position S62 conferred no or relatively modest (≤ 4 -fold) reductions in DCV anti-HCV activity (Table 2). The S62L substitution by itself had no impact on HCV replicon susceptibility to DCV, although in combination with Y93H it reduced HCV susceptibility to DCV by an additional ~ 3 -fold. The impact of the combined S62L + Y93H substitutions on other NS5A inhibitors in development for HCV GT3 infection is unknown.

Table 9. NS5A substitutions at Baseline (BL) and after DCV/SOF treatment failure in HCV GT3 infected subjects with the NS5A Y93H polymorphism. Shown are all positions within NS5A amino acids 1-125 where any change from baseline was observed in any of these 6 subjects with the Y93H polymorphism .

USUBJID	Timepoint	A30*	S54	S62*	T64	M79	H85	Y93*	S103	S116
AI444218-14-97	BL			L	A	T	Y	H	P	
AI444218-14-97	Failure			L	A	T	Y	H	P	
AI444218-24-71	BL			R	S	T		H	P	N
AI444218-24-71	Failure			R	S	T		H	P	N
AI444218-25-74	BL	S	Y	A		T		H	P	N
AI444218-25-74	Failure	S		A		T		H	P	N
AI444218-26-90	BL				S	T	H/Y	H/Y	P/S	D/N
AI444218-26-90	Failure			L	S	T		H	P	N
AI444218-7-98	BL	A/V				T		H/Y	P	N
AI444218-7-98	Failure			L		A		H	P	N
AI444218-9-88	BL			L	A/S	T		H	P	N
AI444218-9-88	Failure			L	A	T		H	P	N

Because the detection of the NS5A Y93H baseline polymorphism was a key factor associated with reduced efficacy of DCV/SOF in HCV GT3 infected subjects in ALLY-3, the review team is recommending that these data are described in Section 12.4 (Microbiology) and also referenced in Section 14 (Clinical Studies) of the DCV prescribing information; see review Section 5. The review team also considered describing these findings as a limitation of use statement, but concluded that the information in Sections 12.4 and 14 would be adequate and appropriate. An assay to identify the Y93H polymorphism in HCV GT3 infected patients is not commercially available, to this reviewer's knowledge, so it is unclear how care providers would use this information in clinical decision making. In addition, HCV GT3 infected patients with the Y93H polymorphism represent a small minority of the U.S. HCV infected patient population, considering that subjects with the Y93H baseline polymorphism are a small subpopulation within the GT3 population, and GT3 infection by itself is relatively uncommon in the U.S. ($\sim 10\%$ of HCV infections). Drug resistance-related risks for subjects with the Y93H polymorphism also appear to be small, given that additional major DCV resistance-associated substitutions did not emerge in subjects with the Y93H polymorphism who experienced virologic failure. Finally, a limitation of use statement describing reduced DCV/SOF efficacy in patients with cirrhosis will likely be included in the DCV prescribing information, and in ALLY-3 only 3 non-cirrhotic subjects with Y93H experienced virologic failure, representing only 2.6% of the noncirrhotic + cirrhosis-not-reported population.

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NS5B (sofosbuvir)

Baseline sofosbuvir resistance analyses focused on known resistance-associated positions in NS5B. Of 150 subjects with available data, no subjects had polymorphisms at positions L159, S282, C316, L320 or V321. This includes the 7 subjects who previously were treated with a SOF-containing regimen.

The sponsor conducted an exploratory next generation sequence analysis (performed by (b) (4) of NS5A and NS5B for the 7 prior SOF failure subjects in an attempt to detect resistance-associated substitutions as minor variants in the viral population, with a sensitivity cutoff of 1%. The sponsor's analysis is summarized in Table 10 ([Report 930085188 v1.0](#), pg. 15). Consistent with the population nucleotide sequence analysis data, SOF resistance-associated substitutions were not detected at baseline or at the time of relapse. Furthermore, the detection of DCV resistance-associated polymorphisms/substitutions in NS5A was consistent with the population sequencing data, with two examples of polymorphisms in minority viral populations that were detected by next generation sequencing but not by population sequencing: M28K in Subject 5-4 and S62A in Subject 33-76.

Table 10. HCV NS5A and NS5B next generation sequence analysis summary for subjects who previously received sofosbuvir-containing treatment.

Subject	Virologic Outcome FUWK12	Sample Visit	Viral Load (IU/mL)	Total Reads	% Q>=30	Average Read Length	% NS5A Variants ^a	% NS5B Variants ^b	
AI444218-5-4	SVR	BL	3467369	2220518	94.16	144.09	M28K 2.0%, S62T 99.5%	None	
AI444218-33-64	SVR	BL	6456542	2788292	93.78	143.76	None	None	
AI444218-23-84	SVR	BL	630957	2118344	88.52	144.94	None	None	
AI444218-17-54	SVR	BL	46774	2118344	88.52	144.94	S62T 99.6%	None	
AI444218-17-94	SVR	BL	389045	2579290	93.32	144.19	None	None	
AI444218-14-97	Relapse	BL	8709636	2554342	92.36	146.58	S62L 99.5%, Y93H 99.6%	None	
		FUWK4	2290868	2676076	92.87	146.08	S62L 99.6%, Y93H 99.6%	None	
AI444218-33-76	Relapse	BL	2884032	3054012	93.64	144.51	S62A 21.2%	None	
		FUWK4	1	1949845	2163314	88.24	145.54	Y93H 99.5%	None
		FUWK4	2	1949845	2623732	91.43	141.50	Y93H 99.6%	None
		FUWK4	3	1949845	2863612	92.13	143.20	Y93H 99.6%	None

BL - baseline, FUWK4 - follow-up Week4, NS5A - non structural protein 5A, NS5B - non structural protein 5B, ID - sample identification, IU/mL - international units per milliliter, Q - quality score, SVR12 - sustained viral response at week 12

^a NS5A, GT-3 amino acid positions closely monitored included M28, P29, A30, L31, P32, P58, S62, E92 and Y93

^b NS5B, GT-3 amino acid positions closely monitored included L159, I179, S282, F289, I293, C316, L320, V321, Q434, and P479

Independent analyses of the next generation sequencing data for these 7 subjects conducted by Dr. Eric Donaldson confirmed the sponsor's findings. See Appendix A for Dr. Donaldson's detailed review, which was also linked to IND 121165 SDN 46.

3.6 Persistence of Daclatasvir Resistance-Associated Substitutions

No data are available from AI444218 regarding the long term persistence of daclatasvir resistance-associated substitutions, as no subjects had resistance data available beyond Follow-up Weeks 4 to 12.

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Interim results from long term follow-up study AI444046, which were included in the Original DCV NDA 206843 submission, included data for 4 HCV GT3 infected subjects who failed treatment with DCV + P/R in clinical trial AI444031. Consistent with results from AI444218, all 4 subjects had NS5A Y93H detected at the time of virologic failure, with one subject also having Y93H at Baseline. Available long term follow-up resistance analysis results for these 4 subjects, focusing on Y93H, are summarized as follows:

- AI444031-14-137: Treatment-emergent Y93H persisted through Follow-up Day 158 (last available)
- AI444031-21-58: Treatment-emergent Y93H persisted through Follow-up Day 536, no longer detected at Follow-up Day 742
- AI444031-8-156: Treatment-emergent Y93H persisted through Follow-up Day 172, no longer detected at Follow-up Day 480
- AI444031-26-91: Y93H baseline polymorphism, which remained detected at all timepoints through Follow-up Day 547 (last available)

The results summarized above, although limited, indicate the potential for persistence of DCV resistance-associated substitutions in HCV GT3. The persistence of daclatasvir resistance-associated substitutions was previously documented for HCV GT1 infected subjects in the same AI444046 long term follow-up study (from the Clinical Virology review of the original DCV and ASV NDA submissions):

A total of 62 HCV genotype 1a subjects and 26 HCV genotype 1b subjects with DCV treatment-emergent substitutions had ≥ 1 year of post-treatment follow-up data in AI444046. Among the 62 genotype 1a subjects, 56 (90%) still had ≥ 1 key DCV treatment-emergent substitution detected through the end of follow-up, which was a median of 684.5 days. Similarly, 25/26 (96%) of genotype 1b subjects still had ≥ 1 key DCV treatment-emergent substitution detected through the end of follow-up, which was a median of 638 days.

3.7 Long-term Clinical Virology Follow-up of Subjects Achieving SVR12

No long-term follow-up data from clinical trial AI444218 are available to assess the durability of SVR12 for HCV GT3 infected subjects treated with DCV/SOF. As noted in the Clinical Virology review of the Original ASV/DCV NDA submissions as well as reviews of other HCV DAAs, virologic relapse after achieving SVR12 generally is rare. For example from the Phase 3 ASV/DCV (DUAL and QUAD) trials, among subjects who achieved SVR12 with available data at Follow-up Weeks 12 and 24, 5/1019 (0.5%) experienced virologic relapse by Follow-up Week 24.

4. CONCLUSIONS

NDA 206843 is approvable from a Clinical Virology perspective for daclatasvir in combination with sofosbuvir for the treatment of adults with chronic HCV genotype 3 infection. See the following review sections for recommended edits to the proposed prescribing information as well as recommended post-marketing studies.

5. PACKAGE INSERT

Clinical virology-related sections of the DCV prescribing information with initial review team-recommended changes are shown below, followed by summaries of the sponsor's responses to these

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initial recommendations. Please see the review by Lalji Mishra, Ph.D., for additional recommended edits to the nonclinical virology-related text in Section 12.4 of the label. Note that the final approved package inserts were not available at the time of finalization of this review.

12.4 Microbiology-Resistance-In Clinical Studies

Of 152 HCV genotype 3 infected subjects treated in the ALLY-3 trial, 17 experienced virologic failure, of whom 12 had cirrhosis. Post-baseline NS5A and NS5B population nucleotide sequencing data were available for virus from 17/17 and 16/17 subjects, respectively. Virus from all 17 subjects at the time of virologic failure harbored one or more of the NS5A resistance-associated substitutions ^{(b) (4)}A30K/S, L31I, S62A/L/P/T, ^{(b) (4)}or Y93H-^{(b) (4)}. The most common substitution at failure was Y93H (15/17 subjects), which was observed at baseline in 6 subjects and emerged in 9 subjects. For NS5B, 1 of 16 subjects had virus with the emergent NS5B resistance-associated substitution S282T at failure.

Sponsor's response in SDN 48: The sponsor agreed with the suggested edits to this section.

12.4 Microbiology-Persistence of Resistance-Associated Substitutions

^{(b) (4)} data from ALLY-3 ^{(b) (4)} on the persistence of daclatasvir resistance-associated substitutions in HCV genotype 3 infected subjects. In a separate long-term follow-up study of predominately HCV genotype 1 infected subjects treated with daclatasvir-containing regimens in phase 2/3 clinical trials, viral populations with treatment-emergent NS5A resistance-associated substitutions persisted at detectable levels for more than ^{(b) (4)} 1 year in most subjects.

Sponsor's response in SDN 48: The sponsor agreed with the suggested edits to this section except that in the first sentence, rather than stating "^{(b) (4)} data" the sponsor proposed stating "Limited data" noting that data are available from one subject. The sponsor did not indicate a subject ID, and to this reviewer's knowledge, no data on the persistence of DCV resistance-associated substitutions from subjects in ALLY-3 have been submitted. Nevertheless, this reviewer concurs with the sponsor's minor edit as it does not change the intended message of this section. We are recommending a post-marketing requirement for the sponsor to conduct a more thorough analysis of the persistence of DCV resistance-associated substitutions in HCV GT3 infected subjects.

12.4 Microbiology-Effect of Baseline HCV Polymorphisms on Treatment Response

In an analysis of ^{(b) (4)}-148 subjects with available baseline resistance data in ALLY-3, virus from 52% ^{(b) (4)} 77/^{(b) (4)} 148) of subjects had baseline NS5A polymorphisms at resistance-associated positions (defined as any change from reference at NS5A amino acid positions 28, 30, 31, 58, 62, 92, or 93) identified by population sequencing. The Y93H polymorphism was detected in 9% (13/148) of subjects receiving DALKINZA and sofosbuvir and was associated with reduced SVR12 rates ^{(b) (4)}. Polymorphisms detected at other NS5A resistance-associated positions were not associated with reduced SVR12 rates; these polymorphisms included M28V (n=1), A30V/K/S/T (n=14), P58R/S (n=3) and S62-any (n=66). ^{(b) (4)} Polymorphisms at positions associated with sofosbuvir resistance or exposure (defined as any change from reference at NS5B positions L159, S282, C316, L320 or V321) were not detected in the baseline NS5B sequence of any subject (n=150) in ALLY-3 by population-based sequencing. Phylogenetic

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analysis of NS5A sequences indicated that all subjects with available data (n=14) were infected with HCV subtype 3a.

Sponsor's response in SDN 48: The sponsor generally agreed with the suggested edits to this section, with minor editorial corrections. Note that the sponsor originally proposed a baseline polymorphism table to go with this section, which the review team had initially modified and moved to Section 14 (see below). After discussion with the sponsor and subsequent internal discussion, the review team decided to place the table back in Section 12.4.

14 Clinical Studies

Add to the description of ALLY-3:

Previous exposure to NS5A inhibitors was prohibited.

Delete (moved to Section 12.4):

~~Phylogenetic analysis of NS5A sequences indicated that all subjects with available data (n=14) were infected with HCV subtype 3a.~~

Add the following baseline Y93H polymorphism efficacy analysis from ALLY-3:

[Redacted content] (b) (4)

Table x. SVR12 Rates in Subjects with HCV Genotype 3 with/without the Baseline NS5A Y93H Polymorphism, by Cirrhosis Status

<u>Study Population</u>	<u>SVR12 with Y93H</u>	<u>SVR12 without Y93H</u>
<u>All Subjects</u>	<u>54%</u> <u>(7/13)</u>	<u>92%</u> <u>(124/135)</u>
<u>No Cirrhosis^a</u>	<u>67%</u> <u>(6/9)</u>	<u>98%</u> <u>(105/107)</u>
<u>With Cirrhosis</u>	<u>25%</u> <u>(1/4)</u>	<u>68%</u> <u>(19/28)</u>

^aIncludes 11 subjects with missing or inconclusive cirrhosis status

Sponsor's response in SDN 48: The sponsor agreed with the suggested edits to this section regarding previous exposure to NS5A inhibitors being exclusionary in ALLY-3, and also moving the subtype 3a description to Section 12.4. The sponsor did not agree with the placement of the table showing SVR12 according to Y93H polymorphism detection, and counter-proposed including only a brief summary statement in the text. We feel it is important that the data are included in table form to illustrate more clearly the association of the Y93H polymorphism with reduced treatment efficacy. After further internal discussion, the review team decided it was appropriate to move the resistance polymorphism table and description back to Section 12.4, consistent with the sponsor's original proposal. In addition, the review team added a statement in Section 14 referring to these data.

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6. RECOMMENDATIONS

We recommend the following studies as post-marketing requirements:

- Conduct a trial to determine if a longer duration of treatment or addition of ribavirin improves the efficacy (i.e., sustained virologic response rate) of daclatasvir plus sofosbuvir for hepatitis C virus genotype 3 infected subjects with cirrhosis.
- Characterize the long-term persistence of treatment-emergent, daclatasvir resistance-associated substitutions in HCV genotype 3 infected subjects.

7. REFERENCES

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8. APPENDIX A: Independent review of next generation sequencing data from AI444218 (ALLY-3) by Dr. Eric Donaldson, Ph.D. (linked to IND 121165 SDN 46)

BACKGROUND AND SUMMARY

IND 121165 covers the development of daclatasvir (DCV, BMS-790052, NS5A inhibitor) in combination with sofosbuvir (SOF, uridine nucleotide analogue prodrug NS5B polymerase inhibitor).

In this submission, the sponsor submitted a supplementary report detailing how they captured and assessed in-house-generated resistance data for samples where the vendor was unable to obtain a result and phenotypic data not available at the time of the SVR12 database lock. This review focused upon the Next Generation Sequencing (NGS) data submitted for seven treatment-experienced subjects infected with HCV GT3a from clinical trial AI444218 (ALLY-3), "*A Phase 3 Evaluation of Daclatasvir and Sofosbuvir in Treatment Naïve and Treatment Experienced Subjects with Genotype 3 Chronic Hepatitis C Infection.*" NGS was performed by (b) (4) using Illumina technology to analyze 7 subjects who had failed prior treatment with SOF-containing regimens (SOF/ribavirin [RBV] and SOF/IFN/RBV) to determine the presence of any minor variants in the virus population (at a sensitivity cut-off of 1%).

NEXT GENERATION SEQUENCING ANALYSIS

NGS analyses were performed on samples derived from 7 subjects who had failed prior treatment with SOF-containing regimens to determine if amino acid substitutions, at a sensitivity cut-off of 1%, in NS5A and/or NS5B could be associated with treatment failure. Five of these subjects achieved SVR12 with the combination of DCV+SOF, while two subjects experienced relapse. Baseline samples from all 7 subjects were sequenced and compared to see if any amino acid polymorphisms previously associated with DCV failure were present in the sequences derived from the subjects who relapsed. In addition, samples were sequenced from the Follow-up Week 4 (FUWK4) timepoint for the two subjects who relapsed, as this was the timepoint sample closest to the time of relapse. For one subject who relapsed, subject AI444218-33-76, analysis of the FUWK4 sample was performed in triplicate to evaluate assay reproducibility.

For resistance assessments, the sponsor focused on specific resistance-associated substitutions known to lead to resistance to DCV, an NS5A inhibitor, and that were associated with resistance to SOF, an NS5B nucleotide analog protease inhibitor. In addition, given that all subjects were infected with HCV GT3a, the sponsor used the HCV GT3a strain S52 sequence as a reference sequence for alignments and comparison (Accession number: GU814263). The amino acid positions that were focused upon were:

- NS5A, GT-3 amino acid positions closely monitored included: M28, P29, A30, L31, P32, P58, S62, E92, and Y93
- NS5B, GT-3 amino acid positions closely monitored included: L159, I179, S282, F289, I293, C316, L320, V321, Q434, and P479

The total number of reads per sample ranged from 2,118,344 to 3,054,012 with average read lengths ranging from 142 to 147. NS5A_Y93H was associated with failure in both subjects who experienced relapse, but no treatment-emergent NS5B amino acid substitutions were identified (Table 1).

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Table 1. GT-3 NS5A and NS5B Next Generation Sequencing (Table 6, page 15, BMS 790052 AI444218 Resistance Data Report).

Subject	Virologic Outcome FUWK12	Sample Visit	Viral Load (IU/mL)	Total Reads	% Q>=30	Average Read Length	% NS5A Variants ^a	% NS5B Variants ^b
AI444218-5-4	SVR	BL	3467369	2220518	94.16	144.09	M28K 2.0%, S62T 99.5%	None
AI444218-33-64	SVR	BL	6456542	2788292	93.78	143.76	None	None
AI444218-23-84	SVR	BL	630957	2118344	88.52	144.94	None	None
AI444218-17-54	SVR	BL	46774	2118344	88.52	144.94	S62T 99.6%	None
AI444218-17-94	SVR	BL	389045	2579290	93.32	144.19	None	None
AI444218-14-97	Relapse	BL	8709636	2554342	92.36	146.58	S62L 99.5%, Y93H 99.6%	None
		FUWK4	2290868	2676076	92.87	146.08	S62L 99.6%, Y93H 99.6%	None
AI444218-33-76	Relapse	BL	2884032	3054012	93.64	144.51	S62A 21.2%	None
		FUWK4 1	1949845	2163314	88.24	145.54	Y93H 99.5%	None
		FUWK4 2	1949845	2623732	91.43	141.50	Y93H 99.6%	None
		FUWK4 3	1949845	2863612	92.13	143.20	Y93H 99.6%	None

BL - baseline, FUWK4 - follow-up Week4, NS5A - non structural protein 5A, NS5B - non structural protein 5B, ID - sample identification, IU/mL international units per milliliter, Q - quality score, SVR12 - sustained viral response at week 12

^a NS5A, GT-3 amino acid positions closely monitored included M28, P29, A30, L31, P32, P58, S62, E92 and Y93

^b NS5B, GT-3 amino acid positions closely monitored included L159, I179, S282, F289, I293, C316, L320, V321, Q434, and P479

The division does an independent analysis of resistance data, and so, the fastq NGS files from the different samples from all 7 subjects were submitted to the FDA and analyzed independently using a bioinformatics pipeline previously described ([Donaldson et al., 2015](#)). Briefly, the sequence reads from each sample were assembled to reference sequences using the HCV GT3a S52 NS5A and NS5B genes as references, and variants were called using the Quality Based Variant Detector (QbVD) or the Probabilistic Variant Detector set to a threshold of 75% (PVD75) as previously described ([Donaldson et al., 2015](#)).

The assemblies produced by (b) (4) workbench had nearly identical coverages and quality scores as those reported by the sponsor (data not shown). In addition, in most cases, the NS5A amino acid substitutions previously associated with resistance to DCV that were detected by BMS were also detected in the independent analysis (Table 1 and Table 2). There were no substitutions in the NS5B that were previously identified as associated with resistance to SOF (Table 1 and Table 2).

Table 2. Detection of candidate NS5A resistance pathways by different variant callers (DAVP Analysis).

USUBJID	VISIT	AAPOS	TCOV	AAREF	AASUB	VCOV	AAFREQ	Protein	VARDECT	Analysis conclusion	Agreement
AI444218-14-97	Baseline	62	15124	S	L	14900	98.52	NS5A	QbVD	S62L present at baseline at >98.5% and persisted at this frequency through W4FU. Y93H was present at baseline at >99.8% frequency and persisted at this frequency through W4FU.	Yes, complete agreement
AI444218-14-97	Baseline	62	117976	S	L	117320	99.44	NS5A	PVD75		
AI444218-14-97	FUWK4	62	8294	S	L	8154	98.31	NS5A	QbVD		
AI444218-14-97	FUWK4	62	94199	S	L	93617	99.38	NS5A	PVD75		
AI444218-14-97	Baseline	93	116594	Y	H	116457	99.88	NS5A	QbVD		
AI444218-14-97	Baseline	93	120570	Y	H	120279	99.76	NS5A	PVD75		
AI444218-14-97	FUWK4	93	89317	Y	H	89201	99.87	NS5A	QbVD		
AI444218-14-97	FUWK4	93	94613	Y	H	94246	99.61	NS5A	PVD75		
AI444218-17-54	Baseline	62	93485	S	T	92962	99.44	NS5A	QbVD	S62T was present at >98.9% at BL	Yes, complete agreement
AI444218-17-54	Baseline	62	99411	S	T	98348	98.93	NS5A	PVD75		
AI444218-33-76	Baseline	62	113112	S	A	24113	21.32	NS5A	QbVD	S62A was present at 21.32% at baseline (QbVD) but not detected at W4FU. Y93H was not detected at BL but was present at >99.5% frequency at W4FU in three replicates.	Yes, complete agreement; however, S62T was detected by only one variant caller in CLC
AI444218-33-76	FUWK4-1	93	97143	Y	H	96944	99.80	NS5A	QbVD		
AI444218-33-76	FUWK4-1	93	100681	Y	H	100260	99.58	NS5A	PVD75		
AI444218-33-76	FUWK4-2	93	77221	Y	H	77081	99.82	NS5A	QbVD		
AI444218-33-76	FUWK4-2	93	79922	Y	H	79599	99.60	NS5A	PVD75		
AI444218-33-76	FUWK4-3	93	99773	Y	H	99652	99.88	NS5A	QbVD		
AI444218-33-76	FUWK4-3	93	103557	Y	H	103242	99.70	NS5A	PVD75		
AI444218-5-4	Baseline	62	54061	S	T	53816	99.55	NS5A	QbVD	S62T was present at >99% frequency at BL	Yes: Not detect M28K (2%)
AI444218-5-4	Baseline	62	61717	S	T	61218	99.19	NS5A	PVD75		

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However, there were two variants detected by BMS that were missed by one or both variant callers in (b) (4):

1. **AI444218-33-76:** S62A was detected at ~20% by BMS and QbVD but missed by PVD75
2. **AI444218-5-4:** M28K was detected in one sample at ~2% by BMS, but was not detected by either (b) (4) variant caller

Two of the three algorithms (BMS and QbVD) identified the S62A substitution at a frequency of ~20%, indicating that this substitution was likely present at baseline in subject AI444218-33-76; however, its impact on treatment outcome is unknown. It appears that this position in the NS5A of HCV GT3a is highly polymorphic. The M28K substitution was only detected by BMS and at a low frequency (~2%). This difference in detection highlights the potential problems associated with NGS data analysis in that different filtering criteria may have removed reads containing the codon for this substitution from the assemblies generated by (b) (4).

Alternatively, the BMS analysis pipeline may have called this variant based upon poor read quality or included reads that contained sequencing errors. Regardless, subject AI444218-5-4 achieved SVR12, so this substitution did not have an impact on treatment outcome with the combination regimen and no later timepoints were sequenced.

The sponsor concluded, based on the NGS data, that the following resistance pathways were associated with treatment failure:

1. **AI444218-14-97**
 - a. NS5A: S62L and Y93H present at >99% frequency at baseline and persisted through FUWK4
 - b. NS5B: No resistance-associated substitutions detected
2. **AI444218-33-76**
 - a. NS5A: S62A present at baseline, but not at FUWK4 and Y93H emerged (>99% at FUWK4, in all replicates)
 - b. NS5B: No resistance-associated substitutions detected

In the independent assessment of the NGS data, we looked to see if any baseline polymorphisms or treatment-emergent substitutions in NS5B could be associated with relapse in the two subjects who failed treatment. Baseline analysis was conducted by comparing the baseline NS5B sequences of the 5 subjects who achieved SVR12 to the baseline NS5B sequences of the 2 subjects who failed. Two NS5B polymorphisms, M82T and I116T, were present in both relapsers and these were detected by both the PVD75 and QbVD variant callers (Figure 1). One subject who achieved SVR12 had an M82T polymorphism at baseline (Figure 1). NS5B positions M82 and I116 were determined to be polymorphic in the resistance analysis for SOF pivotal trials.

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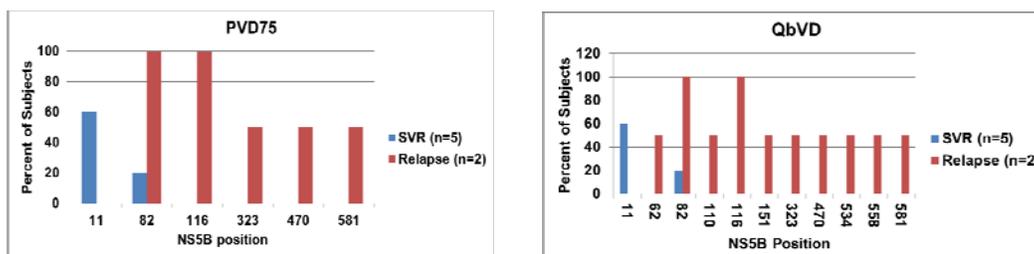


Figure 1. NS5B baseline analysis (DAVP analysis). Baseline NS5B polymorphisms were assessed using the PVD75 (left panel) and QbVD (right panel) variant callers. Two NS5B polymorphisms, M82T and I116T, were present in both relapsers.

There were a few additional NS5B polymorphisms present in one relapser and detected by one or both variant callers (Figure 1); however, none of these were at positions determined to be associated with SOF failure. In fact, the majority of these amino acid substitutions were observed in the SOF clinical trials and determined to be polymorphic sites that were not likely to be associated with resistance.

To determine if any treatment-emergent amino acid substitutions could be associated with treatment failure, the sequences derived from the samples taken at Follow-up Week 4 were assessed and compared to the baseline sequences for each of the subjects who relapsed. In general for NGS analysis, resistance-associated substitutions are defined as those substitutions that are not present at baseline (frequency <1%) but are present at the timepoint closest to failure at a frequency >10%. No substitutions in the FUWK4 samples for the two relapsers met this criterion (Table 3).

All of the amino acid substitutions that increased or decreased to a frequency >10% in the FUWK4 sequence sample compared to the baseline sequence sample for each relapse subject were then assessed to determine if any of these substitutions were detected during the pivotal SOF clinical trials. A total of 14 substitutions were identified that met this definition; however, the majority of these substitutions had been detected in SOF clinical trials and ruled unlikely to be associated with resistance. Two substitutions were of particular interest, including D62N, which was a potential SOF resistance-associated site in HCV GT1a and S470N, which was not observed in the SOF clinical trials; however, given that the numbers were limited (n=1 each), and the substitutions were also present at baseline, no definitive resistance determination could be made.

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Table 3. Detection of NS5B treatment-emergent substitutions in two relapsers by different variant callers (DAVP Analysis).

USUBJID	Increase	Emerge	Explanation	VARDECT	Resistance
AI444218-14-97	-10.40	D62N	Detected at 10.4% at baseline but not detected at FUWK4	QbVD	D61G emerged in three on-treatment failures in P7977-2025, and all three of these had variable substitutions at position 62.
AI444218-14-97	-20.51	S110F	Detected at 20.5% at baseline but not detected at FUWK4	QbVD	Not seen elsewhere
AI444218-14-97	-34.55	K519R	Detected at 34.6% at baseline but not detected at FUWK4	QbVD	Not likely
AI444218-14-97	-34.56	K519R	Detected at 34.6% at baseline but not detected at FUWK4	PVD75	
AI444218-14-97	-20.03	T534I	Detected at 20% at baseline but not detected at FUWK4	QbVD	Not likely
AI444218-14-97	-22.68	V581I	Detected at 22.7% at baseline but not detected at FUWK4	QbVD	Not seen elsewhere
AI444218-14-97	-22.62	V581I	Detected at 22.6% at baseline but not detected at FUWK4	PVD75	
AI444218-33-76	49.92	M82T	Detected at 47.5% at BL increased to ~97% at FUWK4	QbVD	Not likely, probably polymorphic
AI444218-33-76	49.79	M82T	Detected at 47.5% at BL increased to ~97% at FUWK4	PVD75	
AI444218-33-76	19.94	R114K	Detected at 79.4% at BL increased to >99.7% at FUWK4	QbVD	Not likely
AI444218-33-76	19.79	R114K	Detected at 79.7% at BL increased to >99.5% at FUWK4	PVD75	
AI444218-33-76	-79.55	I116T	Detected at 79.6% at baseline but not detected at FUWK4	QbVD	Not likely
AI444218-33-76	20.27	I116T	Detected at 79.4% at BL increased to >99.7% at FUWK4	PVD75	
AI444218-33-76	-99.85	D117N	Detected at 99.9% at baseline but not detected at FUWK4	QbVD	Emerged in two relapsers in SOF phase 3/ unlikely
AI444218-33-76	-19.54	A150T	Detected at 19.5% at baseline but not detected at FUWK4	QbVD	Polymorphic site
AI444218-33-76	-13.09	I184T	Detected at 13% at baseline but not detected at FUWK4	QbVD	Not likely
AI444218-33-76	48.92	R374L	Detected at ~48% at BL increased to >97.2% at FUWK4	QbVD	Not likely
AI444218-33-76	48.73	R374L	Detected at 48.3% at BL increased to >96.4% at FUWK4	PVD75	
AI444218-33-76	21.77	D376N	Detected at ~78.8% at BL increased to ~99.8% at FUWK4	QbVD	Not likely
AI444218-33-76	21.63	D376N	Detected at 79.4% at BL increased to >99.7% at FUWK4	PVD75	
AI444218-33-76	49.51	S470N	Detected at 47.6% at BL increased to >97.1% at FUWK4	QbVD	Not seen elsewhere
AI444218-33-76	49.55	S470N	Detected at 47.6% at BL increased to >96.1% at FUWK4	PVD75	

CONCLUSIONS

- The sponsor performed NGS analysis for 7 subjects infected with HCV GT3a, 5 who achieved SVR12 and two who relapsed.
- The sponsor provided the appropriate information for assessment of NGS data, including the raw fastq sequence files that were assessed independently.
 - Samples run in triplicate produced nearly identical results.
- Y93H was present at baseline in one subject who relapsed and emerged in the second relapser.
- No known NS5B resistance-associated substitutions were detected by NGS for the two subjects who relapsed.
- The analyses performed by the sponsor and by DAVP were in agreement.
- No further regulatory action is required for this submission.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

PATRICK R HARRINGTON
06/26/2015

ERIC F DONALDSON
06/29/2015

JULIAN J O REAR
06/29/2015

VIROLOGY REVIEW
DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
NDA 206843 SDN 36; Review Completed: 06/02/15

Reviewer: LALJI MISHRA, Ph.D.

Date Submitted: 02/13/15

Date Received: 02/13/15

Date Assigned: 02/13/15

Sponsor: Bristol-Myers Squibb Company

5 Research Parkway

P.O. Box 5100

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Charles D. Wolleben, Ph.D.

203-677-3817

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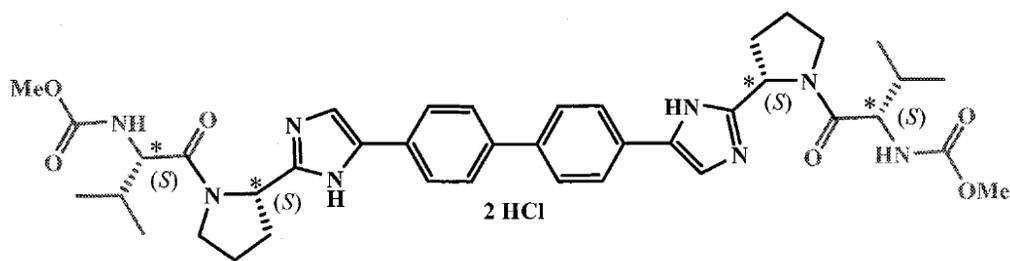
Product Names:

Proprietary: Daklinza

Non-proprietary: BMS-790052, daclatasvir

Chemical Names: [(1,1'-biphenyl)-4,4'-diylbis[1*H*-imidazole-4,2-diyl(2*S*)-2,1-pyrrolidinediyl[(1*S*)-1-(1-methylethyl)-2-oxo-2,1-ethanediyl]]]bis-carbamic acid, dimethyl ester, dihydrochloride

Structure:



Molecular formula: C₄₀H₅₀N₈O₆•2HCl

Molecular weight (free base): 738.88

Drug category: Antiviral

Indication: Treatment of HCV genotype-3 infection in combination with safosbuvir

Dosage Form/Route of administration: Oral solution

Additional submissions reviewed:

Supplement #	Date of Correspondence	Date of Receipt
N206843 SDN 37	03/02/15	03/02/15
N206843 SDN 38	03/06/15	03/06/15
N206843 SDN 40	03/27/15	03/27/15
N206843 SDN 45	05/22/15	05/22/15
N206843 SDN 48	06/24/15	06/24/15

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Abbreviations: A, alanine; AA, amino acid; BL, baseline; CC₅₀, 50% cytotoxic concentration; C_{max}, maximum observed plasma concentration; C_{trough}, trough concentration of drug in plasma; D, aspartate; DAA, direct acting antiviral agent; DCV, Daclatasvir; E, glutamate; EC₅₀, 50% effective concentration; EOT, end of treatment; EVR, early virologic response; F, phenylalanine; FUWK, follow-up week; G, glycine; GT, genotype; H, histidine; HCC, hepato cellular carcinoma (HCC); I, Isoleucine; K, lysine; L, leucine; LLOQ, lower limits of quantification; M, methionine; N, asparagine; P, proline; PDR, protocol defined response; Peg-IFN α , pegylated interferon α ; pM, picomolar; Q, glutamine; R, arginine; RBV, ribavirin; RAS, resistance-associated substitutions; RAV, resistance-associated variants; RVR, rapid virologic response; S, serine; SVR 24, sustained virologic response 24 weeks post-treatment; T, threonine; μ M, micromolar; V, valine; VBT, virologic breakthrough; WK, week; Y, tyrosine

BACKGROUND

Bristol-Myers Squibb Company (BMS) has resubmitted a New Drug Application (NDA # 206843) for daclatasvir (DCV) for use in combination with sofosbuvur (SOF) for the treatment of adults chronically infected with hepatitis C virus (HCV) genotype (GT)-3 (b) (4)

(b) (4). BMS had previously submitted original New Drug Application (NDA # 206843) for daclatasvir for use in combination with asunaprevir for the treatment of genotype 1b chronic hepatitis C virus infection, or in combination with asunaprevir, pegylated inteferon-alfa and ribavirin for the treatment of hepatitis C virus genotype 1a or 4 infection. However, BMS withdrew the original NDA on asunaprevir for safety reasons and could not provide supporting data for the marketing approval of daclatasvir alone. Subsequently a complete response letter (CRL) was issued to the sponsor on November 25, 2014.

BMS has submitted its response to the CR letter dated November 25, 2014. Non-clinical virology and Phase II clinical virology reports submitted in support of the NDA 206843 were previously reviewed (Virology review of NDA 206843 SDN 001 dated 07/08/14).

BMS stated that although effective treatments are available for HCV GT-1, -2, and -4 infections, there are limited treatment options for subjects infected with HCV GT-3. Currently, the combination of Sovladi™ (sofosbuvir [SOF]) and ribavirin (RBV) given for 24 weeks is approved for the treatment of HCV GT-3. This combination produced sustained virologic response (SVR) rates of 77% (treatment-experienced) to 93% (treatment-naive), and the SVR rates can be influenced by the presence of cirrhosis in treatment-experienced subjects (SVR12 of 60% [cirrhosis] vs. 85% [non-cirrhosis]). With SOF/RBV for 12 weeks, lower SVR rates of 37% (treatment-experienced) to 61% (treatment-naive) in non-cirrhotic GT-3 subjects and 19% (treatment-experienced) to 34% (treatment-naive) in cirrhotic GT-3 subjects were achieved. In addition, 24 weeks of (b) (4) (b) (4) is recommended in Europe for the treatment of subjects with HCV GT-3, who have cirrhosis and/or prior treatment failure. Among treatment-naive GT-3 subjects with and without cirrhosis who were administered (b) (4) for 12 weeks, SVR12 rates were 64% (16/25) and 100% (26/26), respectively. However, the clinical data to support the use of the (b) (4) regimen in subjects infected with HCV GT-3 are limited. Despite these

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treatment advancements, there remains an unmet medical need for HCV treatments, particularly for GT-3 subjects. Subjects with GT-3 tend to have lower response rates than subjects with other HCV genotypes. GT-3 subjects show faster progression of fibrosis and liver disease, and are likely to have steatosis (fatty liver), which is thought to contribute to the accelerated disease progression and low SVR rates. These subjects also have a higher risk of advanced/late-stage liver disease conditions such as hepatocellular carcinoma (HCC). Therefore, there is a need for safe and effective HCV therapies (preferably without RBV) that can be given for shorter treatment durations to both treatment-naïve and treatment-experienced GT-3 subjects, including those with compensated cirrhosis.

In support of marketing application of DCV in combination with SOF for the treatment of HCV genotype-3 infection, BMS has submitted efficacy and safety data from a Phase 3 study AI444218 (ALLY-3) conducted in subjects with HCV GT-3 (n = 152), who were either treatment-naïve or treatment-experienced. Additional support of the DCV (60 mg QD)/SOF ± RBV 12 or 24 week regimen comes from a Phase 2 study AI444040 (n = 211, including 18 GT-3 infected subjects). Please see the review of Virology Reviewer Pat Harrington, Ph.D. for analysis of clinical virology and resistance data for study AI444218, AI444040 and other supportive studies.

Mechanism of action of DCV, antiviral activity of DCV in HCV replicon cell lines, cytotoxicity of DCV in cell culture, effect of serum binding on anti-HCV activity of DCV, anti-HCV activity of DCV in combination with interferon- α , selection of HCV variants resistant to DCV in cell culture replicon cells, genotypic and phenotypic analyses of DCV resistant mutants, and activity of DCV against GT-1a, -1b, and -2a replicons, GT-3a, -4a, -5a and -6a hybrid replicons were previously reviewed (See Virology Review of NDA 206843 SDN 001 dated 07/08/14).

BMS has submitted additional data on the antiviral activity of DCV against HCV genotypes 1a, 1b, 2, 3a, 3b, 4, 5 and 6 in the resubmitted NDA 206843. These HCV isolates were derived from the clinical specimens. Since BMS is seeking an indication for treatment of HCV genotype 3 infection, the antiviral activity data submitted against genotype 3a, 3b are reviewed first followed by data for other HCV genotypes.

I. Prevalence of HCV genotypes in HCV infected subjects

HCV is classified into at least six different genotypes and multiple subtypes based on phylogeny. GT-3 is endemic in south-east Asia and is variably distributed in different countries. For example GT-3 is the predominant genotype in India, Pakistan and Brazil and accounts for >30% cases in Greece, Poland, Netherlands and China ([Hernandez et al., 2013](#)). In the United States genotype-1 accounts for 77% of infections, genotype-2 for 11.3%, and genotype-3 for 9.6% ([Nainan et al., 2006](#); see Figure 1, Page 4). Genotypes-2, -4, and -6 are clustered predominately in Southeast Asia, Africa, and the Middle East, and are found less commonly in the USA (NDA 206843, SDN 36, Module 2.2). For a large cohort of subjects with HCV infection (n=110,484) in the USA, [Kanwal et. al. \(2014\)](#) reported that the subjects with HCV genotype 3 infection were 31% and 80% more likely to develop cirrhosis and HCC, respectively, compared to subjects with the most common HCV genotype 1 infection.

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HCV Genotype Distribution

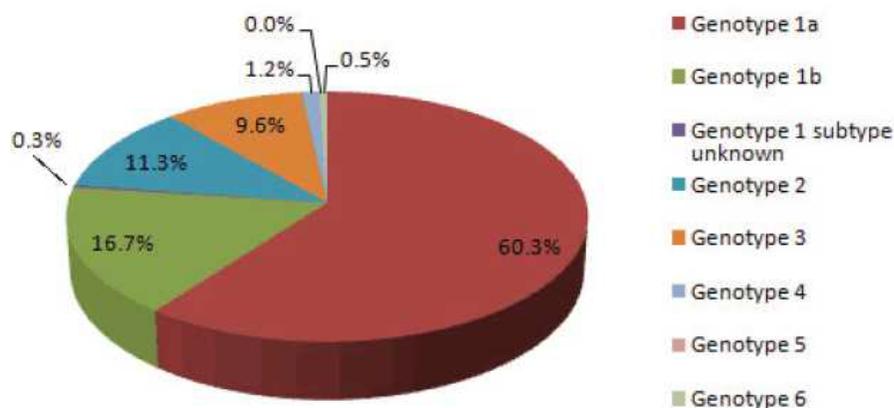


Figure 1: Distribution of HCV genotypes in the United States (Source: (b) (4), NDA 206843, SDN 36 dated 02/13/15)

The sponsor stated that the improvement in assay performance by inclusion of the core region, in addition to the 5' nontranslated region, has led to more accurate assessment of the distribution of subtypes 1a (60%) and 1b (17%) as compared with some of the earlier literature. Genotype 1b was significantly more common in individuals >50 years of age (P<0.0001) whereas genotype 3 was more likely to be seen in the younger population (<30 years old) (P<0.0001).

II (a). DCV susceptibility of genotype 3a, 3b subject-derived baseline NS5A hybrid replicons

Subject derived hybrid replicons were evaluated for their susceptibility to DCV using the luciferase transient HCV replication assay as described in Methodology. If no transient replication was detected, a stable cell line was selected after transfection of HCV RNA by selection with 0.5 mg/mL G418. The median EC₅₀ values for each subtype are shown in Table 1. Of the 25 tested GT-3 NS5A sequences, 21 segregated with GT-3a, 3 with GT-3b, and 1 with GT-3i. The median EC₅₀ value of DCV in hybrid replicons with GT-3a NS5A (n=21) was 0.256 nM and >5,000 nM against GT-3b (n=3) NS5A hybrid replicons and 3,650 nM against the GT-3i NS5A (n=1) hybrid replicon.

Table 1: Susceptibility of HCV GT-3 subject-derived NS5A hybrid replicons to inhibition by DCV (Source: NDA 206843 SDN 38, Response to FDA Comment Page 2 Table 1)

GT	Total			Without NS5A Polymorphisms ^a			With NS5A Polymorphisms ^a		
	No of NS5A sequences	Median EC ₅₀ (nM)	Range of EC ₅₀ values (nM)	No of NS5A sequences	Median EC ₅₀ (nM)	Range of EC ₅₀ values (nM)	No of NS5A sequences	Median EC ₅₀ (nM)	Range of EC ₅₀ values (nM)
			>5000						
3a	21	0.256	0.006 - 50	17	0.2	0.006 - 3.2	4	13.5	1.3 - 50
3b	3	>5000	NA	0	NA	NA	3	>5000	NA
3i	1	3620	NA	0	NA	NA	1	3620	NA

NA - not applicable, No - number,

^a GT-1a NS5A polymorphisms at M28 (excluded M28V), A30, L31 and Y93

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Table 1 shows that the median EC₅₀ value of DCV against 17 GT-3a subject-derived NS5A hybrid replicons without NS5A polymorphisms (at amino acid position 28, 30, 31, 62, 93) was 0.2 nM, and against 4 GT3a subject-derived NS5A hybrid replicons with NS5A polymorphisms, the median EC₅₀ value was 13 nM. Similarly, the median EC₅₀ value of DCV against 3 GT-3b subject-derived NS5A hybrid replicons with NS5A polymorphisms was >5,000 nM. EC₅₀ values were not available for GT-3b subjects without NS5A polymorphisms.

Comment

The median EC₅₀ values of DCV against 25 GT-3 subject-derived NS5A hybrid replicon are also included in Table 2, Page 6.

II (b). DCV susceptibility of genotype 1a, 1b, 2, 3, 4, 5, and 6 subject-derived baseline NS5A hybrid replicons

Table 2 shows the median EC₅₀ values of DCV against the genotype 1a, 1b, 2, 3, 4, 5 and 6 infected subject-derived baseline NS5A hybrid replicons. The median and range of DCV EC₅₀ values of GT-1a baseline NS5A sequences were calculated from DCV susceptibility data from 40 subjects. BMS stated that all except one EC₅₀ value were calculated from transient replication replicon assays while one EC₅₀ value was determined from a stable cell line assay.

Table 2: Susceptibility of HCV GT-1a, 1b, 2, 3,4, 5, 6 subject-derived NS5A hybrid replicons to inhibition by DCV, median EC₅₀ values (Source: NDA 206843, SDN 36, integrated resistance summary report; DCN-930077713, Page 16, Table 3)

GT	Total			Without NS5A Polymorphisms ^a			With NS5A Polymorphisms ^a		
	No of NS5A sequences	Median EC50 (nM)	Range of EC50 values (nM)	No of NS5A sequences	Median EC50 (nM)	Range of EC50 values (nM)	No of NS5A sequences	Median EC50 (nM)	Range of EC50 values (nM)
1a	40	0.008	0.002 - 2409	35 ^b	0.008	0.002 - 0.03	5	76	4.6 - 2409
1b	42	0.002	0.0007 - 10	30	0.002	0.0007 - 0.006	12 ^c	0.05	0.002 - 10
2	21	16	0.005 - 60	5	0.008	0.005 - 0.02	16	17.5	0.3 - 60
3	25	0.4	0.006 - >5000	17	0.2	0.006 - 3.2	8	1835	1.3 - >5000
4	14	0.025	0.001 - 158	4	0.003	0.001 - 0.007	10	0.035	0.007 - 158
5	3	0.004	0.003 - 0.019	0	NA	NA	3	0.004	0.003 - 0.019
6	1	NA	0.054	0	NA	NA	1	NA	0.054

^aGT-1a NS5A polymorphisms at M28 (excluded M28V), Q30, L31 and Y93; GT-1b at L31 and Y93H; GT-2 at F28 (excluded F28L), K30, L31 and Y93; GT- 3 at M28 (excluded M28V), A30, L31 and Y93; GT-4 at L28, L30, M31 (excluded M31L) and Y93.

^b1 subject had an NS5A sequence that more closely aligned with GT-1a H77c (80% sequence identity) than GT-1b Con1 (78% sequence identity); in this current analysis, the NS5A sequence was shown to more closely segregate with a GT-1c NS5A sequence (94% sequence identity with NS5A accession number D14853) than GT-1a.

^c2 subjects who were determined as GT-1b by the line probe genotyping assay had NS5A sequences that aligned with GT-1c and GT-11.

The mean EC₅₀ values of DCV for each genotype and each genotype subtype are shown in Table 3. The total mean EC₅₀ values significantly differ from the total median EC₅₀ values for subject-derived genotype 1a, 1b, 3, and 4 NS5A sequences reported in Table 1 and Table 2. The sponsor

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stated that the mean EC₅₀ values are impacted by the few subject-derived outlier sequences with and without baseline NS5A polymorphisms at amino acid positions 28, 30, 31, or 93. A Table of the EC₅₀ values of DCV for each of the tested subject-derived NS5A sequences is included in the Appendix.

Table 3: Susceptibility of HCV GT-1a, 1b, 2, 3,4, 5 , 6 subject derived-NS5A hybrid replicons to inhibition by DCV: mean EC₅₀ values (Source: NDA 206843, SDN 40) Response to DAVP comment Page 2, Table 1)

GT	Total			Without NS5A Polymorphisms ^a			With NS5A Polymorphisms ^a		
	No of NS5A sequences	Mean EC50 (nM)	Range of EC50 values (nM)	No of NS5A sequences	Mean EC50 (nM)	Range of EC50 values (nM)	No of NS5A sequences	Mean EC50 (nM)	Range of EC50 values (nM)
1a	40	66	0.002 - 2409	35 ^b	0.009	0.002 - 0.03	5	531	4.6 - 2409
1b	42	0.3	0.0007 - 10	30	0.002	0.0007 - 0.006	12 ^c	0.9	0.002 - 10
2	21	14	0.005 - 60	5	0.01	0.005 - 0.02	16	18	0.3 - 60
3a	21	4	0.006-50	17	0.5	0.006-3.2	4	20	1.3-50
3b	3	>5000	NA	0	NA	NA	3	>5000	NA
4	14	11	0.001 - 158	4	0.004	0.001 - 0.007	10	16	0.007 - 158
5	3	0.009	0.003 - 0.019	0	NA	NA	3	0.009	0.003 - 0.019
6	1	NA	0.054	0	NA	NA	1	NA	0.054

^aGT-1a NS5A polymorphisms at M28 (excluded M28V), Q30, L31 and Y93; GT-1b at L31 and Y93H; GT-2 at F28 (excluded F28L), K30, L31 and Y93; GT- 3 at M28 (excluded M28V), A30, L31 and Y93; GT-4 at L28, L30, M31 (excluded M31L) and Y93.

^b subjects had an NS5A sequence that more closely aligned with GT-1a H77c (80% sequence identity) than GT-1b Con1 (78% sequence identity); in this current analysis, the NS5A sequence was shown to more closely segregate with a GT-1c NS5A sequence (94% sequence identity with NS5A accession number D14853) than GT-1a.

^c2 subjects who were determined as GT-1b by the line probe genotyping assay had NS5A sequences that aligned with GT-1c and GT-1l.

Summary of median and mean EC₅₀ values of DCV reported in Table 2 and Table 3

- For isolates from 35 GT-1a subject-derived NS5A sequences without detectable polymorphisms at 28, 30, 31, or 93 known to be associated with resistance to DCV, the median and mean EC₅₀ values of DCV were 0.008 nM and 0.009 nM, respectively (range 0.002-0.03 nM).
- For isolates from 5 GT-1a subject-derived NS5A sequences with detectable polymorphisms at 28, 30, 31, or 93 associated with resistance to DCV, the median and mean EC₅₀ values were 76 nM and 531 nM, respectively (range 4.6-2,409 nM).
- For isolates from 30 GT-1b subject-derived NS5A sequences without detectable polymorphisms at 31 or 93 known to be associated with resistance to DCV, the median and mean EC₅₀ values of DCV were 0.002 nM and 0.002 nM, respectively (range 0.0007-0.006 nM).
- For isolates from 12 GT-1b subject-derived NS5A sequences with detectable polymorphisms at 31 or 93 associated with resistance to DCV, the median and mean EC₅₀ values of DCV were 0.05 nM and 0.9 nM, respectively (range 0.002-10 nM).

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- For isolates from 5 GT-2 subject-derived NS5A sequences without detectable polymorphisms at 28, 30, 31, or 93 known to be associated with resistance to DCV, the median and mean EC₅₀ values of DCV were 0.008 nM and 0.01 nM, respectively (range 0.005-0.020 nM).
- For isolates from 16 GT-2 subject-derived NS5A sequences with detectable polymorphisms at 28, 30, 31 or 93 associated with resistance to DCV, the median and mean EC₅₀ values of DCV were 17.5 nM and 18 nM, respectively, (range 0.3-60 nM).
- For isolates from 17 GT-3a subject-derived NS5A sequences without detectable polymorphisms at 28, 30, 31 or 93 known to be associated with resistance to DCV, the median and mean EC₅₀ values of DCV were 0.2 nM and 0.5 nM, respectively (range 0.006-3.2 nM).
- For isolates from 4 GT-3a subject-derived NS5A sequences with detectable polymorphisms at 28, 30, 31 or 93 associated with resistance to DCV, the median and mean EC₅₀ values of DCV were 13.5 nM and 20 nM, respectively (range 1.3-50 nM)
- For isolates from 3 GT-3b subject-derived NS5A sequences with detectable polymorphisms at 28, 30, 31 or 93 associated with resistance to DCV, the median and mean EC₅₀ values of DCV were >5,000 nM, respectively.
- For isolates from 4 GT-4 subject-derived NS5A sequences without detectable polymorphisms at 28, 30, 31, or 93 known to be associated with resistance to DCV, the median and mean EC₅₀ values of DCV were 0.003 nM and 0.004 nM, respectively (range 0.001 - 0.007 nM). Of the 4 GT-4 subjects, 3 were determined as GT-4a, and 1 was GT-4 (GT-4a by phylogenetic analysis) using the LiPA genotyping kit.
- For isolates from 10 GT-4 subject-derived NS5A sequences with detectable polymorphisms at 28, 30, 31, or 93 associated with resistance to DCV, the median and mean EC₅₀ values of DCV were 0.035 nM and 16 nM, respectively (range 0.007-158 nM). Of the 10 GT-4 subjects, 6 were determined as GT-4 (2 GT-4a, 2 GT-4d, 1 GT-4f and 1 GT-4n by phylogenetic analysis), 3 were GT-4a (2 GT-4a and 1 GT-4e by phylogenetic analysis) and 1 was GT-4e/o (GT-4o by phylogenetic analysis) using the LiPA genotyping kit.
- For isolates from 3 GT-5 subject-derived NS5A sequences with detectable polymorphisms at 28, 30, 31, or 93 associated with resistance to DCV, the median and mean EC₅₀ values were 0.004 nM and 0.009 nM, respectively (range 0.003-0.019 nM).
- The EC₅₀ value of DCV for isolates from 1 GT-6a NS5A hybrid replicon derived from a commercial sample (^{(b) (4)}) was 0.054 nM.

Comments

1. NS5A polymorphisms V/T28, H/R/30, I/M/V31, C/N/Y54, Q/P/R58, D/G/Q/V62, C/H/N/S/Y93 are known to be associated with resistance to DCV for baseline GT-1a isolates (Virology Review of NDA 206843 SDN 001 dated 07/08/14).
2. NS5A polymorphisms V28, Q30, I/M31, H/Y54, S58, D62, H93 are known to be associated with resistance to DCV for baseline GT-1b isolates (Virology Review of NDA 206843 SDN 001 dated 07/08/14).
3. NS5A polymorphisms C/L28, H/R30, M31, S8S, A/G/H/I/S/T62, 92S, H93 are known to be associated with resistance to DCV for baseline GT-2 isolates (Virology Review of IND 79599 SDN 655 dated 02/16/14).

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4. NS5A polymorphisms E/K/S/T/V30, A/T54, A/R/S58, A/L/P/T/V62, and H93 are known to be associated with resistance to DCV for baseline GT-3 isolates (Virology Review of IND 79599 SDN 655 dated 02/16/14).
5. NS5A polymorphisms 28M, R/S30, V31, N/R54, A/T58, E/K/Q62 are known to be associated with resistance to DCV for baseline GT-4 isolates (Virology Review of IND 79599 SDN 655 dated 02/16/14).
6. Data for baseline GT-5a and GT-6 isolates for NS5A polymorphisms associated with resistance to DCV are limited. However, DCV resistance-associated substitutions in GT-5a replicons frequently mapped to L31 and K56 of NS5A. For GT-6a, DCV resistance-associated substitutions frequently mapped to amino acid positions 24 (Q24H), 31 (L31M), 32 (P32L/S) and 58 (T58A/N/S) of NS5A (Virology Review of NDA (b)(4) SDN001 dated 07/08/14).

III. Selection of DCV-resistant GT-3a variants in cell culture

HCV GT-3a replicons with reduced susceptibility to DCV were selected in cell culture, and the genotype and phenotype of DCV resistant variants characterized. DCV resistant GT-3a variants contained NS5A amino substitutions A30K, L31I, S62L and Y93H. GT-2aJFH-1/ GT-3a hybrid replicons containing NS5A substitutions A30K, A30T, L31F, S42L and Y93H were tested for susceptibility to DCV using the renilla luciferase assay. Since some variants replicated poorly in the transient replication assay (Table 4) and accurate EC₅₀ values could not be obtained for the poorly replicating clones, EC₅₀ values were also derived from stable replicon cell lines. Phenotypic analysis in a stable cell line (Table 4) showed that variants containing A30K, A30T, L31F, S62L and Y93H substitutions exhibited 56-, 1-, 603-, 1.75-, and 2737-fold reduced susceptibility to DCV, respectively.

Table 4: Resistance profile of DCV resistant GT-3a replicons (Source: Addendum 02: In vitro efficacy of the HCV NS5A inhibitor, BMS-790052 (NDA206843 SDN 45, dated 05/22/15 and Virology Review of NDA 206843 SDN 000 dated 07/08/14)

Replicon ^a	Replication Level (%) ^b		EC ₅₀ (nM) ^{b,c}		EC ₅₀ (nM) ^{c,d}	
	Average	SD	Average	SD	Average	SD
GT-3a1 WT	100		0.25	0.10	0.53	0.15
A30K	13.8	2.0	15.4	7.3	29.6	4.4
A30T	103.3	4.5	0.11	0.05	0.57	0.013
L31F	152.8	4.1	80	35	320	5.9
S62L	88.1	3.1	0.53	0.039	0.93	0.17
Y93H	29.3	5.3	688	253	1451	344

^a GT-3a hybrid replicon with cell culture replication enhancing substitution S2204I

^b Data derived from transient replication assays

^c EC₅₀ - median effective concentration, SD - standard deviation, WT - wild type

^d Data derived from replicon cell lines

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Comment

Data on the cell culture susceptibility of DCV resistant GT-3a replicons were reviewed previously (see Virology Review of NDA 206843, SDN 000 dated 07/08/14 and Virology Review of IND 79599 SDN 647 dated 12/19/13).

IV. Cross-resistance

The anti-HCV activity of SOF against DCV-resistant variants containing DCA-resistance associated substitutions (M28T, Q30E, L31M/V, Y93H, M28D and H58D, L31M/V and Y93H) was determined to assess cross-resistance using a JFH1 hybrid replicon harboring H77cNS5A and 1b (con1) replicons (Table 4). The fold-change in susceptibility of DCV-resistant variants to SOF was less than 2-fold compared with the wild-type control indicating that SOF was fully active against the variants containing DCV resistance-associated substitutions in NS5A. As expected variants containing DCV resistance-associated substitutions were resistant (3 to >51,000-fold reduced susceptibility) to DCV.

Table 5: Anti-HCV activity of SOF against key DCV-resistant variants (Source: NDA 206843 SDN 36, Addendum 01 to Resistance Summary Report, Pages 20-21, Table 4)

NS5B genotype	NS5A genotype	NS5A substitutions	SOF		DCV	
			EC50 nM	FC over WT	EC50 nM	FC over WT
2a (JFH1) ^a	1a (H77c)	WT	202		0.006	
		M28T	130	0.64	3	500
		Q30E	110	0.54	111	18500
		L31V	258	1.28	15	2500
		Y93H	244	1.21	34	5667
		M28T,H58D	114	0.56	311	51833
NS5B genotype	NS5A genotype	NS5A substitutions	SOF		DCV	
			EC50 nM	FC over WT	EC50 nM	FC over WT
1b (Con1)	1b (Con1)	Q30E,L31M	127	0.63	492	82000
		L31M,Y93H	201	1.00	576	96000
		WT	143		0.003	
		L31M	138	0.97	0.008	3
		L31V	95	0.66	0.1	33
		Y93H	84	0.59	0.09	30
		L31M,Y93H	129	0.90	48	16000
L31V,Y93H	91	0.64	101	33667		

^a GT-1a NS5A substitutions were evaluated using a JFH1 hybrid replicon harboring H77c NS5A.

Comment

BMS stated that cross-resistance analyses have not been performed using GT-3 NS5A constructs. An addendum report to the non-clinical efficacy report will be prepared to capture the susceptibility of GT-3 DCV-resistant substitutions and wild-type to inhibition by sofosbuvir (SOF) in vitro.

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CONCLUSIONS

The median and mean EC₅₀ values of DCV against 17 GT-3a subject-derived NS5A sequences without detectable polymorphisms at 28, 30, 31 or 93 known to be associated with resistance to DCV were 0.2 nM and 0.5 nM, respectively (range 0.006-3.2 nM). However, the median and mean EC₅₀ values of DCV against isolates from 4 GT-3a subject-derived NS5A sequences with detectable polymorphisms at 28, 30, 31 or 93 associated with resistance to DCV were 13.5 nM and 20 nM, respectively (range 1.3-50 nM). Similarly, the median and mean EC₅₀ values of DCV against isolates from 3 GT-3b subject-derived NS5A sequences with detectable polymorphisms at 28, 30, 31 or 93 associated with resistance to DCV were >5,000 nM, respectively.

The median and mean EC₅₀ values of DCV for isolates from 35 GT-1a subject-derived NS5A sequences without detectable polymorphisms at 28, 30, 31, or 93 known to be associated with resistance to DCV were 0.008 nM and 0.009 nM, respectively (range 0.002-0.03 nM). However, the median and mean EC₅₀ values of DCV for isolates from 5 GT-1a subject-derived NS5A sequences with detectable polymorphisms at 28, 30, 31, or 93 associated with resistance to DCV were 76 nM and 531 nM, respectively (range 4.6-2409 nM).

The median and mean EC₅₀ values of DCV for isolates from 30 GT-1b subject-derived NS5A sequences without detectable polymorphisms at 31 or 93 known to be associated resistance to DCV were 0.002 nM and 0.002 nM, respectively (range 0.0007-0.006 nM). However, the median and mean EC₅₀ values of DCV for isolates from 12 GT-1b subject-derived NS5A sequences with detectable polymorphisms at 31 or 93 associated with resistance to DCV were 0.05 nM and 0.9 nM, respectively (range 0.002-10 nM).

The median and mean EC₅₀ values of DCV for isolates from 5 GT-2 subject-derived NS5A sequences without detectable polymorphisms at 28, 30, 31, or 93 known to be associated with resistance to DCV were 0.008 nM and 0.01 nM, respectively (range 0.005-0.020 nM). However, the median and mean EC₅₀ values of DCV for isolates from 16 GT-2 subject-derived NS5A sequences with detectable polymorphisms at 28, 30, 31 or 93 associated with resistance to DCV were 17.5 nM and 18 nM, respectively, (range 0.3-60 nM).

The median and mean EC₅₀ values of DCV for isolates from 4 GT-4 subject-derived NS5A sequences without detectable polymorphisms at 28, 30, 31, or 93 known to be associated with resistance to DCV were 0.003 nM and 0.004 nM, respectively (range 0.001-0.007 nM). However, the median and mean EC₅₀ values of DCV for isolates from 10 GT-4 subject-derived NS5A sequences with detectable polymorphisms at 28, 30, 31, or 93 associated with resistance to DCV were 0.035 nM and 16 nM, respectively (range 0.007-158 nM).

The median and mean EC₅₀ values of DCV for isolates from 3 GT-5 subject-derived NS5A sequences with detectable polymorphisms at 28, 30, 31, or 93 known to be associated with DCV resistance were 0.004 nM and 0.009 nM, respectively (range 0.003-0.019 nM).

The EC₅₀ value of DCV for isolates from 1 GT-6a NS5A hybrid replicon derived from a commercial sample was 0.054 nM.

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HCV GT-3a replicons with reduced susceptibility to DCV were selected in cell culture, and the genotype and phenotype of DCV resistant variants characterized. DCV resistant GT-3a variants contained NS5A amino substitutions A30K, L31I, S62L and Y93H. GT-2aJFH-1/ GT-3a hybrid replicons containing NS5A substitutions A30K, A30T, L31F, S42L and Y93H were tested for susceptibility to DCV using the renilla luciferase assay. Phenotypic analysis in a stable replicon cell line showed that variants containing A30K, A30T, L31F, S62L and Y93H substitutions exhibited 56-, 1-, 603-, 1.75-, and 2737-fold reduced susceptibility to DCV, respectively.

Results from a cross-resistance study showed that SOF was fully active against GT-1a and GT-1b variants containing DCA resistance-associated NS5A substitutions. However, cross-resistance data for DCV resistant GT-3a variants were not available. Additionally, susceptibility data for variants containing NS5B-S282T substitution to daclatasvir were not available.

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METHODOLOGY

Cell lines and viral constructs, cell culture assays, HCV replicon luciferase and FRET assays, transient replicon replication assays, quantification of HCV RNA, assays for cytotoxicity and cell culture inhibitor combination effect are described in BMS study report 930023288 and previously reviewed (Microbiology review of IND 79599 SDN 000 dated 11/08/07).

Construction of HCV hybrid replicons, chimeric replicons, HCV replicon luciferase assay, transient replicon replication assays, and infectious HCV assays were performed as described in Addendum 1 to Scientific Report 930023288 (NDA 206843; see Virology Review of IND 79599 SDN 557 dated 04/23/13).

Methods for the selection of BMS-790052 resistant HCV genotype 1a and 1b replicon cells are described in BMS Scientific Report 930023286 and previously reviewed (Microbiology Review of IND 79599 SDN 000 dated 11/08/07).

Methodology for the selection of DCV resistant GT 2a, 3a, 4a, 5a and 6a hybrid replicons are described in addendum 1, 2 and 3 to BMS Scientific Report 930023288, (NDA 206843) and and previously reviewed (Virology Review of IND 79599 SDN 557 dated 04/23/13, SDN 647 dated 12/19/13 and SDN 655 dated 02/06/14).

The parental GT-1a H77c, GT-1b Con1 and GT-2a JFH-1 replicon constructs have been described previously (Virology Review of IND 79599 SDN 000 dated 11/08/07). A recombinant PCR was performed to introduce amino acid substitutions of interest to the NS5A coding region of replicons as described previously for study AI444014 (Virology Review of IND 79599 SDN 605 dated 11/20/13). The GT-1b [REDACTED] (b) (4) to flank the NS5A region. The replicon NS5A region was replaced with subject-derived NS5A sequences; PCR amplicons infused onto either GT-1b [REDACTED] (b) (4)

[REDACTED]

The resulting hybrid replicons were evaluated for their susceptibility against DCV using the luciferase transient HCV replication assay described previously (BMS 2013, Document Control Number 93006888, Virology Review of IND 79599 SDN 605 dated 11/20/13). If no transient replication was detected, a stable cell line was selected after transfection of HCV RNA by selection with 0.5-mg/mL G418.3 The 50% effective concentration (EC₅₀) was calculated as the concentration of inhibitor required for a 50% reduction in luciferase activity.

Label

Note: The sponsor has accepted DAVP's version of label for Mechanism of Action and Resistance sections. The sponsor has added a Table for describing Antiviral Activity. The proposed DAVP version is shown in blue font (See Antiviral Activity)

CLINICAL PHARMACOLOGY

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12.1 Mechanism of Action

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

12.4 Microbiology

Mechanism of Action

Daclatasvir is an inhibitor of NS5A, a non-structural protein encoded by HCV. Daclatasvir binds to the N-terminus of NS5A and inhibits both viral RNA replication and virion assembly. (b) (4)
Characterization of daclatasvir resistant viruses, biochemical studies and computer modeling data indicate that daclatasvir interacts with the N- terminus within Domain 1 of the protein, which may cause structural distortions that interfere with NS5A functions.

Antiviral Activity

(b) (4)

Daclatasvir had a median EC₅₀ value of 0.2 nM (range 0.006-3.2 nM, n=17) against hybrid replicons containing genotype-3a subject-derived NS5A sequences without detectable daclatasvir resistance-associated polymorphisms at NS5A amino acid positions 28, 30, 31 or 93. Daclatasvir activity was reduced against genotype-3a subject-derived replicons with resistance-associated polymorphisms at positions 28, 30, 31 or 93, with a median EC₅₀ value of 13.5 nM

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(range 1.3-50 nM). Similarly, the EC₅₀ values of daclatasvir against 3 genotype-3b and 1 genotype-3i subject-derived NS5A sequences with polymorphisms (relative to a genotype-3a reference) at positions 30 or 31 were ≥3,620 nM.

The median EC₅₀ values of daclatasvir for genotypes-1a, -1b, -2, -4, and -5 subject-derived NS5A hybrid replicons were 0.008 nM (range 0.002 - 2,409 nM, n = 40), 0.002 nM (range 0.0007 - 10 nM, n = 42), 16 nM (range 0.005 - 60 nM, n = 16), 0.025 nM (range 0.001 - 158 nM, n = 14), and 0.004 nM (range 0.003 - 0.019 nM, n =3), respectively. The EC₅₀ value against a single HCV genotype-6 derived replicon was 0.054 nM.

Daclatasvir was not antagonistic with interferon alfa, HCV NS3/4A protease inhibitors, HCV NS5B nucleoside analog inhibitors, and HCV NS5B non-nucleoside inhibitors in cell culture combination antiviral activity studies using the cell-based HCV replicon system.

Resistance

In Cell Culture

HCV genotype-3a replicon variants with reduced susceptibility to daclatasvir were selected in cell culture, and the genotype and phenotype of daclatasvir resistant variants characterized. Phenotypic analysis of stable replicon cell lines showed that variant replicons containing A30K, A30T, L31F, S62L and Y93H substitutions exhibited 56-, 1-, 603-, 1.75-, and 2737-fold reduced susceptibility to daclatasvir, respectively.



Cross Resistance



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(b) (4)

(b) (4)

Based on resistance patterns observed in cell culture replicon studies and HCV genotype 3-infected subjects, cross-resistance between daclatasvir and other NS5A inhibitors is expected. Cross-resistance between daclatasvir and other classes of direct-acting antivirals is not expected. The impact of prior daclatasvir treatment experience on the efficacy of other NS5A inhibitors has not been studied. Conversely, the efficacy of daclatasvir (b) (4) sofosbuvir has not been studied in subjects who have previously failed treatment with regimens that include an NS5A inhibitor.

Revised version of the Microbiology Label (6/18/15)

BMS has made edits to the following sections of the previous version of the Microbiology Label. Sponsor's edits shown in red font in the Antiviral Activity section is not acceptable and is marked as strikethrough. Sponsor's edit in Cross-Resistance section shown in red font is not acceptable and marked as strikethrough. Sponsor's edit shown in blue strikethrough is acceptable.

Antiviral Activity

(b) (4)

Daclatasvir had a median EC₅₀ value of 0.2 nM (range 0.006-3.2 nM, n=17) against hybrid replicons containing genotype-3a subject-derived NS5A sequences without detectable daclatasvir resistance-associated polymorphisms at NS5A amino acid positions 28, 30, 31 or 93. Daclatasvir activity was reduced against genotype-3a subject-derived replicons with resistance-associated polymorphisms at positions 28, 30, 31 or 93, with a median EC₅₀ value of 13.5 nM (range 1.3-50 nM). Similarly, the EC₅₀ values of daclatasvir against 3 genotype-3b and 1 genotype-3i subject-derived NS5A sequences with polymorphisms (relative to a genotype-3a reference) at positions 30 or 31 were $\geq 3,620$ nM.

The median EC₅₀ values of daclatasvir for genotypes-1a, -1b, -2, -4, and -5 subject-derived NS5A hybrid replicons were 0.008 nM (range 0.002 - 2,409 nM, n = 40), 0.002 nM (range 0.0007 - 10 nM, n = 42), 16 nM (range 0.005 - 60 nM, n = 16), 0.025 nM (range 0.001 - 158 nM, n = 14), and 0.004 nM (range 0.003 - 0.019 nM, n =3), respectively. The EC₅₀ value against a single HCV genotype-6 derived replicon was 0.054 nM.

Daclatasvir was not antagonistic with interferon alfa, HCV NS3/4A protease inhibitors, HCV NS5B nucleoside analog inhibitors, and HCV NS5B non-nucleoside inhibitors in cell culture combination antiviral activity studies using the cell-based HCV replicon system.

Cross Resistance

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(b) (4)

Based on resistance patterns observed in cell culture replicon studies and HCV genotype 3-infected subjects, cross-resistance between daclatasvir and other NS5A inhibitors is expected. Cross-resistance between daclatasvir and other classes of direct-acting antivirals is not expected. The impact of prior daclatasvir treatment experience on the efficacy of other NS5A inhibitors has not been studied. Conversely, the efficacy of **DAKLINZA in combination with** sofosbuvir has not been studied in subjects who have previously failed treatment with regimens that include an NS5A inhibitor.

Microbiology Label (06/24/15)

12.1 Mechanism of Action

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

12.4 Microbiology

Mechanism of Action

Daclatasvir is an inhibitor of NS5A, a non-structural protein encoded by HCV. Daclatasvir binds to the N-terminus of NS5A and inhibits both viral RNA replication and virion assembly (b) (4). Characterization of daclatasvir resistant viruses, biochemical studies and computer modeling data indicate that daclatasvir interacts with the N-terminus within Domain 1 of the protein, which may cause structural distortions that interfere with NS5A functions.

Antiviral Activity

Daclatasvir had a median EC₅₀ value of 0.2 nM (range 0.006-3.2 nM, n=17) against hybrid replicons containing genotype-3a subject-derived NS5A sequences without detectable daclatasvir resistance-associated polymorphisms at NS5A amino acid positions 28, 30, 31 or 93. Daclatasvir activity was reduced against genotype-3a subject-derived replicons with resistance-associated polymorphisms at positions 28, 30, 31 or 93, with a median EC₅₀ value of 13.5 nM (range 1.3-50 nM). Similarly, the EC₅₀ values of daclatasvir against 3 genotype-3b and 1 genotype-3i subject-derived NS5A sequences with polymorphisms (relative to a genotype-3a reference) at positions 30 or 31 were ≥3,620 nM.

The median EC₅₀ values of daclatasvir for genotypes-1a, -1b, -2, -4, and -5 subject-derived NS5A hybrid replicons were 0.008 nM (range 0.002 - 2,409 nM, n = 40), 0.002 nM (range 0.0007 - 10 nM, n = 42), 16 nM (range 0.005 - 60 nM, n = 16), 0.025 nM (range 0.001 - 158 nM, n = 14), and 0.004 nM (range 0.003 - 0.019 nM, n =3), respectively. The EC₅₀ value against a single HCV genotype-6 derived replicon was 0.054 nM.

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Daclatasvir was not antagonistic with interferon alfa, HCV NS3/4A protease inhibitors, HCV NS5B nucleoside analog inhibitors, and HCV NS5B non-nucleoside inhibitors in cell culture combination antiviral activity studies using the cell-based HCV replicon system.

Resistance

In Cell Culture

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Cross Resistance

Based on resistance patterns observed in cell culture replicon studies and HCV genotype 3-infected subjects, cross-resistance between daclatasvir and other NS5A inhibitors is expected. Cross-resistance between daclatasvir and other classes of direct-acting antivirals is not expected. The impact of prior daclatasvir treatment experience on the efficacy of other NS5A inhibitors has not been studied. Conversely, the efficacy of DAKLINZA in combination with sofosbuvir has not been studied in subjects who have previously failed treatment with regimens that include an NS5A inhibitor.

RECOMMENDATION

With respect to virology, NDA 206843 is approvable.

Lalji Mishra, Ph.D.
Microbiologist, HFD-530

Concurrence

Julian O'Rear, Ph.D. _____ Signature _____ Date _____
HFD-530/Micro TL

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Appendix 1

Table 1: EC₅₀ values of DCV for subject-derived NS5A Sequences (Source: NDA 206843 SDN 38, Response to FDA Comment Pages 3-6 ,Table 1)

Subject ID	Genotype by commercial kit	Subtype by phylogeny	Visit	EC50 (nM)	NS5A variants ^a
AI447011-3-6	1a	1a	BL	0.006	H58P
AI447011-7-26	1a	1a	BL	0.008	
AI447011-13-5	1a	1a	BL	0.008	
AI444010-12-179	1a	1a	BL	0.007	
AI444010-18-3	1a	1a	BL	0.009	
AI444010-19-436	1a	1a	BL	76	Y93S
AI444010-26-287	1a	1a	BL	0.01	
AI444010-41-114	1a	1a	BL	0.02	
AI444010-58-254	1a	1a	BL	0.009	
AI444010-71-171	1a	1a	BL	0.008	
AI444010-73-255	1a	1a	BL	0.008	
AI444010-73-312	1a	1a	BL	0.007	
AI444010-7-246	1a	1a	BL	0.006	
AI444010-8-514	1a	1a	BL	0.005	
AI444010-30-257	1a	1a	BL	19	H54Y,E62E/G,Y93Y/H
AI444010-40-143	1a	1a	BL	0.03	
AI444010-42-327	1a	1a	BL	0.01	M28M/V
AI444011-18-328	1a	1a	BL	0.008	
AI444011-15-338	1a	1a	BL	0.01	
AI444011-58-44	1a	1a	BL	0.009	H58H/P
AI444011-29-264	1a	1a	BL	0.007	
AI444011-4-322	1a	1a	BL	0.008	
AI444011-52-362	1a	1a	BL	0.01	
AI444011-74-187	1a	1a	BL	0.03	M28M/V
AI444040-7-54	1a	1a	BL	4.6	M28M/V,Q30H/R
AI444040-19-134	1a	1a	BL	147	Y93N
AI444040-14-126	1a	1a	BL	2409	Q30E,Y93N
AI444014-4-42	1a	1a	BL	0.005	
AI443014-3-160	1a	1a	BL	0.007	M28M/V
AI444004-1-23	1a	1a	BL	0.007	
AI444004-5-60	1a	1a	BL	0.002	
AI444004-1-91	1a	1a	BL	0.008	
AI444004-1-93	1a	1a	BL	0.009	
AI444004-1-98	1a	1a	BL	0.006	E62D
AI444004-6-117	1a	1a	BL	0.02	
AI444004-10-118	1a	1a	BL	0.007	
AI444004-2-157	1a	1a	BL	0.008	
AI444004-4-141	1a	1a	BL	0.003	
AI444004-5-115	1a	1a	BL	0.01	
AI444010-11-284	1a	1c	BL	0.002	M28V,H58P,E62E/D
AI447011-7-2	1b	1b	BL	0.001	
AI447011-7-192	1b	1b	BL	0.006	
AI447011-12-14	1b	1b	BL	0.003	
AI447011-14-203	1b	1b	BL	0.002	
AI447011-20-208	1b	1b	BL	0.02	Y93S
AI447011-23-154	1b	1b	BL	0.004	
AI447011-23-191	1b	1b	BL	0.08	Y93H
AI447011-26-139	1b	1b	BL	0.003	P58T
AI447017-1-1001	1b	1b	BL	0.002	Y93Y/H
AI447017-1-1011	1b	1b	BL	0.003	P58L
AI447017-3-3003	1b	1b	BL	0.002	

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Subject ID	Genotype by commercial kit	Subtype by phylogeny	Visit	EC50 (nM)	NS5A variants ^a
AI447017-3-3004	1b	1b	BL	0.001	
AI447017-3-3011	1b	1b	BL	0.005	
AI447017-3-3013	1b	1b	BL	0.002	Y93Y/H
AI447017-4-4003	1b	1b	BL	0.004	
AI447017-4-4008	1b	1b	BL	0.004	
AI447017-4-4009	1b	1b	BL	0.002	P58P/S,Y93Y/H
AI447017-4-4012	1b	1b	BL	0.2	Y93H
AI444010-66-117	1b	1b	BL	0.001	
AI444010-38-392	1b	1b	BL	0.001	
AI444010-33-207	1b	1b	BL	0.001	P58S
AI444010-63-399	1b	1b	BL	0.001	
AI444010-73-273	1b	1b	BL	0.002	
AI444010-41-109	1b	1b	BL	0.002	
AI444010-28-542	1b	1b	BL	0.3	P58P/S,A92A/T,Y93Y/H
AI444011-53-372	1b	1b	BL	10	R30H,Y93H
AI444011-63-300	1b	1b	BL	0.002	R30Q
AI444011-74-154	1b	1b	BL	0.003	Q62Q/E
AI444011-75-232	1b	1b	BL	0.003	P58P/S
AI444021-1-21217	1b	1b	BL	0.002	
AI444021-4-21204	1b	1b	BL	0.0007	
AI444021-5-21201	1b	1b	BL	0.002	
AI444021-1-21214	1b	1b	BL	0.002	
AI444021-2-21208	1b	1b	BL	0.2	L31V
AI444021-2-21213	1b	1b	BL	0.007	L31L/M
AI444022-4-22206	1b	1b	BL	0.001	Q62E
AI444014-7-58	1b	1b	BL	0.002	P58T,Q62E
AI444004-4-127	1b	1b	BL	0.002	
AI444004-2-65	1b	1b	BL	0.001	
AI444004-6-16	1b	1b	BL	0.002	
AI444052-38-710	1b	1c	BL	0.2	L28V,R30Q,L31M
AI443014-7-119	1b	1l	BL	0.02	L28M,R30Q,L31M
AI444031-4-13	2a	2	BL	28	F28L,L31M
AI444031-10-186	2a	2a	BL	22	L31M
AI444031-28-193	2a	2a	BL	16	L31M
AI444031-29-75	2a	2a	BL	17	L31M
AI444031-31-119	2a	2a	BL	0.3	L31M,N62G
AI444031-3-29	2a	2a	BL	20	L31M
AI452017-158-552	2a/c	2a	BL	15	L31M
2a (HC-J6 in JFH-1 replicon)	2a	2a		18	L31M
2a (13-4 in JFH-1 replicon)	2a	2a		19	L31M
2a (14-1 in JFH-1 replicon)	2a	2a		8.8	F28L,L31M
AI444031-18-118	2b	2b	BL	0.005	F28L
AI444031-20-120	2b	2b	BL	17	F28L,L31M
AI444031-18-98	2b	2b	BL	6.8	F28L,L31M
AI444031-30-47	2b	2b	BL	0.006	F28L
AI444040-10-103	2b	2b	BL	0.008	F28L
AI444040-1-113	2b	2b	BL	18	F28L,L31M
AI444040-14-117	2	2b	BL	20	F28L,L31M

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Subject ID	Genotype by commercial kit	Subtype by phylogeny	Visit	EC50 (nM)	NS5A variants ^a
AI452017-23-311	2b	2b	BL	60	F28L,L31M,C92S
AI452017-8-235	2b	2b	BL	0.01	F28F/L
AI444031-28-172	2a	2c	BL	7	F28C
AI444031-9-143	2a	2c	BL	0.02	K30R
AI444031-16-147	3a	3a	BL	50	A30K,S62S/L
AI444031-30-154	3a	3a	BL	0.256	
AI444040-11-28	3a	3a	BL	0.2	
AI444040-9-20	3	3a	BL	1.3	A30S
AI444031-12-103	3a	3a	BL	0.1	
AI444031-12-121	3a	3a	BL	0.6	
AI444031-13-125	3a	3a	BL	0.2	P58S
AI444031-18-70	3a	3a	BL	0.3	
AI444031-21-61	3a	3a	BL	0.2	
AI444031-4-106	3a	3a	BL	10	E92E/A,Y93H
AI444031-4-31	3a	3a	BL	0.09	
AI444031-6-1	3a	3a	BL	0.4	S62T
AI444031-6-6	3a	3a	BL	0.1	S62S/T
AI444040-1-111	3	3a	BL	0.2	
AI444040-14-30	3	3a	BL	3.2	S62I
AI452017-139-262	3a	3a	BL	17	A30K
AI452017-98-1194	3a	3a	BL	0.006	M28V
3a (1-100 in Con1 replicon)	3a	3a		0.19	E92A
3a-1 (in JFH-1 replicon)	3a	3a		0.53	E92A
3a-2 (in JFH-1 replicon)	3a	3a		0.14	E92A
3a-4 (in JFH-1 replicon)	3a	3a		1.25	E92A
AI444040-8-47	3	3b	BL	>5000	A30K,L31M,S62D/E
AI452017-10-1168	3b	3b	BL	>5000	A30K,L31M
AI452017-46-887	3b	3b	BL	>5000	A30K,L31M
(b) (4)					
AI444010-68-118	4a	4a	BL	0.002	D62E
AI444010-26-102	4a	4a	BL	0.001	D62E
AI444010-27-309	4	4a	BL	0.004	D62E
AI444010-68-300	4	4a	BL	0.08	L30R,D62E
4a-20 (full-length in Con1 replicon)	4a	4a		0.007	
4a-21 (full-length in Con1 replicon)	4a	4a		0.007	L30R
4a-23 (full-length in Con1 replicon)	4a	4a		0.013	L28M
(b) (4)					
AI444010-31-516	4e/o?	4o	BL	158	L28M,L30S,M31V,D62K
5a (1-100 in JFH-1 replicon)	5a	5a		0.019	T62E

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Subject ID	Genotype by commercial kit	Subtype by phylogeny	Visit	EC50 (nM)	NS5A variants^a
5a-7 (in JFH-1 replicon)	5a	5a		0.004	T62E
5a-9 (in JFH-1 replicon)	5a	5a		0.003	T62E
6a (in JFH-1 replicon)	6a	6a		0.054	F28L.V62E

^a NS5A amino acid position monitored: 28, 30, 31, 54, 58, 62, 92 and 93

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

LALJI MISHRA
06/25/2015

JULIAN J O REAR
06/25/2015

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NDA 206843 SDN 001; Review Completed: 07/08/14

Reviewer: LALJI MISHRA, Ph.D.

Date Submitted: 02/28/14

Date Received: 02/28/14

Date Assigned: 02/28/14

Sponsor: Bristol-Myers Squibb Company

5 Research Parkway

P.O. Box 5100

Wallingford, CT 06492-7660

Charles D. Wolleben, Ph.D.

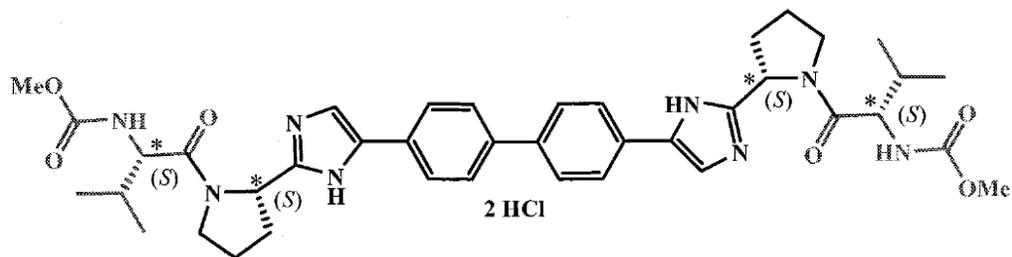
203-677-3817

203-677-3818 (FAX)

Product Names: BMS-790052, daclatasvir

Chemical Names: [(1,1'-biphenyl)-4,4'-diylbis[1*H*-imidazole-4,2-diyl(2*S*)-2,1-pyrrolidinediyl][(1*S*)-1-(1-methylethyl)-2-oxo-2,1-ethanediyl]]bis-carbamic acid, dimethyl ester, dihydrochloride

Structure:



Molecular formula: C₄₀H₅₀N₈O₆•2HCl

Molecular weight (free base): 738.88

Drug category: Antiviral

Indication: Treatment of chronic HCV infection

Dosage Form/Route of administration: Oral solution

Additional submissions reviewed:

Supplement #	Date of Correspondence	Date of Receipt
N206843 SDN 002	03/29/14	03/31/14
N206843 SDN 006	04/29/14	04/29/14
N206843 SDN 012	06/26/14	06/26/14
N206843 SDN 013	06/27/14	06/27/14

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Abbreviations: A, alanine; AA, amino acid; BL, baseline; CC₅₀, 50% cytotoxic concentration; C_{max}, maximum observed plasma concentration; C_{trough}, trough concentration of drug in plasma; D, aspartate; DAA, direct acting antiviral agent; DCV, Daclatasvir; E, glutamate; EC₅₀, 50% effective concentration; EOT, end of treatment; EVR, early virologic response; F, phenylalanine; FUWK, follow-up week; G, glycine; GT, genotype; H, histidine; I, Isoleucine; K, lysine; L, leucine; LLOQ, lower limits of quantification; M, methionine; N, asparagine; P, proline; PDR, protocol defined response; Peg-IFN α , pegylated interferon α ; pM, picomolar; Q, glutamine; R, arginine; RBV, ribavirin; RAS, resistance-associated substitutions; RAV, resistance-associated variants; RVR, rapid virologic response; S, serine; SVR 24, sustained virologic response 24 weeks post-treatment; T, threonine; μ M, micromolar; V, valine; VBT, virologic breakthrough; WK, week; Y, tyrosine

Note: In this review daclatasvir (DCV) is used interchangeably for BMS-790052

Executive Summary

1. Summary of Virology Assessments
 - 1.1. Nonclinical Virology
 - 1.2. Clinical Virology

Bristol-Myers Squibb Company (BMS) has submitted a New Drug Application (NDA # 206843) for daclatasvir (DCV) for use in combination with asunaprevir (ASV, BMS-650032) for the treatment of genotype 1b chronic hepatitis C virus (HCV) infection, (b) (4)

Currently approved treatments for HCV infection in the U.S. include the NS3/NS4 protease inhibitors boceprevir, simprevir and telaprevir, and NS5B polymerase inhibitor sofosbuvir. These compounds are direct-acting antiviral agents which are administered in combination with pegylated interferon and/or ribavirin for the treatment of HCV genotype 1a, 1b infection. HCV GT-1a is the most prevalent HCV genotype in the United States and GT-1b in Europe.

DCV is a first-in-class HCV NS5A inhibitor and is a new molecular entity. DCV has demonstrated anti-HCV activity against HCV genotype 1a, 1b and against genotypes 2, 3, 4, 5 and 6. DCV inhibits viral RNA replication and virion assembly.

DCV binds to the N-terminus of NS5A (b) (4)

Characterization of DCV resistant replicons provides in vivo validation of the proposed target. DCV was shown to suppress production of the hyperphosphorylated form of NS5A (b) (4).

The antiviral activity of DCV was determined in HCV replicon cell lines. EC₅₀ values of DCV ranged from 50 pM to 3 nM against genotype 1a replicons and 1.2 to 9 pM against HCV genotype 1b replicons depending on the assay used. EC₅₀ values of DCV against genotype 2a, 2b, 3a, 4a and 5a replicons ranged from 12 to 146 pM. CC₅₀ values (concentration of DCV which inhibited cell viability by 50%) ranged from 17 to 90 μ M. The

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therapeutic index (TI) for DCV against HCV genotype 1a and 1b were calculated to be 3.4×10^5 and 1.9×10^6 , respectively.

The anti-HCV activity of DCV was reduced by less than 2-fold in the presence of 40% human serum in a cell culture replicon system. The combination of DCV with IFN- α , the NS3/4A protease inhibitor BMS-650032 or rhIFN λ 1 in antiviral activity assays was not antagonistic.

DCV resistant replicons selected in cell culture were expanded for resistance testing and RT-PCR analysis. For genotype 1a, M28T, Q30H/R, L31M/V and Y93C/H/N substitutions conferred reduced susceptibility to DCV. For genotype 1b, DCV resistance-associated substitutions identified were L31F/V and Y93H/N. The residue Q54H was also selected but did not confer reduced susceptibility to DCV in cell culture. Overall, genotype 1a and 1b replicons had similar substitution patterns with L31 and Y93 being common resistant sites. Genotypic and phenotypic analysis indicated that the resistance patterns observed in the clinical isolates are very similar to the patterns generated in cell culture replicon except that linked substitutions are more complex in clinical specimens.

In addition to GT-1a, -1b, and -2a replicons, GT-3a, -4a, -5a and -6a hybrid replicons were constructed to evaluate the genotype coverage of DCV. EC₅₀ values of DCV for these replicons ranged from 0.003-19.0 nM. An L31M substitution resulted in a 1,000 fold decrease in susceptibility to DCV in the 2a (JFH-1) replicon clone. The loss in DCV potency was attributed directly to the methionine at position 31.

DCV resistance-associated substitutions in GT-5a replicons frequently mapped to L31 and K56 of NS5A. Resistance was observed for variants with single amino acid substitutions L31F/R/V/R or K56R (shift in EC₅₀ values of 0.012 nM to 6.9 nM) with 2.5- to 1,438-fold decreases in susceptibility to DCV.

For GT-6a, DCV resistance-associated substitutions frequently mapped to amino acid positions 24 (Q24H), 31 (L31M), 32 (P32L/S)) and 58 (T58A/N/S) of NS5A. The L31M and P32L substitutions conferred >850-fold and 5,000-fold decreases in susceptibility to DCV, respectively. Other GT-6a variants (Q24H, P32S, T58A/N/S) conferred 41- to 532-fold decreases in susceptibility to DCV.

Replicon cell lines containing substitutions that confer high levels of resistance to DCV (Q30E, Q30K, Y93N, Y93H, M28T-Q30H) were tested for susceptibility to the NS3/4A protease inhibitor BMS-650032, the NS5B thumb-1 polymerase inhibitor BMS-791325, as well as peg-IFN α . These replicon cell lines were resistant to DCV, as expected, but were as sensitive as wild type replicon to inhibitors of NS3/4A protease, NS5B polymerase and peg-IFN α . DCV resistant replicons were not cross-resistant to inhibitors of NS3 protease, NS5B polymerase and peg-IFN α . DCV resistant replicons were also not cross-resistant to cyclosporine which also inhibits HCV NS5A activity.

1.2 Clinical Virology

Clinical Studies

Study AI444010

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AI444010 was a randomized, double-blind, placebo-controlled, multicenter, Phase 2b study. Chronically infected HCV GT-1 and GT-4 treatment naïve (NV) subjects received daclatasvir at doses of 20 and 60 mg QD, respectively, and combined with Pegasys[®]/Copegus[®] (IFN α /RBV) for 24 or 48 weeks.

Sequence analysis of baseline (BL) samples from 113 GT-1a subjects in 60 mg cohort showed that NS5A polymorphisms at amino acid positions previously shown to be associated with resistance to DCV (M28V/T, Q30H/R, L31M/V, H54C/N/Y, H58P/R, E62D/Q/V, Y93H/N) were detected in 26% (29/113) of these subjects. Forty-five percent (13/29) of the subjects with BL NS5A polymorphisms experienced virologic breakthrough, relapse, met the protocol Week 4 stopping rule, or were partial responders. NS5A resistance-associated polymorphisms included M/V28, M/V31, C/Y54, R58, V62, and N93. L31M/V and Y93N substitutions conferred 250- and 34,833-fold decrease in susceptibility to DCV in cell culture replicon respectively, as compared to wild type GT-1a (H77c) replicon. Approximately 48% (14/29) of the subjects with BL NS5A resistance-associated polymorphisms achieved SVR24; NS5A polymorphisms detected included T/V28, H/R30, M31, N/Y54, P58, D62, and H93. Virologic outcome for 7% (2/29) of the subjects BL NS5A resistance-associated polymorphisms (I28, D62 and Q62) was not known.

There were 45% (51/113) subjects in the 60 mg cohort who did not achieve SVR24 and 45/51 subjects met the criteria for resistance testing. NS5A resistance-associated substitutions were detected in plasma samples of 42/45 subjects at or close to the time of virologic failure. Emergent NS5A resistance-associated substitutions included M28A/T, Q30E/ H/K/R/, L31M/V, H54Y, H58D and Y93H/N.

DCV resistance-associated substitutions (M28A/T/V, Q30E/H/K/R, L31M/V, H54C/Y, H58D/R, E62V, Y93C/H/N) present in baseline isolates or on WK 2 or 4 on treatment isolates from 22/45 virological failure subjects persisted till follow-up weeks 24, 36 or 48.

Sequence analysis of BL samples from 31 GT-1b subjects in 60 mg cohort demonstrated that NS5A polymorphisms at amino acid positions previously shown to be associated with resistance to DCV (L28V, R30Q, L31I/M, Q54H/Y, P58S, Q62D/E, A92T, Y93H) were detected in 71% (22/31) of these subjects. Thirty-two percent (7/22) of the subjects with BL NS5A resistance-associated polymorphisms experienced virologic breakthrough, relapse, or were lost to follow up and 68% (15/22) of the subjects with BL NS5A resistance-associated polymorphisms achieved SVR24.

There were 27% (7/31) of GT-1b subjects in 60 mg who did not achieve SVR24; 1/7 experienced virologic breakthrough, 4/7 were relapsers, and 2/7 had a HCV RNA value missing at a critical visit or were lost to follow up. Five of the 7 subjects who met the criteria for resistance testing, DCV resistant variants were detected in 5/5 subjects at or close to the time of virologic failure. Emergent NS5A resistance-associated substitutions included L28A, R30Q, L31M/V, P58S, Q62D and Y93C/H.

DCV resistance-associated substitutions (L/M28A/V, L31M/V, Q54H, P58S, H58D, Q62D/E, Y93C/H) present in baseline isolates or on WK 2 or 4 on treatment isolates from 4/7 virological failure subjects persisted till follow-up weeks 24, 36 or 48.

Sequence analysis of BL samples from 13 GT-4 subjects in 60 mg cohort showed that NS5A polymorphisms at amino acid positions (M28, L30R, V31, R54, A/T58, E/Q62) previously shown to be associated with resistance

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to DCV were detected in 100% (13/13) of these subjects. None of the subjects (0/13) with BL NS5A resistance-associated polymorphisms experienced virologic failure.

Study AI444011

In the randomized, double-blind, placebo-controlled, multicenter, Phase 2b study AI444011, chronically infected HCV GT-1 subjects who failed prior IFN-based therapy (null responders [NR] and partial responders [PR]) were treated with DCV at doses of 20 and 60 mg QD, respectively, and combined with PegIFN α /RBV for 24 or 48 weeks based on response-guided therapy. Subjects treated with placebo received PegIFN α /RBV for 48 weeks.

Pre-existing NS5A polymorphisms at amino acid positions associated with DCV resistance (amino acid positions 28, 30, 31, 54, 58, 62, and 93) were detected by population sequencing in samples from 15% (36/247) of GT-1a subjects included in the DCV analysis. The NS5A substitutions M28T, Q30H, L31M, H58D, and Y93C each conferred a significant decrease in susceptibility to DCV (250- to 1667-fold) compared with the reference GT-1a (H77c). Of those 36 subjects with NS5A resistance-associated polymorphisms, 86% (31/36) did not achieve SVR24.

There were ~84% (219/261 subjects; 150 null responder and 69 partial responder) of subjects who were classified as not achieving SVR24. Of those 219 subjects, 6/219 failures were not included in the analysis due to misgenotyping or incorrect sample identifiers. GT-1a resistance data were analyzed at or close to the time of virologic failure where possible and examined for 197 of the 213 subjects. GT-1a NS5A resistance-associated substitutions detected at or close to virologic failure included M28A/G/S/T/V, Q30D/E/G/H/K/N/R/T, L31I/M/V, H54R/Y, H58D/N/P/Q/V, A92P, and Y93C/H/N/R/S. Q30 variants were detected most frequently (91%; 180/197 failures) and were detected as the only variant in 25% (50/197) of GT-1a failures. The most prevalent Q30 variants contained Q30E substitution which was detected in 27% (53/197) of GT-1a failures and Q30R substitution which was detected in 24% (48/197) of GT-1a failures. The Q30R substitution was associated with a second NS5A resistance-associated substitution (M28T, L31M, H58D, and/or Y93N). Other NS5A variants to be detected included Y93 variants (Y93C/H/N/R/S detected in 36% [71/197] of failures), L31 variants (L31M/V) detected in 58% [114/197] failures). M28A/G/S//T/V, and H58D/N/P/Q/V were detected in 29% (58/197) and 25% (49/197) of virologic failure subjects.

Of the 131 confirmed GT-1b subjects treated with DCV (82 prior null responders and 49 prior partial responders) BL NS5A sequences were available from samples from 127/131 subjects. Pre-existing NS5A polymorphisms at amino acid positions associated with DCV resistance (amino acid positions 28, 30, 31, 54, 58, 62, 92, and 93) were detected by population sequencing in samples from 65% (82/127) of subjects (null responder, 73% [58/80]; partial responder, 51% [24/47]). The GT-1b BL NS5A resistance-associated polymorphisms included M/V28, H/Q30, M31, H/N/Y54, A/Q/S58, E/K/N/R/S62, T/V92, and F/H93.

Genotypes for baseline matched treatment failure isolates were available only for 50 subjects. GT-1b NS5A resistance-associated substitutions detected at or close to virologic failure included L28M, P29X, R30H/K/L/P/Q/S, L31F/I/M/V, P32X, Q54H/Y, P58S, A92E/K/T, and Y93H. Variants with L31I/M/V 96% (48/50), and Y93H 90% (45/50) substitutions emerged in isolates from most of the virologic failure subjects. In

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addition, Q54H and R30Q substitutions also emerged in isolates from 54 % (27/50) and 22% (11/50) of virologic failure subjects, respectively.

There was an association with the emergence of L31M and Y93H substitutions in GT1b isolates and virologic failure. L31M/V and Y93 substitutions emerged in isolates from most GT1b virologic failure subjects (95-98%).

Study AI444014

In study AI444014, 48 subjects from 14 centers in the United States and France were randomized 1:1:1:1 to placebo or once daily DCV 3 mg, 10 mg, or 60 mg in combination with PegIFN α -2a, 180 μ g/week, and RBV, 1.0 to 1.2 gm QD, for 48 weeks.

Emergent NS5A resistance substitutions were detected by population sequencing at or close to the time of virologic failure. Of the 31% (11/36; 7/11 receiving 3-mg, 2/11 receiving 10-mg, 2/11 receiving 60-mg) subjects who failed treatment with DCV, emerging DCV-resistant variants were detected in isolates of 82% (9/11) of these subjects; the 2 subjects with no emerging detectable NS5A resistance-associated variants discontinued therapy early (\leq 2 weeks of treatment) and had detectable HCV RNA at the time of discontinuation. In GT-1a failures, detected DCV-resistant variants included substitutions M28A, Q30E/R/H/G, L31M, H58D, and Y93C/H. In GT-1b failures, variants with L28M, L31M and Y93H substitutions were detected. The fold loss in susceptibility to DCV associated with these substitutions, when introduced into reference HCV GT-1 replicons in cell culture, suggest that the NS5A variants emerged as a result of the selective pressure exerted by DCV, particularly at suboptimal exposures of DCV (3-mg cohort).

Study AI444021

In this double-blind study, 45 Japanese subjects were randomized equally to receive once daily DCV, 10 mg or 60 mg, or placebo in combination with PegIFN α -2b/RBV. Subjects receiving DCV + PegIFN α -2b/RBV who achieved a protocol-defined response (PDR) were treated for 24 weeks. PDR was defined as HCV RNA $<$ LLOQ (15 IU/mL) at Week 4 and undetectable at Week 12. Subjects not achieving PDR received DCV + PegIFN α -2b/RBV for 48 weeks.

Virologic failure was detected more frequently in the non-responder cohorts (72%, 13/18 subjects) than in the treatment naïve cohorts (21%, 4/19 subjects). In the non-responder cohorts, the failures tended to be prior null responders rather than partial responders to PegIFN α -2b/RBV (56% prior partial responders failed DCV treatment vs. 89% prior null responders).

The GT-1b NS5A resistance associated polymorphisms included M/V28, Q30, M/V31, H/Y54, E/H/R62, T92 and H93. Of the 26 subjects whose virus had detectable resistance-associated polymorphisms, 46% experienced virologic failure. There were 11 subjects whose virus had no detectable NS5A resistance-associated polymorphisms; 46% were virologic failures. Further analysis of signature NS5A resistance-associated polymorphisms such as M/V31 and H93 indicated an association with virologic failure. Signature DCV-resistance-associated polymorphisms M/V31 and H93 were detected in 5 subjects' viruses at BL; 80% (4/5) of these subjects failed treatment and for the 1/5 subjects who achieved SVR24, a PDR was not achieved.

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The emergence of linked NS5A resistance variants were detected in the viruses from 100% (17/17) of subjects (9 virologic breakthrough, 1 partial responder, and 7 relapsers) who failed treatment with DCV combined with PegIFN α -2b/RBV. In the viruses from these 17 GT-1b failures, L28M, R30Q/H, L31F/I/M/V, Δ P32, Q54H, A92K, and/or Y93H were detected at or close to the time of virologic failure. The signature GT-1b DCV-resistant variants L31I/M/V-Y93H were detected in 88% (15/17) of treatment failures' viruses.

Persistence of GT-1b NS5A resistance variants was observed. NS5A resistance variants were still detected at FUWK4 in 9 subjects experiencing virologic breakthrough and 1 subject who was a partial responder.

Study AI444022

In the double-blind, placebo-controlled, Phase 2a study AI444022, a total of 42 chronically infected HCV GT-1 (2 GT-1a, 40 GT-1b) Japanese subjects were randomized 1:1:1 in treatment naïve and 1:1 in non-responder to receive DCV at 10 and 60 mg QD (or placebo in treatment naïve subjects) with PegIFN α -2a/RBV for 24 or 48 weeks and followed up to 24 weeks post-treatment. Virologic failure was detected more frequently in the non-responder cohorts (35%, 6/17 subjects) than in the treatment naïve cohorts (6%, 1/17 subjects). In the non-responder cohorts, the failures tended to be prior null responders rather than partial responders to PegIFN α /RBV (100%, 6/6 prior null responders).

In subjects infected with GT-1a, NS5A resistance-associated polymorphisms included L30 and H93; while for GT-1b NS5A resistance-associated polymorphisms included M28, Q30, C/H/N/Y54, S58, E/G/H/R62, E/T92 and H93. Of the 25 subjects with virus having detectable resistance-associated polymorphisms, 24% (6/25) experienced virologic failure compared with 11% (1/9) with no detectable NS5A resistance-associated polymorphisms. The signature NS5A resistance polymorphism H93 was detected in 5 (1 GT-1a, 4 GT-1b) subjects' viruses at BL (3 in the 10 mg NV cohort; 1 in the 60 mg NV cohort and 1 in the 60 mg NR cohort) and all 5 subjects achieved SVR24.

The emergence of linked NS5A resistance substitutions were detected in the viruses from 100% (7/7) of subjects who failed treatment (3 virologic breakthrough, 4 relapsers) with DCV combined with PegIFN α -2a/RBV. In the viruses from 7 GT-1b failures, L28M, R30H, L31M/V and/or Y93H substitutions were detected at or close to the time of virologic failure.

Recommendations

1.3 Recommendation and Conclusion on Approvability

With respect to virology, NDA 206843 is approvable.

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

2. Administrative

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2.1 Reviewer's Signature(s)

Lalji Mishra, Ph.D.
Microbiologist, HFD-530

2.2 Concurrence

Julian O'Rear, Ph.D. _____ Signature _____ Date _____
HFD-530/Micro TL

BACKGROUND

Bristol-Myers Squibb Company (BMS) has submitted a New Drug Application (NDA # 206843) for daclatasvir (DCV) for use in combination with asunaprevir (ASV) for the treatment of genotype 1b chronic hepatitis C virus (HCV) infection, _____ (b) (4)

DCV is an HCV NS5A inhibitor and ASV is an HCV NS3/4A protease inhibitor. The DCV/ASV DUAL regimen was granted Breakthrough Therapy Designation (FDA communication dated 02/03/2014). The sponsor submitted on 02/28/2014 nonclinical virology reports as a part of rolling submission for NDA 206843 and clinical studies reports on 03/31/14. Non-clinical virology and Phase II clinical virology reports submitted in support of the NDA 206843 are reviewed here.

HCV is known to be associated with cirrhosis and hepatocellular carcinoma, and is the most common cause for liver transplantation in the United States. HCV is an enveloped virus which belongs to the Flaviviridae family. Its genome is a 9.6 kb positive sense, single-stranded RNA that encodes a polyprotein precursor of ~3000 amino acids. This polyprotein precursor is proteolytically processed by both cellular and viral proteases to 10 individual proteins: the structural proteins C, E1, E2, and p7, and the nonstructural proteins NS2, NS3, NS4A, NS4B, NS5A and NS5B ([Simmonds et al., 2005](#)). The nonstructural protein, NS3, comprises an N-terminal protease domain of 181 amino acids and a C-terminal helicase domain. The serine protease activity of NS3 in complex with the NS4A cofactor is responsible for the proteolytic cleavage at four junctions of the HCV polyprotein precursor: NS3-NS4A (self-cleavage), NS4A-NS4B, NS4B-NS5A, and NS5A-NS5B.

Nonstructural protein 3 (NS3), NS4A, NS4B, NS5A, and NS5B are sufficient for replication of HCV RNA as a replicon in cell culture. NS3-4A is the primary viral protease, and NS5B is an RNA dependent RNA polymerase. NS4B, a hydrophobic protein with multiple *trans*-membrane domains, induces an endoplasmic reticulum-derived membranous web that harbors the HCV replication complex.

NS5A is an RNA binding protein that interacts with other HCV nonstructural proteins and is capable of altering NS5B polymerase activity in cell culture ([Fridell et al., 2011](#)). NS5A also interacts with a variety of cellular proteins and is potentially involved in modulating multiple aspects of the cellular environment. In addition to its role(s) in RNA replication, NS5A is also required for viral assembly ([Fridell et al., 2011](#)).

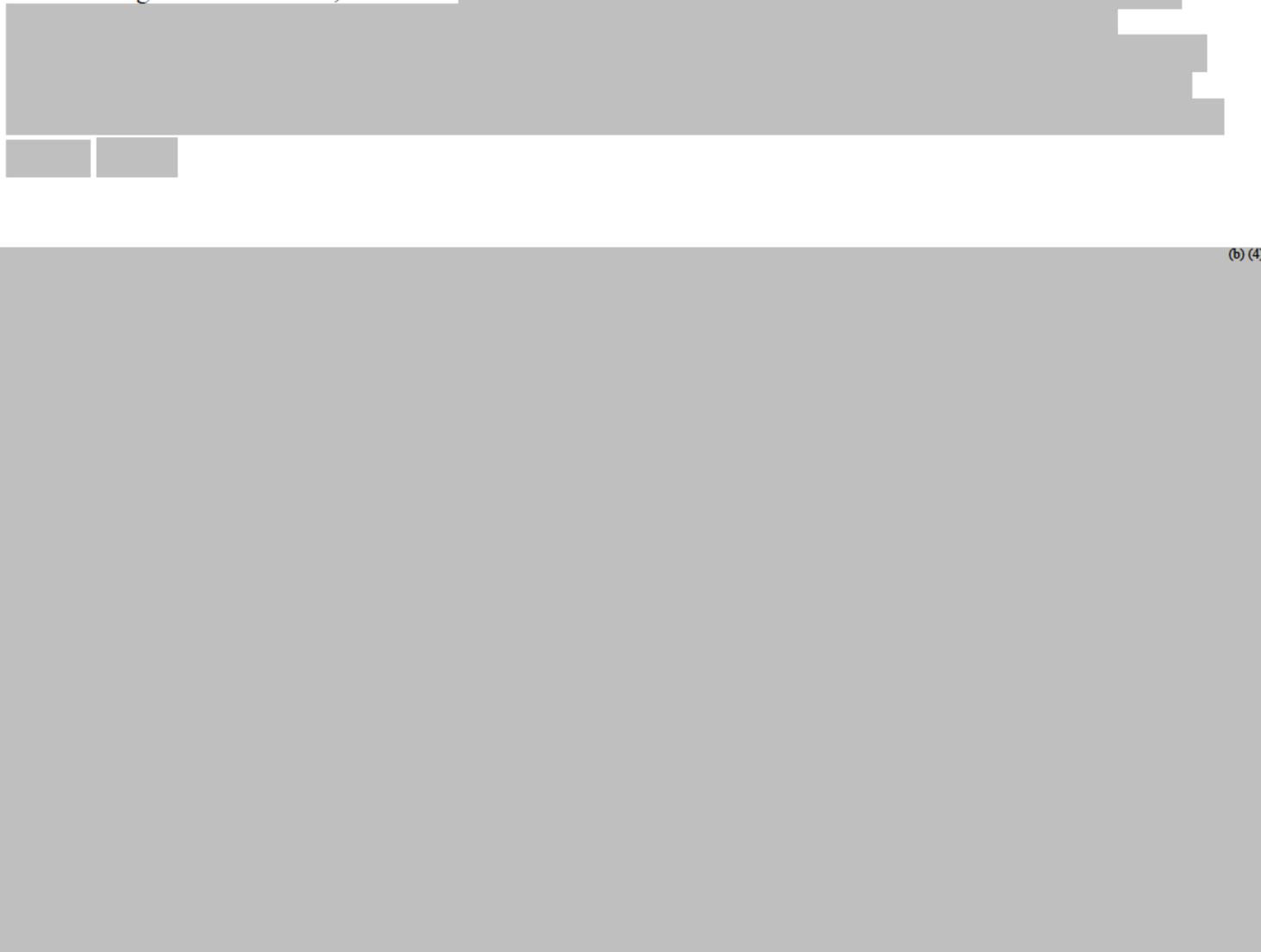
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NS5A is a modular protein with an N-terminal amphipathic α -helix membrane anchor (amino acids [aa] 5 to 25) and three distinct structural domains. Domain I (aa 28 to 213) is essential for RNA replication and has been crystallized as a dimer in two different configurations, suggesting the potential for distinct functional conformations and/or higher-order multimers ([Tellinhuisen et al., 2005](#)). Domains II (aa 250 to 342) and III (aa 356 to 447) are natively unfolded, and roles for these domains in RNA replication and viral assembly are still emerging.

I. Mechanism of action

I (a). Binding of DCV to NS5A region (b) (4)

Binding studies with HCV replicon cells revealed that DCV binds to the N-terminus of recombinant NS5A in the region of (b) (4) (Figure 1). A crystal structure of the N-terminal domain indicates that NS5A exists as a dimer. Using this information, DCV was (b) (4)



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(b) (4)

I (b). Effect of DCV on Phosphorylation of NS5A

NS5A is highly phosphorylated and is expressed in cell culture as basally phosphorylated (p56) and hyperphosphorylated (p58) forms. Basal phosphorylation is believed to occur at residues in the central and C-terminal parts of the protein, while several highly conserved serine residues in the central part of the protein (aa 214 to 249) are required for NS5A hyperphosphorylation. Phosphorylation has been implicated as a regulatory switch, modulating multiple NS5A functions ([Tellinghuisen et al., 2008](#)).

NS5A inhibitor was shown to suppress production of the hyperphosphorylated form of NS5A expressed from a genotype 1b replicon. To determine if DCV has a similar activity, genotype 1b (con1) and 2a (JFH1) replicon clones were expressed in cell culture using a vaccinia virus-T7 expression system (Figure 2). Treatment of the cells with DCV resulted in a substantial decrease in the expression of hyperphosphorylated NS5A in both genetic backgrounds (Figure 2), indicating that this activity is conserved among inhibitors of this class and is not restricted to genotype 1b replicons.

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Figure 2: Suppression of NS5A hyperphosphorylation by DCV. Con1 and JFH1 subgenomic replicon clones without cell culture adaptive mutations were transiently expressed in a vaccinia virus T7 expression system. Cells were treated with 500 nM DCV (+) or DMSO (-). After 16 h, cell lysates were fractionated by SDS PAGE, followed by immunoblot analysis with anti-NS5A antibody. hyper-PO₄, hyperphosphorylated NS5A; basal-PO₄, basally phosphorylated NS5A (Source: [Fridell et al., 2011](#)).

Comment:

The mechanism by which NS5A inhibitors alter NS5A hyperphosphorylation remains unclear. It has been suggested that functions of NS5A that are dependent on hyperphosphorylation are inhibited by DCV. BMS has not provided any information on the affinity of NS5A for DCV.

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II (a). Anti-HCV activity of DCV

The antiviral activity of DCV was determined in HCV replicon cell lines. Construction of replicon cell lines and assay for the measurement of anti-HCV activity are described in the Methodology Section. Replication competent genotype 1a or 1b replicons have been used to generate hybrid replicons. A series of hybrid replicons with the entire NS5A or the first 100 amino acids of NS5A derived from genotypes 2a, 3a, 4a, and 5a have been constructed to address genotype coverage of NS5A inhibitors. Table 1 shows EC₅₀ values (concentration required to inhibit 50% HCV replication) of DCV and its major metabolite BMS-805215 as well as BMS-795853 (major metabolite in dogs) against 1a, 1b and other HCV genotype replicons. Similar EC₅₀ values of DCV were reported by [Gao et al., 2010](#).

Table 1: Anti-HCV activity of DCV, BMS-805215 and BMS-795853 in replicon cell lines (Source: NDA 206843, Study Report 930023288, Page 24, Table 1).

HCV Replicon Genotype	Assay Method	BMS-790052 (EC₅₀ value)	BMS-805215 (EC₅₀ value)	BMS-795853 (EC₅₀ value)
1a (H77c)	FRET	50 ± 13 pM	19 ± 5 nM	14 ± 2 nM
1a (H77c)	Taqman	3 ± 0.6 nM	17 ± 4.5 nM	14 ± 2 nM
1b (Con 1)	FRET	9 ± 4 pM	820 ± ±230 pM	70 ± 30 pM
1b (Con 1)	Taqman	1.2 ± 0.7 pM	630 ± 100 pM	ND ^c
1b (Con1)	Luciferase	1.8 ± 054 pM	400 ± 202 pM	ND ^c
2a (JHF-1)	FRET	71 ± 17 pM	56 ± 15 nM	9 ± 2.5 nM
2a ^a (JFH-1) hybrid	FRET	103 ± 36 pM	19 ± 3 nM	2 ± 1 nM
2a ^a (HC-J6CF) hybrid	FRET	7.6 ± 2.8 nM	1.6 ± 0.5 μM	364 ± 90 nM
3a ^a , hybrid	FRET	146 ± 34 pM	3.8 ± 1 nM	50 ± 30 nM
4a ^a , hybrid	FRET	12 ± 4 pM	657 ± 130 pM	1.6 ± 1.4 nM
5a ^a , hybrid	FRET	33 ± 10 pM	1.9 ± 0.4 pM	416 ± 65 pM

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1 b (Con1) CC ₅₀	Fluorescence	17 ± 1 μM	17 ± 1 μM	>30 μM
Therapeutic Index ^b	CC ₅₀ value/ EC ₅₀ value	1,900,000	21,000	>330,000

^a Results derived from hybrid replicons

^b Therapeutic index = CC₅₀/EC₅₀ in 1b (Con1) replicon cells

^c Not determined.

Data presented in Table 1 for anti-HCV activity of BMS-790052 are summarized as follows:

1. DCV exhibited anti-HCV activity at pM concentrations against all genotypes except genotype 2a (HC-J6CF). The EC₅₀ values of DCV ranged from 50 pM to 3 nM against genotype 1a replicons and 1.2 to 9 pM against HCV genotype 1b replicons. EC₅₀ values of DCV against genotype 2a, 2b, 3a, 4a and 5a replicons ranged from 12 to 146 pM.
2. The strain 2a (HC-J6CF) exhibited a 1,000-fold decrease in susceptibility to BMS-790052. The sponsor stated that in the 2a (JFH-1) replicon clone an L31M substitution resulted in a 1,000-fold decrease in susceptibility to DCV.
3. The 50% cytotoxic concentration (CC₅₀) value of DCV in the 1b (Con1) replicon cell line (Huh-7) was 17 μM.
4. The therapeutic index (TI) for DCV against HCV 1 GT1a and 1b were calculated to be 3.4x10⁵ and 1.9 x10⁶, respectively

II (b). Cytotoxicity of DCV in cell culture

Cytotoxicity of DCV was determined in Huh-7, Vero, MDBK, MRC-5 and MT-2 cells derived from various tissues. Cells were incubated with serially diluted DCV for up to five days and cell viability quantified by using either MTS assay for MT-2 or an by Alamar blue assay for other cell lines. CC₅₀ values (concentration of DCV which inhibited cell viability by 50%) ranged from 17 to 90 μM (Source: NDA 206843, Study Report 930023288, Page 30, Table 8).

II (c). Effect of serum binding on anti-HCV activity of DCV

The anti-HCV activities of DCV and its metabolites BMS-805215 and BMS-795853 were determined in the presence of 40% human serum (HS) and compared to their activities in presence of standard 10% fetal bovine serum. Results showed that the anti-HCV activities of DCV and its' metabolites were reduced by less than 2-fold in the presence of 40% human serum (Source: NDA 206843, Study Report 930023288, Page 30, Table 7).

II (d). Anti-HCV activity of DCV in combination with interferon α (IFN-α)

The anti-HCV activity of DCV in combination with IFN-α was determined in an HCV replicon system using the Dual-Glo luciferase assay as described (See Methodology Section). The cytotoxicities of these combined

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agents were also measured in parallel by Alamar blue staining. The degree of antagonism, additivity, or synergy was determined over a range of drug concentrations and combination response curves were fit to assess the antiviral effects of drug treatment combinations. The concentration ratios were analyzed using the method of Chou.

Results indicated that DCV in combination with IFN- α was not antagonistic and were consistent with synergistic anti-HCV activity at 50%, 75% and 90% effective concentrations. Additivity was observed at a 1:2.5 ratio of IFN- α to DCV at the 50%, 75% and 90% effective concentrations in experiment 3 and only at 50% effective concentration in experiment 2 (Source: NDA 206843, Study Report 930023288, Page 33, Table 10).

II (e). Anti-HCV activity of DCV in combination with BMS-650032

The anti-HCV activity of DCV in combination with the HCV NS3 inhibitor BMS-650032 was not antagonistic in the HCV replicon system. Results indicated at a ratio of 1:1, 2.5:1 and 1:2.5, BMS-650032 in combination with DCV exhibited additive to synergic effects in a cell culture replicon system (Source: NDA 206843, Study Report 930023288, Page 34, Table 11).

II (f). Anti-HCV activity of rIFN λ 1 in combination with DCV

Interleukin 29 (IL-29, IFN λ 1) is a member of the recently discovered Type III IFN family. IFN λ 1 is one of three closely related IFN λ type III cytokines distantly related to the type I IFNs and IL-10 family members. Combination of rIFN λ 1 with DCV yielded a mixture of synergistic and additive effects in three independent experiments. At the 50% effective level, a mixture of synergy and additivity was observed in all experiments. At the 75 and 90% effective levels, a mixture of synergy and additivity was observed in experiment 1 while additivity resulted in experiments 2 and 3. Importantly, no drug antagonism was observed at the 50, 75, or 90% effective doses.

III. Selection of HCV variants resistant to DCV (BMS Scientific Report 930023286)

DCV selected replicon containing cells were expanded for resistance testing and RT-PCR analysis as described in BMS Scientific Report 930023286. Amino acid substitutions that contribute to BMS-790052 resistance were identified by nucleotide sequence analysis.

To examine the resistance phenotype, changes identified by nucleotide sequence analysis were introduced into replicon clones by using recombinant overlapping RT-PCR. Clones were verified by sequence analysis. Since the first 100 amino acids of NS5A determine inhibitor susceptibility and genotype 1a replicons replicate relatively poorly in transient assays, a hybrid replicon containing a genotype 1b genetic background with the first 100 amino acids of genotype 1b NS5A replaced by 1a NS5A was used for genotype 1a evaluation (NDA 206843 Scientific Report 930023286, Page 17). This methodology was validated by evaluating a subset of changes in a genotype 1a replicon clone and similar results were observed from both replicons.

III (a). Genotypic and phenotypic analysis of DCV resistant mutants

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Amino acid substitutions that confer reduced susceptibility to DCV are summarized here (Source: NDA 206843, Scientific Report 930023286, Page 23, Table 1). For genotype 1a, M28T, Q30H/R, L31M/V and Y93C/H/N substitutions conferred reduced susceptibility to DCV. For genotype 1b, DCV resistance-associated substitutions identified were L31F/V and Y93H/N. The residue Q54H was also selected but by itself did not confer reduced susceptibility to DCV. Overall, genotype 1a and 1b replicons had similar substitution patterns with L31 and Y93 being common resistant sites. This similarity in resistance-associated substitutions indicates conservation of the inhibitor binding pocket in the N-terminal region of NS5A. Phenotypic analysis results are summarized below (Source: NDA 206843, Scientific Report 930023286, Page 23, Table 1).

- Substitutions at amino acid residues L31 and Y93 were common in DCV resistant mutant viruses from both genotype 1a and 1b replicons.
- Amino acid substitutions M28T, Q30H, Q30R, L31V, Y93C and M28T/Y93C were observed in DCV resistant HCV 1a replicons. These substitutions conferred 43- to 1130-fold reduced susceptibility to DCV in HCV 1a replicons.
- Substitutions L31F/V conferred 4- to 16-fold reduced susceptibility to DCV in the HCV genotype 1b replicon.
- Y93N substitution conferred approximately 25-fold reduced susceptibility to DCV in the HCV 1b replicon.
- The substitutions L31V/Y93H conferred more than 4,600-fold reduced susceptibility to DCV in HCV 1b replicon.

III (b). Correlation of variants identified in cell culture replicon and in vivo

Viral breakthrough or relapse was observed in subjects treated with DCV monotherapy in the clinic. Genotypic and phenotypic analysis indicated that the major HCV variants observed in clinical studies were at amino acid positions identified in the cell culture replicon selection analyses. The resistance patterns observed in the clinical isolates are very similar to the patterns generated in cell culture replicon except that linked substitutions are more complex in clinical specimens (Table 2 and Table 3). In these Tables, resistant variants selected in replicon cells are indicated by plain letters, resistant variants selected in replicon cells and observed in clinical specimens are indicated by bold letters, and resistant variants identified in clinical specimens are indicated by bold italic letters.

The resistance substitutions in GT-1a frequently mapped to residues M28, Q30, L31, and Y93 (Table 2). In general, larger reductions in susceptibility were observed from single substitutions in GT-1a compared to GT-1b. For example, a Q30E variant with an EC₅₀ value of 150 nM in transient assays and 212 nM in a cell line conferred a large reduction in susceptibility. Some variants displayed poor replication ability, especially variants with substitutions at residue Y93. However, other variants, such as Q30E and L31V, replicated as well as wild type replicon. In general, variants with double amino acid substitutions conferred large reduction in susceptibility with poor replication ability in the transient replication assays.

Comment

EC₅₀ values for genotype 1a and 1b replicon shown in Table 2 and Table 3 are published ([Fridell et. al., 2011](#)).

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The resistance substitutions in GT-1b frequently mapped to residues L31 and Y93 (Table 3). In general, single amino acid substitutions conferred minimal reductions in susceptibility. For example, variants with L31V or Y93H have EC₅₀ values < 0.41 nM. However, some double amino acid substitutions, such as L31V-Y93H with EC₅₀ values of = 37.9 nM in transient assays and 241.6 nM in a cell line, conferred large shifts in susceptibility. The deletion of proline at residue 32 of NS5A (P32del) generated a replicon that did not replicate well in the transient replication assay, but displayed a large reduction in susceptibility to DCV, with an EC₅₀ value ≥1 μM. Replicon cell lines were isolated for some commonly observed variants, such as L31V, Y93H, and L31V/Y93H and have been used routinely in the laboratory to evaluate DCV. Generally, EC₅₀ values derived from GT-1a and GT-1b cell lines are slightly higher than EC₅₀ values derived from transient assays (Table 2 and Table 3).

Table 2: Resistance profile of DCV in cell culture replicon system: GT1a replicon (Source: NDA 206843, Module 2.6.2 Pharmacology, Written Summary, Pages 17-19, Table 2.3.2-2)

Replicon ^a	Replication Level (%) ^b		EC ₅₀ (nM) ^{b,c}		EC ₅₀ (nM) ^{c,d}	
	Average	SD	Average	SD	Average	SD
WT	100		0.0059	0.0038	0.020	0.008
T21I	169	9	0.0046	0.0004	ND	
M28A	27	25	27.3	17.9	ND	
M28I	200	38	0.0074	0.0004	ND	
M28T	31	23	4.05	0.4	13.6	4.03
M28V	16	11	0.0074	0.0026	ND	
Q30D	171	134	>2000		ND	
Q30E	130	56	149.7	89.1	212	76
Q30G	54	22	51.03	15	ND	
Q30H	75	31	8.8	1.9	ND	
Q30K	19	9	145	70	ND	
Q30L	37	11	0.022	0.0041	ND	
Q30P	114	19	0.009	0.0009	ND	
Q30R	41	16	7.3	1.08	ND	
delQ30	ND		ND		ND	
L31M	55	15	2	0.7	ND	

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Replicon ^a	Replication Level (%) ^b		EC50 (nM) ^{b,c}		EC50 (nM) ^{c,d}	
L31P/Q/R	ND		ND		ND	
L31V	117	29	20.1	5.9	56.97	16.9
P32L	18	18	1.4	0.2	ND	
<i>V37A</i>	13	2	0.005	0.0008	ND	
<i>V37M</i>	117	5	0.005	0.001	ND	
H58D	92	9	2.9	0.4	ND	
<i>H58P</i>	266	261	0.0072	0.0008	ND	
<i>E62D</i>	103	32	0.023	0.007	ND	
Y93C	11	7	11.1	4.1	ND	
Y93H	18	11	32.3	9.4	66.8	36.6
Y93N	13	8	282	64.7	350	153
<i>T21I-L31M</i>	82	5	3.23	0.024	ND	
<i>M28A-Q30R</i>	45	11	1704	369	ND	
M28T-Q30H	31	22	622	315	787	209
<i>M28T-Q30K</i>	15	2	>2000		ND	
M28T-Q30R	76	23	356	12	ND	
M28T-Y93H	POOR REPLICATION		ND		482	231
<i>M28V-Q30R</i>	147	55	1.9	0.01	ND	
Q30E-Y93H	6	1	404	61.6	ND	
<i>Q30H-H58D</i>	28	2	>2000		ND	
Q30H-Y93C	ND		ND		ND	
Q30H-Y93H	20	6	553.2	207.4	308	144
Q30K-Y93H	ND		ND		ND	
Q30R-L31M	54	15	1171	917	ND	
Q30R-H58D	60	12	2520	62	>1000	
<i>Q30R-E62D</i>	30	14	151.1	9.0	ND	
Q30R-Y93H	6	1	340.9	40.8	427	13.5
<i>L31M-H58D</i>	41	4	397.6	14.2	ND	
<i>L31V-V37A</i>	10	0.1	63.6	8.9	ND	
<i>L31V-H58P</i>	100	0	72.9	8.1	ND	
L31V-Y93H	20	2	>1000		ND	
<i>H58P-Y93H</i>	22	0.7	7.2	0.08	ND	

^a Genotype 1a replicon: H77C with cell culture replication enhancing mutations P1496L and S2204I.

^b Data derived from transient replicon replication assays.

^c Values can be converted to ng/mL using the conversion factor ~1.35 for DCV. For example 1 ng/mL = 1.35 nM.

^d Data derived from replicon cell lines.

SD = Standard deviation; ND = Not determined.

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Plain type: Resistant variant selected in replicon cells

Bold type: Resistant variant selected in replicon cells and identified in clinic

Bold and italic type: Resistant variant identified in clinic

Table 3: Resistance profile of DCV in cell culture replicon system:GT-1b replicon (Source: NDA 206843, Module 2.6.2, Pharmacology Written Summary, Pages 16-17, Table 2.3.2-1)

Replicon ^a	Replication Level (%) ^b		EC50 (nM) ^{b,c}		EC50 (nM) ^{c,d}	
	Average	SD	Average	SD	Average	SD
WT	100		0.0026	0.0003	0.004	0.0019
R30Q	91	1	0.0015	0.0003	ND	
L31F	146	44	0.012	0.0012	ND	
<i>L31I</i>	54	12	0.0036	0.0015	ND	
L31M	99	23	0.0084	0.0019	ND	
L31V	158	54	0.072	0.02	0.27	0.26
L31W	191	9	0.236	0.064	ND	
P32L	18	6	0.042	0.022	ND	
<i>P32del</i>	29.1	3.2	>1000		ND	
<i>F37L</i>	151	32	0.0018	0.0001	ND	
Q54H	83	18	0.0032	0.0004	ND	
<i>Q54N</i>	83	29	0.0036	0.0008	ND	
P58S	121	17	0.0023	0.0004	ND	
<i>Y93C</i>	61.9	4.4	0.0085	0.0019	ND	
Y93H	27	16	0.062	0.027	0.409	0.165
Y93N	19	5	0.129	0.09	ND	
L23F-L31F	65	5	0.034	0.005	ND	
R30Q-L31F	224	39	0.229	0.099	ND	
<i>L31I-Y93H</i>	43	11	6.48	3.38	ND	
L31M-Y93H	70	68	18.2	16.5	133.6	18.9
L31V-Y93H	49.9	38	37.9	33.3	241.6	93.2
<i>P32del-Y93H</i>	Poor replication		ND		ND	
F37L-Y93H	34	4	0.049	0.007	ND	
Q54H-Y93H	22	7	0.024	0.007	ND	
L31V-Q54H-Y93H	189	25	48.7	10.4	ND	

^a Genotype 1b replicon: Con1 with cell culture replication enhancing substitution S2204I.

^b Data derived from transient replicon replication assays.

^c Values can be converted to ng/mL. The conversion factor for DCV is ~1.35. For example 1 ng/mL = 1.35 nM.

^d Data derived from replicon cell lines.

SD = Standard deviation; ND = Not determined.

Plain type: Resistant variants selected in replicon cells.

Bold and italic type: Resistant variants identified in the clinic.

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Bold type: Resistant variants selected in replicon cells and identified in the clinic

III (c). Activity of DCV against genotype 2a virus and genotype 2a, 3a, 4a, 5a, and 6a hybrid replicons

An HCV cell culture system with the GT-2a virus JFH-1 strain ([Wakita et al., 2005](#)) has been established. Since the JFH-1 virus produced in cell culture is capable of infecting chimpanzees and the virus collected from chimpanzees retains the ability to re-infect cells, this system provides a biologically relevant system to evaluate inhibitors in cell culture replicon system ([Lindenbach et al., 2006](#)). DCV is a potent inhibitor of JFH-1 cell culture virus (Table 4).

In addition to GT-1a, -1b, and -2a hybrid replicons, GT-3a (4 different isolates), -4a (3 different isolates), -5a (3 different isolates), and -6a (1 isolate) hybrid replicons were constructed to evaluate the genotype coverage of DCV more broadly. EC₅₀ values of DCV for these replicons ranged from 0.003-19.0 nM (Table 4).

Table 4: Activity of DCV against different HCV genotypes (Source: NDA 206843, Addendum 1 to BMS Scientific Report 930023288, Pages 5-6, Table 1)

HCV Replicon Genotype	DCV (nM)
1a (H77, wildtype) ¹	0.020 +/- 0.009
1b (Con1, wildtype) ¹	0.004 +/- 0.002
2a (JFH-1) virus	0.020 +/- 0.004
2a (JFH-1) ¹ replicon	0.034 +/- 0.019
2a (HC-J6; 1-429 in JFH-1 replicon) ¹	18 +/- 5.1
2a (13-4; 1-429 in JFH-1 replicon)*	19 +/- 9
2a (14-1; 1-429 in JFH-1 replicon)*	8.8 +/- 4.7
3a (1-100 in Con1 replicon) ¹	0.19 +/- 0.07
3a-1(1-429 in JFH-1 replicon)	0.53 +/- 0.15
3a-2 (1-429 in JFH-1 replicon)	0.14 +/- 0.04
3a-4 (1-429 in JFH-1 replicon)	1.25 +/- 0.5
4a-20 (full-length in Con1 replicon) ¹	0.007 +/- 0.003
4a-21 (full-length in Con1 replicon)	0.007 +/- 0.0002
4a-23 (full-length in Con1 replicon)	0.013 +/- 0.0001
HCV Replicon Genotype	DCV (nM)
5a (1-100 in JFH-1 replicon) ¹	0.019 +/- 0.007
5a-7 (1-429 in JFH-1 replicon)	0.004 +/- 0.0007
5a-9 (1-429 in JFH-1 replicon)	0.003 +/- 0.0003
6a (1-429 in JFH-1 replicon)	0.054 +/- 0.008

The length of the NS5A sequence cloned from each clinical isolate (1-100 or 1-429 or full-length) and substituted for the NS5A of a replicon (Con1 or JFH-1) is specified for each hybrid replicon.

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¹Cell lines used routinely in the lab; these are EC₅₀ ± SD values as of 6-Nov-2012, (n) > 20.

*Values derived from transient replication assay; unmarked values are derived from stable cell lines.

Comment

In Table 1 and in previous review (Virology Review of IND 79599, SDN 000 dated 11/08/07), the EC₅₀ values of DCV reported for genotype 1a ranged from 50 pM to 3 nM in the cell culture replicon system and 1.2 to 9 pM against genotype 1b replicons. In the present study, the EC₅₀ values of DCV against HCV genotype 1a and 1b replicons were 20 pM and 4 pM, respectively. The EC₅₀ values of DCV against genotype 2a, 2b, 3a, 4a and 5a replicons previously reported ranged from 12 to 146 pM. However, the EC₅₀ value of DCV against strain 2a previously reported (HC-J6CF) was 7.6 nM. The sponsor stated that in the 2a (JFH-1) replicon clone an M31 polymorphism resulted in 1,000 fold decrease in susceptibility to BMS-790052 (Virology Review of IND 79599, SDN 000 dated 11/08/07).

The loss in DCV potency was attributed directly to the methionine at position 31 by introducing the L31M substitution into the JFH-1 replicon. The EC₅₀ value of DCV against the L31M JFH-1 variant was 4.4 +/- 0.2 nM, compared to 0.034 nM for wild type JFH-1 (Table 5; Source: NDA 206843, Addendum 1 to BMS Scientific Report 930023288, Pages 6-7, Table 2).

For genotypes 2a, 3a, clinical isolates in a hybrid JFH -1 replicon, the median EC₅₀ values of DCV were 18,000 pM (n=3; range 8,800 to 19,000 pM), 530 pM (n=3, range 140 to 1,250 pM) and 3 pM (n=3; range 3 to 72 pM), respectively. The median EC₅₀ value of DCV for genotype 4a clinical isolates in replicon Con1 was 7 pM (n=3; range 7 to 13 pM) and for 5a clinical isolates in a hybrid JFH -1 replicon, the median EC₅₀ value was 3.6 pM (n=3; range 3 to 7.2 pM), respectively. EC₅₀ values of DCV against HCV genotype 6 (n=1) ranged from 3 to 54 pM.

IV. Activity of DCV against HCV genotype 2, 3 and 4 replicon variants containing DCV resistance-associated substitutions

Results are presented in Table 5 and are summarized here.

1. Genotype 2a replicons containing amino acid substitutions F28S, L38M, C92R, and Y93H exhibited 440- to 56,300-fold reduced susceptibility to DCV compared to wild-type replicons.
2. Genotype 3a replicons containing amino acid substitutions A30K, L31F, S62L and Y93H conferred 1.75- to 2,749-fold reduced susceptibility to DCV.
3. Genotype 4a-21 replicons (wild-type L28) containing amino acid substitutions R30G/H/S exhibited 133- to 1,728-fold reduced susceptibility to DCV.
4. Genotype 4a-23 replicons (wild-type M28) containing amino acid substitutions L30H/R, Y93H/R, M28-L30H, and L30I-Y93R exhibited 6.4- to 9,077-fold reduced susceptibility to DCV.

Comment

Genotype 4a-23 replicons containing amino acid substitution L30H alone conferred 1,215-fold reduced susceptibility to DCV. However, in combination with amino acid substitution M28L, the L30H substitution

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only conferred 64-fold reduced susceptibility to DCV. It's not known how and why the M28L substitution reduced the effect of L30H substitution in conferring reduced susceptibility to DCV.

Table 5: Activity of DCV against wild-type and DCV resistant replicons of GT- 2, 3, and 4 (Source: NDA 206843, Addendum 1 to BMS Scientific Report 930023288, Pages 6-7, Table 2)

Replicons	DCV (EC₅₀, nM)
GT-2a (wildtype, JFH-1)*	0.010 +/- 0.002
F28S*	563 +/- 20
L31M*	4.4 +/- 0.2
C92R*	4.7 +/- 0.4
Y93H*	35 +/- 9
GT-3a1 (wildtype)	0.53 +/- 0.15
A30K	29.6 +/- 4.4
A30T	0.57 +/- 0.013
L31F	320 +/- 5.9
S62L	0.93 +/- 0.17
Y93H	1451 +/- 244
GT-4a21 (wildtype, L28)	0.007 +/- 0.0002
R30G	12.1 +/- 2.0
R30H	0.93 +/- 0.1
R30S	1.0 +/- 0.053
<hr/>	
Replicons	DCV (EC₅₀, nM)
GT-4a23 (wildtype, M28)	0.013 +/- 0.0001
L30H	15.8 +/- 7.2
L30R	2.4 +/- 0.064
Y93H	2.2 +/- 0.06
Y93R	5.9 +/- 0.4
M28L-L30H	0.83 +/- 0.17
L30I-Y93R	118 +/- 43

*Values derived from transient replication assays; unmarked values are derived from stable cell lines.

V. Anti-HCV activity of DCV against GT-5a and -6a resistant replicons

The sponsor stated that when the Y93H substitution was introduced into the HCV GT 5a or -6a replicons, the Y93H substitution conferred minimal reductions in susceptibility to DCV, with EC₅₀ values ranging from 0.14 to 0.16 nM (4.1 to 4.3-fold reduction in susceptibility)). In contrast, a Y93H substitution in GT-1a replicon conferred a large reduction in susceptibility with an EC₅₀ value of 110 nM (4,583-fold) and EC₅₀ value of 0.82 nM in GT-1b replicon (91-fold) reduced susceptibility to DCV compared to wild type genotype 1a and genotype 1b replicons, respectively (Virology Review of IND 79599 SDN 655 dated 02/06/14).

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HCV GT-5a and -6a replicons with reduced susceptibility to DCV were selected in cell culture replicon. Genotype analysis showed that all resistance substitutions, like those for GT-1a and 1b replicons, mapped to the first 100 amino acids of NS5A. Resistance phenotype was evaluated by introducing the substitutions observed in selected cells into GT-5a or -6a NS5A hybrid replicons with the GT-2a (JFH) replicon backbone. EC₅₀ values for GT-5a variants were determined from stable cell lines, since the GT-5a variants replicated poorly in transient assays and EC₅₀ values and replication level could not be determined by this method. EC₅₀ values for GT-6a variants were derived from both transient and stable replicon cell line assays, as described previously (Virology Review of IND 79599 SDN 557 dated 04/23/13).

The sponsor stated that DCV resistance-associated substitutions in GT-5a replicons frequently mapped to L31 and K56 of NS5A (Table 6). As small shifts in susceptibility (EC₅₀ value of 0.12nM; 2.5 fold decrease) was observed for variants with single amino acid substitution K56 R, whereas variants with L31F/V substitutions conferred a large (EC₅₀ value of 6.9 nM; 1,438-fold) decrease in susceptibility to DCV.

For GT-6a, DCV resistance-associated substitutions frequently mapped to amino acid positions 24 (Q24H), 31 (L31M), 32 (P32L/S) and 58 (T58A/N/S) of NS5A. The L31M and P32L substitutions conferred >850-fold and 5,000-fold decreases in susceptibility to DCV, respectively. Other GT-6a variants (Q24H, P32S, T58A/N/S) conferred 41- to 532-fold decreases in susceptibility to DCV.

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Table 6: Resistance profile of DCV in genotypes 5a and 6a replicons (Source: NDA206843, Addendum 03 to BMS Scientific Report 930023288, Page 7, Table 3)

Replicon	Replication Level (%)		EC50 (nM) ^a			EC50 (nM) ^b		
	Average	SD	Average	SD	Fold Resist.	Average	SD	Fold Resist.
GT-5a (parent, subject 7)	ND		ND			0.0048	0.0005	
L31F	ND		ND			6.9	1.0	1438
L31V	ND		ND			2.2	0.81	458
K56R	ND		ND			0.012	0.0013	2.5
L31F- K56R	ND		ND			39.3	13.1	8188
L31V- K56R	ND		ND			29.4	2.2	6125
GT-5a (parent, subject 9)	ND		ND			0.0031	0.0005	
L31V	ND		ND			2.2	0.05	710
GT-6a (parent)	100		0.026	0.0027		0.050	0.0048	
Q24H	28.9	8.6	0.657	0.076	25.3	2.04	0.5	40.8
L31M	44.7	11.2	52.6	14.4	2023.1	43.9	12.4	878
P32L	28.3	8.6	65.1	2.8	2503.8	250	69.4	5000
P32S	9.2	0.9	2.7	1.5	103.8	19.1	1.43	382
T58A	79.6	11.3	2.4	0.11	92.3	2.36	0.69	47.2
T58N	106	35.2	11.8	3.7	453.8	26.6	3.76	532
T58S	50.2	10.9	1.0	0.44	38.5	2.4	0.4	48

^a Data from transient replicon assay

^b Data from stable cell lines

SD = standard deviation; Resist. = resistance; ND = not determined.

VI. Cross-resistance with HCV inhibitors of other targets

To determine if HCV NS5A resistance variants are as sensitive as wild type to inhibitors of other HCV targets, replicon cell lines bearing substitutions that confer large reductions in susceptibility to DCV (Q30E, Q30K, Y93N, Y93H, M28T-Q30H) were established and tested for susceptibility to the NS3/4A protease inhibitor BMS-650032, the nonnucleoside analog thumb-1 NS5B polymerase inhibitor BMS-791325, as well as peg-

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IFN α . These replicon cell lines were resistant to DCV, as expected, but are as sensitive as wild type replicon to inhibitors of NS3/4A protease, NS5B polymerase and peg-IFN α (Table 7).

Table 7: Profile of anti-HCV agents on wild type and DCV resistant variants in the cell culture replicon system: 1a replicon cell lines (Source: NDA 206893, Addendum 1 to BMS Scientific Report 930023288, Page 8, Table 3)

Cell Line	EC ₅₀				
	BMS-790052 (nM)	NS3 Protease Inhibitor (nM)	NS5B Site I Inhibitor (nM)	NS5B Site II Inhibitor (nM)	pegIFN- α (ng/mL)
1a WT	0.009-0.050	(b) (4)	2.7-7.5	43.2	1.7
Q30E / K / D	217-239 / 82 / >2000	(b) (4)	3.3-4.6 / 2.6 / 2.7	27.8 / nd / nd	2.3 / nd / nd
Y93N / H	354-465 / 52	(b) (4)	1.8-3.6 / 4.1	17.4 / nd	1.7 / nd
M28A-Q30R	>2000	(b) (4)	3.2	nd	nd
M28T-Q30H	768- >1000	(b) (4)	2.4-3.2	25.2	2.9
Q30R-L31M	442	(b) (4)	5.6	nd	nd
Q30H-Y93H	471-561	(b) (4)	1.5-2.8	15.5	3.0
Q30R-H58D	>2000	(b) (4)	5.4	nd	nd

nd = not determined

Table 7 shows that replicons containing amino acid substitutions (listed above) which confer a very high level of reduced susceptibility to DCV were susceptible to BMS-650032 (NS3/4A protease inhibitor), BMS-791325 (NS5B site 1) and HCV 796 (NS5B site II inhibitor) and peg IFN- α .

VII. Susceptibility of DCV resistant replicons to cyclosporine

HCV NS5A resistant replicons containing amino acid substitutions (M28T, Q30H, Q30R, L31V, Y93H) were tested for their susceptibilities to cyclosporine (NS5A inhibitor). Table 8 shows that the DCV resistant replicons tested were as sensitive as wild type replicons to cyclosporine A.

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Table 8: Susceptibility of DCV resistant variants replicons to cyclosporine in 1a replicon cell lines (Source: NDA 206893, Addendum 1 to BMS Scientific Report 930023288, Page 8, Table 4)

Agent	1aWT	M28T	Q30H	Q30R	L31V	Y93H
DCV EC ₅₀ (nM)	0.0049	4.1	9.7	6.2	21	29
CsA EC ₅₀ ± sd (nM)	230 ± 20	290 ± 20	270 ± 20	290 ± 50	310 ± 70	320 ± 20
CsA CC ₅₀ ± sd (nM)	3030 ± 960	>20,000	>20,000	>20,000	>20,000	5190 ± 2120

Comment

DCV resistant replicons were not cross-resistant to cyclosporine which also inhibits HCV NS5A activity. The sponsor did not test the activity of DCV against replicons expressing alisporivir resistance substitutions, i.e. D320E, R347W, A349V, T324R, P350L.

Clinical Studies

Study AI444010

Title: Genotypic and Phenotypic Analysis of Viral Variants in Treatment-Naïve Subjects Infected with HCV Genotype 1 and Genotype 4 and Treated with Daclatasvir (BMS-790052) plus PegInterferon Alfa-2a (Pegasys[®]) and Ribavirin (Copegus[®]) in the Phase 2 B Study AI444010.

In the randomized, double-blind, placebo-controlled, multicenter, Phase 2b study, AI444010, chronically infected HCV GT-1 and GT-4 treatment naïve (NV) subjects were treated with DCV at doses of 20 and 60 mg QD, respectively, and combined with Pegasys[®]/Copegus[®] (IFN α /RBV) for 24 or 48 weeks based on response-guided therapy. Subjects treated with placebo received IFN α /RBV for 48 weeks.

Resistance Testing

On-treatment plasma specimens were collected on Day 1 (BL) and at WKs 2, 4, 6, 8, 12, 16, 20, 24 and 48; and were collected post-treatment at follow-up Weeks (FUWKs) 4, 12, 24, and 48. Resistance testing was performed by BMS using population sequencing on (1) all BL samples, and (2) samples in subjects with HCV RNA \geq 1,000 IU/mL at or close to the time of virologic failure, defined by the following criteria:

- Virologic breakthrough, defined as confirmed >1 log₁₀ increase in HCV RNA over nadir or confirmed HCV RNA \geq LLOQ after confirmed HCV RNA $<$ LLOQ, TND while on treatment.
- Measurements were confirmed at the next scheduled visit.
- <1 log₁₀ decrease in HCV RNA from baseline at Week 4 of treatment.

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- Failure to achieve EVR: $<2 \log_{10}$ decrease in HCV RNA from baseline and HCV RNA \geq LLOQ at Week 12 of treatment.
- HCV RNA \geq LLOQ at Week 12 and HCV RNA >50 IU/mL at Week 24 of treatment.
- HCV RNA \geq LLOQ at EOT (including early discontinuation).
- Relapse, defined as HCV RNA $<$ LLOQ, TD or \geq LLOQ during follow-up after HCV RNA $<$ LLOQ, TND at EOT.

I. Resistance analysis of isolates from subjects infected with HCV GT-1a

Resistance analysis of isolates from subjects infected with HCV genotype 1a are summarized here from data presented in NDA 206843, BMS Study Report 930071097, Pages 29-49, Table 6.

Comment

This report was previously reviewed and is summarized here. For a complete detail, please see Virology Review of IND 79599 SDN 605 dated 11/20/13.

I (a). GT-1a NS5A resistance-associated polymorphisms at baseline (BL)

Pre-existing NS5A polymorphisms at amino acid positions associated with resistance to DCV (amino acid positions 28, 30, 31, 54, 58, 62, and 93) were detected by population sequencing in samples from 27% (29/106) of subjects from the cohort receiving DCV 20 mg, and from 25% (28/113) of subjects from the 60 mg cohort. The GT-1a baseline NS5A polymorphisms included V/T28, H/R30, I/M/V31, C/N/Y54, Q/P/R58, D/G/Q/V62, C/H/N/S93. Of those 57 subjects having viruses with NS5A resistance-associated polymorphisms, 44% (25/57) did not achieve SVR24. Of those 25 subjects who did not achieve SVR24, 12% (3/25) had missing data points or an AE, 16% (4/25) experienced virologic breakthrough, 12% (3/25) met the Week 4 stopping rule, 28% (7/25) were partial responders, and 32% (8/25) experienced relapse.

In the subjects without these resistance-associated polymorphisms, 46% (75/162) did not achieve a protocol-defined SVR24. Of those 75 subjects who did not achieve SVR24, 19% (14/75) had missing data points, lost to follow up, an AE, withdrew consent or died, 28% (21/75) experienced virologic breakthrough, 1% (1/75) had detectable HCV RNA at the end of treatment, 4% (3/75) met the Week 4 stopping rule, 11% (8/75) were partial responders, and 37% (28/75) experienced relapse.

20 mg Cohort

Thirty-four percent (10/29) of the subjects with BL NS5A resistance-associated polymorphisms experienced virologic breakthrough, relapse, met the protocol Week 4 stopping rule, or were partial responders. NS5A polymorphisms included V28, M31, Y54, P/Q/R58, G62, and H/S93 substitutions. Variants containing M28T, Q30H, L31M, Q30R/L31M and Y93C/N/S substitutions conferred 250- to 1.4×10^6 -fold decrease in susceptibility to DCV in cell culture replicons, respectively in comparison to wild type GT-1a (H77c) replicon (NDA 206843, BMS Study Report 930071097, Pages 49-51, Table 7).

Comment

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Variants with amino acid substitutions M28T, Q30H, L31M, Y93C/N/S, Q30R/L31M, Q30H/Y93H, H54Y/Y93H conferred large decreases in susceptibility to DCV in cell culture replicon.

Fifty-nine percent (17/29) of the subjects with BL NS5A resistance-associated polymorphisms achieved SVR24; NS5A polymorphisms detected included V28, R/H30, M31, Y54, P/R58, D62, and C93 substitutions. Variants containing NS5A substitutions Q30R/H, L31M, and Y93C conferred 1,083, 250 and 1,366-fold decrease in susceptibility to DCV in cell culture, respectively as compared to wild type GT-1a (H77c) replicon (NDA 206843, BMS Study Report 930071097, Pages 49-51, Table 7).

To determine whether a specific NS5A resistance-associated polymorphism was associated with virologic outcome, the effects of key variants on virologic outcome was examined:

- V28 polymorphism was detected in the viruses from 28% (8/29) of subjects' viruses with NS5A resistance-associated polymorphisms. Of those 8 subjects, 6/8 subjects' viruses had only V28. Of these 6, 3/6 relapsed (AI444010-19-353, AI444010-23-513 and AI444010-42-327). The other 3 subjects whose viruses had only V28 polymorphisms present in baseline isolates achieved SVR24. Two of 8 subjects whose viruses had a baseline V28 polymorphism in combination with other NS5A resistance-associated polymorphisms also achieved SVR24.
- H/R30 polymorphisms were detected in the viruses from 14% (4/29) of subjects' viruses with NS5A resistance-associated polymorphisms. Of those 4 subjects, 25% (1/4) of the subjects whose viruses had a H30 polymorphisms experienced virologic failure (missed critical visits) and 3/4 of the subjects whose viruses had V28, H30, or R30, M31 achieved SVR 24.
- M31 was detected in the viruses from 14% (4/29) of subjects whose viruses had NS5A resistance-associated polymorphisms. Of those 4 subjects, 50% (2/4) experienced virologic failure (virologic breakthrough [AI444010-69-360] or WK4 stopping rule [AI444010-28-50] and 2/4 subjects achieved SVR24.
- A Y54 polymorphisms was detected in the viruses from 10% (3/29) of subjects whose viruses had NS5A resistance-associated polymorphisms. Of those 3 subjects, 1/3 of the subjects (AI444010-30-257) experienced relapse and 2/3 of the subjects achieved SVR24.
- An P58 polymorphisms was detected in the viruses from 24% (7/29) of the subjects whose viruses had NS5A polymorphisms. Of those 7 subjects, 57% (4/7) experienced virologic failure (virologic breakthrough, partial response or relapse) and 3/7 subjects achieved SVR24. Four subjects experienced virologic failure; virologic breakthrough (AI444010-29-11; AI444010-69-360), partial response (AI444010-38-427), or relapse (AI444010-18-425).
- C93 variants were detected in the viruses from 14% (4/29) of subjects whose viruses had NS5A resistance-associated polymorphisms. Of those 4 subjects, 50% (2/4) experienced virologic failure (WK4 stopping rule [AI444010-19-436] or relapse [AI444010-30-257]) and 2/2 subjects with C93 substitution achieved SVR24.
- H/R30 polymorphisms were detected in the viruses from 14% (4/29) of subjects whose viruses had NS5A resistance-associated polymorphisms. Of those 4 subjects, 25% (1/4) of the subjects whose viruses had a H30 substitution experienced virologic failure (missed critical visits) and 3/4 of the subjects whose viruses had V28, H30, or R30, M31 achieved SVR24.

60 mg Cohort

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Sequence analysis of BL samples from 113 GT-1a subjects showed that NS5A polymorphisms at amino acid positions previously shown to be associated with resistance to DCV (M28V/T, Q30H/R, L31M/V, H54C/N/Y, H58P/R, E62D/Q/V, Y93H/N) were detected in 26% (29/113) of these subjects' viruses.

- 45% (13/29) of the subjects whose viruses had BL NS5A polymorphisms experienced virologic breakthrough, relapse, met the protocol Week 4 stopping rule, or were partial responders. NS5A resistance-associated polymorphisms included V28, M/V31, C/Y54, R58, V62, and N93 substitutions. L31M/V and Y93N substitutions conferred 250- and 34,833-fold decreases in susceptibility to DCV in cell culture replicon, respectively, as compared to wild type GT-1a (H77c) replicon.
- The virologic outcome for 7% (2/29) of the subjects (A444010-26-270 and AI444010-75-504) whose virus had BL NS5A resistance-associated polymorphisms (I28, D62 and Q62) was not known.
- ~48% (14/29) of the subjects virologic outcome BL NS5A resistance-associated polymorphisms achieved SVR24; NS5A polymorphisms detected included T/V28, H/R30, M31, N/Y54, P58, D 62, and H93 (NDA 206843, BMS Study Report 930071092, Pages 29-49, Table 6). Variants containing substitutions M28T, Q30H/R, and L31M conferred 500-, 1083- and 250-fold decreases in susceptibility to DCV, respectively, in cell culture replicon compared to wild type GT-1a (H77c) replicon (NDA 206843, BMS Study Report 930071092, Pages 49-51, Table 7). Cell culture susceptibility data for variants containing Y93H substitution were not provided.

To determine whether a specific NS5A resistance-associated polymorphism was associated with virologic outcome, the effects of key variants on virologic outcome was examined:

- V/T/I28 polymorphisms were detected in the viruses from 38% (11/29) of subjects whose viruses had NS5A resistance-associated polymorphisms. Of those 11 subjects, 50% (5/11) experienced virologic failure (virologic breakthrough, partial response or relapse). Five of 11 subjects whose viruses had V28, or T28, P58, V28, R30 polymorphisms achieved SVR24. Virologic response for 1 subject whose viruses had baseline polymorphisms I28, D62 was not reported.
- H/R30 polymorphisms were detected in the viruses from 10% (3/29) of subjects whose viruses had NS5A resistance-associated polymorphisms. All 3 subjects achieved SVR24. H30 or V28, R30, or H30, H93 substitutions were detected, respectively.
- M31 polymorphisms were detected in the viruses from 10% (3/29) of subjects whose viruses had NS5A resistance-associated polymorphisms. Of those 3, 67% (2/3) subjects (AI444010-2-195, AI444010-20-156) experienced virologic failure (partial response or WK4 stopping rule). One of 3 subjects whose viruses had an M31 polymorphism at baseline achieved SVR24 (AI444010-10-454).
- N/Y54 polymorphisms were detected in the viruses from 17% (5/29) of subjects whose viruses had NS5A resistance-associated polymorphisms. Of those 5 subjects, 40% (2/5) experienced virologic failure (partial response or relapse) and 3/5 achieved SVR.
- An P/R58 polymorphism was detected in the viruses from 17% (5/29) of subjects whose viruses had NS5A resistance-associated polymorphisms; 4/5 subjects whose viruses had a P58 polymorphism achieved SVR24 and 1 subject whose viruses had an R58 substitution at baseline experienced virologic failure (partial responder).
- D62 polymorphisms were detected in the viruses from 14% (4/29) of subjects whose viruses had NS5A resistance-associated polymorphisms. Of those 4 subjects, 50% (2/4) experienced virologic failure (virologic breakthrough or missed a critical visit) and 2/4 subjects achieved SVR24.

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- H93 polymorphisms were detected in the viruses from 10% (3/29) of subjects whose viruses had NS5A resistance-associated polymorphisms. Of those 3 subjects, 67% (2/3) experienced virologic failure (partial response or relapse) and 1/3 subjects whose viruses had H30, H93 substitutions achieved SVR24.

I (b). NS5A resistance-associated substitutions in isolates from virologic failure GT-1a subjects

20 mg Cohort

There were 46% (49/106) subjects who did not achieve SVR24; 11/49 experienced virologic breakthrough, 1/49 had detectable HCV RNA at the end of treatment, 6/49 were partial responders, 17/49 were relapsers, 3/49 subjects met the Week 4 stopping rule, and 11/49 failed for other reasons such as lost to follow-up, had a HCV RNA value missing at a critical visit, had HCV RNA <LLOQ (TD) for one visit, withdrew consent, had an AE, or died. These 11 subjects did not meet the criteria for resistance testing in that their HCV RNA levels did not increase above LLOQ (TD).

Of the 38/49 subjects who met the criteria for resistance testing, resistance-associated substitutions were detected in 34/38 subject plasma samples at or close to the time of virologic failure. Emergent NS5A resistance-associated substitutions included M28T, Q30E/G/H/K/N/R/T, L31M/V, H54Y, H58D and Y93C/H/N (NDA 206843, BMS Study Report 930071097, Pages 29-49, Table 6).

- 11/38 subjects experienced virologic breakthrough;
 - Q30 variants (Q30E/H/K or Q30E, L31M, M28T-Q30H, Q30H-Y93C/H) were detected in isolates from 9/11 virologic failure subjects.
- 1/38 subjects had detectable HCV RNA at EOT; Q30E, L31M substitutions were detected at FUWK 4.
- 6/38 subjects were partial responders. Q30K/R/H, L31M/V, Y93H substitutions were detected in failure isolates.
- 17/38 subjects were relapsers. Q30 variants (Q30E/K/H/R), Q30H, L31V, Y93H, M28V, Q30K, Q30R, L31M variants detected in isolates from 10/17 relapsers. M28V-L31M substitutions were detected from 1/17 relapsers and Y93 variants (Y93C/H) were detected in isolates from 3/17 failure subjects. Three of 3/17 relapsers did not meet the criteria for resistance testing as the HCV RNA was \leq LLOQ (TD).
- 3/38 subjects met the Week 4 stopping rule; DCV resistance-associated substitutions L31M, Q30H or Y93S were detected in isolates from subjects who met stopping rule.;

Assessment of the DCV susceptibility of the detected variants from the 34 failures, using the replicon reference GT-1a H77c NS5A sequence indicated that EC_{90} values were higher than the plasma C_{trough} values for 25/34 of these subjects (NDA 206843, BMS Study Report 930071097, Pages 51-66, Table 8). For 8/34 subjects (6 were relapsers, 1 was a partial responder, and 1 met the WK4 stopping rule criteria), EC_{90} values were approximately 2-to 4-fold lower than the of the plasma C_{trough} values. The sponsor stated that this level of discrepancy has been observed between transient replicon replication assays and stable cell line assays, and the subject NS5A background sequence can also impact the EC_{90} value. One of the 34 subjects (relapser) had plasma DCV C_{trough} values that were approximately 10-fold higher than the cell culture replicon EC_{90} value for the observed substitutions.

60 mg Cohort

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There were 45% (51/113) subjects who did not achieve SVR24; 14/51 experienced virologic breakthrough, 9/51 were partial responders, 19/51 were relapsers, 3/51 subjects met the Week 4 stopping rule, and 6/51 failed for other reasons such as lost to follow-up, had a HCV RNA value missing at a critical visit, withdrew consent, or had an AE. These 6 subjects did not meet the criteria for resistance testing in that their HCV RNA did not increase above LLOQ (TD).

Of the 45/51 subjects who met the criteria for resistance testing, NS5A resistance-associated substitutions were detected in plasma samples of 42/45 subjects at or close to the time of virologic failure. Emergent NS5A resistance-associated substitutions included M28A/T, Q30E/ H/K/R/, L31M/V, H54Y, H58D and Y93H/N (NDA 206843, BMS Study Report 930071097, Pages 29-49, Table 6).

- 14/45 subjects experienced viral breakthrough.
 - Q30E, Q30R-L31M, Q30R-H58D, Q30R-L31M-H58D, Q30H-Y93H substitutions were detected in the viruses from 12/14 failures.
 - Y93N substitution was detected in the virus from 1/14 failures.
 - 1/14 virologic breakthrough subject had variants with M28T substitution.
- 9/45 subjects were partial responders;
 - Variants with Q30 substitutions were detected in the viruses from 7/9 partial responders; Q30E/R/H, H58P, L31M, M28A/T, Y93H. Variants with L31 substitutions were detected in the viruses from 2/9 failures; M28T, L31M/V, Y93H.
- 19/45 subjects were relapsers;
 - Variants with Q30 substitutions (Q30E/H/K/R), Q30H, Y93H, L31M were detected in the viruses from 11/19 relapsers.
 - Variants with L31 substitutions were detected in the viruses from 3/19 failures; L31M; M28V-L31M.
 - Variants with Y93 substitutions were detected in the viruses from 2/19 failures: Y93H/N
- For 3/19 subjects classified as relapsers, resistance testing was not possible as HCV RNA was \leq LLOQ (TD) or $<1,000$ IU/mL.
- 3/45 subjects met the Week 4 stopping rule; variants with Q30 substitutions were detected in the viruses from all 3; Q30E, Q30R, L31M and Q30R/L31M/H58D/Y93C

Assessment of the DCV susceptibility of the detected variants in the 42 failures, using the replicon reference GT-1a H77c NS5A sequence indicated that EC_{90} values were greater than the plasma DCV C_{trough} values for 39/42 of these subjects (NDA 206843, BMS Study Report 930071092, Pages 51-66, Table 8). For 2/42 of the subjects (1 partial responder and 1 relapser), the cell culture EC_{90} values of DCV for the detected variants were 2-to 3-fold lower than the C_{trough} plasma concentrations.

I (c). Persistence of GT1-a NS5A resistance-associated variants

Eighty-three virological failure subjects were monitored for persistence of DCA resistance-associated substitutions till follow up Weeks 24 to 48 (FUWK 24 to 48). Persistence of baseline NS5A polymorphism and the treatment emergent DCA resistance-associated substitutions are briefly summarized here.

20 mg Cohort

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DCA resistance-associated substitutions (M28T/V, Q30H/K/R, L31M/V, H58P, Y93C/H/S) present in baseline isolates or on WK 2 or 4 on treatment isolates from 17/38 virological failure subjects persisted till follow-up weeks 24, 36 or 48 (NDA 206843, BMS Study Report 930071097, Pages 29-49, Table 6).

60 mg cohort

DCA resistance-associated substitutions (M28A/T/V, Q30E/H/K/R, L31M/V, H54C/Y, H58D/R, E62V, Y93C/H/N) present in baseline isolates or on WK 2 or 4 on treatment isolates from 22/45 virological failure subjects persisted till follow-up weeks 24, 36 or 48 (NDA 206843, BMS Study Report 930071097, Pages 29-49, Table 6).

I. Resistance Analysis of Subjects Infected with HCV GT-1b

II (a). DCV resistance-associated NS5A polymorphism in isolates from GT-1b infected subjects at baseline

20 mg Cohort

Sequence analysis of BL samples from 41 GT-1b subjects showed that NS5A polymorphisms at amino acid positions previously shown to be associated with resistance to DCV were detected in 61% (25/41) of these subjects (L28I, P29L, R30Q, L31M/V, Q54H/Y, P58S/T, Q62E, A92T/V, Y93H) [NDA 206843, BMS Study Report 930071097, Pages 79-82, Table 10].

- 16% (4/25) of the subjects whose viruses had BL NS5A resistance-associated polymorphisms experienced virologic breakthrough, relapse, were partial responders or missed a visit. NS5A polymorphisms included substitutions R30Q, Q54H, P58S, A92V, and Y93H. Only Y93H substitution conferred 30-fold decrease in susceptibility to DCV in cell culture replicon as compared to wild type GT-1b (Con1) replicon (NDA 206843, BMS Study Report 930071092, Pages 83-84, Table 11).
- 76% (19/25) of the subjects whose viruses had BL NS5A resistance-associated polymorphisms achieved SVR24; NS5A polymorphisms detected included amino acid substitutions L28I, P29L, R30Q, L31M/V, Q54H/L/Y, P58S/T, Q62E, A92T/V, and Y93H. The NS5A resistance-associated substitutions L31M/V and Y93H, when introduced into a HCV GT-1b Con1 replicon construct, respectively, conferred 33- and 30-fold decrease in susceptibility to DCV in cell culture replicon as compared to wild type GT-1b Con1 replicon.

To determine whether a specific NS5A polymorphism was associated with virologic outcome, the effects of key variants on virologic outcome was examined:

- Q30 polymorphisms (R30Q) were detected in the viruses from 20% (5/25) of subjects whose viruses had NS5A resistance-associated polymorphisms. Of those 5 subjects, 40% (2/5) experienced virologic failure (partial response or missed critical visits) and 3/5 subjects achieved SVR24.
- M/V31 polymorphisms were detected in the viruses from 8% (2/25) subjects whose viruses had NS5A resistance-associated polymorphisms. Both subjects achieved SVR24.

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- H/L/Y54 polymorphism was detected in the viruses from 64% (16/25) of subjects whose viruses had NS5A resistance-associated polymorphisms. Of the 16 subjects, 12.5% (2/16) experienced virologic failure (partial response or relapse) and 14/16 subjects with Q54H substitution alone or in combination with other substitutions achieved SVR24.
- H93 polymorphism was detected in the viruses from 12% (3/25) of subjects whose viruses had NS5A resistance-associated polymorphisms. Of those 3 subjects, 33% (1/3) experienced virologic failure (relapse) and 2/3 subjects achieved SVR24.

60 mg Cohort

Sequence analysis of BL samples from 31 GT-1b subjects demonstrated that that NS5A polymorphisms at amino acid positions previously shown to be associated with resistance to DCV (L28V, R30Q, L31I/M, Q54H/Y, P58S, Q62D/E, A92T, Y93H) were detected in 71% (22/31) of these subjects (NDA 206843, BMS Study Report 930071097, Pages 79-82 , Table 10].

- 32% (7/22) of the subjects with BL NS5A resistance-associated polymorphisms experienced virologic breakthrough, relapse, or were lost to follow up. NS5A resistance-associated polymorphisms included V28, Q30, H54, S58, D/E62, T92, and H93.
- 68% (15/22) of the subjects whose viruses had BL NS5A resistance-associated polymorphisms achieved SVR24; NS5A resistance-associated polymorphisms detected included substitutions Q30, I/M31, H/Y54, S58, and H93.

To determine whether a specific NS5A resistance-associated polymorphism was associated with virologic outcome, the effects of key variants on virologic outcome were examined:

- V28 variants were detected in the viruses from 9% (2/22) of subjects whose viruses had NS5A resistance-associated polymorphisms. Both experienced virologic failure (virologic breakthrough or relapse).
- I/M31 polymorphisms were detected in the viruses from 9% (2/22) of subjects whose viruses had NS5A resistance-associated polymorphisms. Both subjects achieved SVR24.
- H/Y54 polymorphisms were detected in the viruses from 54% (12/22) of subjects whose viruses had NS5A polymorphisms. Of the 12 subjects, 25% (3/12) experienced virologic failure (relapse or lost to follow up) and 9/12 subjects achieved SVR24.
- S58 polymorphisms were detected in the viruses from 32% (7/22) of subjects whose viruses had NS5A resistance-associated polymorphisms. Of the 7 subjects, 43% (3/7) experienced virologic failure (relapse) and 4/7 subjects achieved SVR24.
- An H93 polymorphism was detected in the viruses from 18% (4/22) subjects whose viruses had NS5A polymorphisms. Of those 4, 25% (1/4) experienced virologic failure (relapse) and 3/4 subjects achieved SVR24.

II (b). DCV resistance-associated substitutions in GT-1b virologic failure subjects

20 mg Cohort

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There were 27% (11/41) of subjects who did not achieve SVR24; 1/11 experienced virologic breakthrough, 1/11 had detectable HCV RNA at the end of treatment, 3/11 were partial responders, 5/11 were relapsers, and 1/11 had a HCV RNA value missing at a critical visit.

Of the 10/11 subjects who met the criteria for resistance testing, DCV resistance-associated substitutions were detected in 7/10 subjects at or close to the time of virologic failure. Emergent DCV resistance-associated substitutions included R30H, L31I/M, Q54H, P58S and Y93H (NDA 206843, BMS Study Report 930071097, Pages 79-82, Table 10).

- 1/10 subjects experienced virologic breakthrough at WK20 although HCV RNA was <1,000 IU/mL through WK2 to WK24 of treatment. At the last visit tested, viral load was 1176 IU/mL; no NS5A sequence could be amplified.
- 1/10 subjects had detectable HCV RNA at EOT and Y93H substitution was detected at FUWK4 in isolates from this subject.
- 3/10 subjects were partial responders. R30H, Y93H substitutions were detected at follow up Week 12 from 1 of the partial responders.
- 5/10 subjects relapsed. L31I/M, P28S and Y93H substitutions were detected at follow-up Week 12-48 in isolates from 5/5 subjects.

Assessment of DCV susceptibility of the detected variants using the replicon reference GT-1b Con1 NS5A sequences from 8 virologic failure subjects in the 20 mg cohort showed that that EC₉₀ values for 4/8 of these subjects were greater than the plasma DCV C_{trough} values (NDA 206843, BMS Study Report 930071097, Page 84-86, Table 12). However, EC₉₀ values of the emergent variants from 2/8 virologic failure subjects were lower than the DCV C_{trough} values. EC₅₀ values of emergent variants from the remaining 2/8 virologic failure subjects were not available.

60 mg Cohort

There were 27% (7/31) of subjects who did not achieve SVR24; 1/7 experienced virologic breakthrough, 4/7 were relapsers, and 2/7 had a HCV RNA value missing at a critical visit or were lost to follow up. These subjects did not meet the criteria for resistance testing in that their HCV RNA did not increase above LLOQ (TND). Of the 5/7 subjects who met the criteria for resistance testing, DCV resistant variants were detected in 5/5 subjects' viruses at or close to the time of virologic failure. Emergent NS5A resistance-associated substitutions included L28A, R30Q, L31M/V, P58S, Q62D and Y93C/H (NDA 206843, BMS Study Report 930071097, Pages 79-82, Table 10).

- 1/5 subjects experienced viral breakthrough. L28A, L31V, R30Q substitutions in combination with BL polymorphism D62 emerged during therapy in isolates from breakthrough subject.
- 4/5 subjects were relapsers. Variants with amino acid substitutions L28V, L31M/V, Q54H, P58S, Q62E/D, A92T, Y93H developed during treatment and at the time of follow-up. Baseline isolates from these 4 relapsers also contained V28, H54, S58, D/E62, T92 and H93 polymorphisms which persisted at follow-up therapy at FUWK 4 through FUWK 48 (NDA 206843, BMS Study Report 930071097, Pages 79-82, Table 10).

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Assessment of the DCV susceptibility of the detected variants in the 5 failures in 60 mg cohort using the replicon reference GT-1b Con1 NS5A sequence indicated that EC₉₀ values were greater than the plasma DCV C_{trough} values for 3/5 relapsers. [NDA 206843, BMS Study Report 930071092, Page 84-86, Table 12). For the 2 failures (both relapsers) where plasma DCV C_{trough} values were greater, 1 of the 2 had variants that resulted in EC₉₀ values that were 2-to 3-fold lower than the plasma DCV C_{trough} value. For 1/5 subjects, EC₉₀ values of the detected variant did not provide an explanation as to why the subject experienced virologic failure.

II (c). Persistence of GT-1b NS5A resistance variants

There were 18 subjects who did not achieve SVR24. Of those 18 subjects, 15/18 had a defined reason for virologic failure, and of those 15 subjects, resistance testing was possible in 12/15 subjects.

20 mg Cohort

DCA resistance-associated substitutions (R30H, 93H/) present in baseline isolates from 2/11 virological failure subjects persisted till follow-up weeks 24, 36 or 48 (NDA 206843, BMS Study Report 930071097, Pages 79-82, Table 10).

60 mg cohort

DCA resistance-associated polymorphisms (L/M28A/V, L31M/V, Q54H, P58S, H58D, Q62D/E, Y93C/H) present in baseline isolates or emerging in WK 2 or 4 on treatment isolates from 4/7 virological failure subjects persisted till follow-up weeks 24, 36 or 48.

II. Resistance Analysis of Subjects Infected with HCV GT-4

III (a) GT-4 NS5A resistance-associated polymorphisms at baseline

Pre-existing NS5A polymorphisms at amino acid positions associated with DCV resistance (amino acid positions 28, 30, 31, 54, and 62) were detected by population sequencing in samples from 83% (10/12) of subjects receiving 20 mg DCV and 100% (13/13) of subjects receiving 60 mg DCV [NDA 206843, BMS Study Report 930071097, Pages 91-92, Table 14]. Sequence data were not available for 2/12 subjects (20 mg cohort). The GT-4 BL NS5A resistance-associated polymorphisms included M28, R/S30, V31, N/R54, A/T58, E/K/Q62.

20 mg Cohort

Sequence analysis of BL samples from 12 GT-4 subjects showed that NS5A polymorphisms at amino acid positions (L28M, L30R/S, M31V, H54N, D62E/K) previously shown to be associated with resistance to DCV were detected in 83% (10/12) of these subjects (NDA 206843, BMS Study Report 930071097, Pages 91-92, Table 14).

- 30% (3/10) of the subjects whose viruses had BL NS5A E/K/Q s experienced virologic breakthrough (AI444010-26-27), met the Week 4 stopping rule criteria (AI444010-26-102), or relapse (AI444010-33-

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382). NS5A polymorphisms included E62. The D62E variant did not negatively impact the cell culture potency of DCV given that the EC₅₀ value of the reference GT-4a hybrid replicon was 0.002 nM (NDA 206843, BMS Study Report 930071097, Page 93, Table 15).

- 70% (7/10) of the subjects whose viruses had BL NS5A resistance-associated polymorphisms achieved SVR24; NS5A resistance-associated polymorphisms detected included M28, R/S30, V31, N54, and E/K62.

60 mg Cohort

Sequence analysis of BL samples from 13 GT-4 subjects showed that NS5A polymorphisms at amino acid positions (L28M, L30R, M31V, H54R, P58A/T, D62E/Q) previously shown to be associated with resistance to DCV were detected in 100% (13/13) of these subjects (NDA 206843, BMS Study Report 930071097, Pages 91-92, Table 14).

- 0% (0/13) of the subjects whose viruses had BL NS5A resistance-associated polymorphisms experienced virologic failure.

III (b). DCV resistance-associated substitutions in isolates from GT4 virologic failure subjects

20 mg Cohort

There were 33% (4/12) of subjects who did not achieve SVR24; 1/4 experienced virologic breakthrough, 2/4 were relapsers, and 1/4 subjects met the Week 4 stopping rule criteria. Of the 4 subjects who met the criteria for resistance testing, NS5A resistance variants were detected in 3/4 subjects at or close to the time of virologic failure. Emergent NS5A resistance-associated substitutions included L28L/M, L30H/P/S/Y, D62E, Y93Y/H (NDA 206843, BMS Study Report 930071097, Pages 91-92, Table 14).

Assessment of the DCV susceptibility of the detected variants to DCV in the 2 failures (AI444010-26-27, AI444010-26-102) using the replicon reference GT-4a NS5A (AI444010-68-118) sequence, indicated that EC₉₀ values were 2- to 4-fold higher than the plasma DCV C_{trough} values for these 2 subjects (NDA 206843, BMS Study Report 930071097, Pages 93-94, Table 16).

60 mg Cohort

All subjects (13/13 in DCV 60-mg cohort achieved SVR 24 (NDA 206843, BMS Study Report 930071097, Page 92, Table 14).

III (c). Persistence of GT-4 NS5A resistance variants

The persistence of resistance variants to DCV in the 3 subjects in the 20 mg cohorts were monitored till follow-up Week 48 (FUWK48). These subjects were virologic failure. Amino acid substitutions L28L/M, L30H/P/S/Y, D62E detected at Weeks 2 to 4 of treatment persisted till follow-up Week 4 thru Week 48 in isolates from 2/3 virologic failure subjects. Post-treatment isolates from another subjects at follow-up Weeks 4 developed L28M and L30P/S substitutions in addition to baseline substitution D62E. These substitutions persisted till follow-up

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Week 24 through Week 48. Thus, amino acid substitutions from 3 failure isolates in 20 mg cohort persisted till follow-up Week 48.

Comment

BMS has also submitted a report (930073428) entitled, “Genotypic and Phenotypic Analysis of Daclatasvir in Hepatitis C Virus Genotype-4 NS5A Sequences and Comparison with Genotype 1.”

Resistance data for GT 4 subjects treated with 20 mg and 60 mg DCV submitted in this report (BMS Report # 930073428) are the same as reviewed for study AI444010 above for genotype 4 subjects and also have been previously reviewed (Virology Review of IND 79599 SDN 655 Dated 02/06/14).

BMS stated (NDA206843, BMS Report # 930073428, Page 18) that they have sequenced 132 subject-derived GT-4 NS5A sequences from different clinical studies including AI444010, AI444042 and AI447016. Forty sequences from the EUHCV DB were also obtained. All 172 NS5A sequences were aligned against the GT-4a NS5A reference sequence ED43. Sequence analysis (NDA206843, BMS Report # 930073428, Page 20, Table 6) showed that the predominant NS5A resistance-associated polymorphisms were similar in both databases (L28, R30, M31, H54, P58, E62, A92, and Y93). The predominant NS5A resistance-associated polymorphisms were L28M, L30R/C/Q, M31L/V, H54N/R, P58T, D62E/Q in both the in-house and EU databases.

Study AI444011

Title: Genotypic and Phenotypic Analysis of Viral Variants in HCV Genotype 1 Subjects Who Were Null or Partial Responders to Prior Treatment with Peginterferon Alfa plus Ribavirin Therapy and Treated with Daclatasvir (BMS-790052) plus Peginterferon Alfa-2a (Pegasys[®]) and Ribavirin (Copegus[®]) in the Phase 2 B Study AI444011

In the randomized, double-blind, placebo-controlled, multicenter, Phase 2b study AI444011, chronically infected HCV GT-1 subjects who failed prior IFN-based therapy (null responders [NR] and partial responders [PR]) were treated with DCV at doses of 20 and 60 mg QD, respectively, and combined with PegIFN α /RBV for 24 or 48 weeks based on response-guided therapy. Subjects treated with placebo received PegIFN α /RBV for 48 weeks.

Subjects eligible for the study included those with chronic HCV GT-1 infection who were nonresponders (defined as those subjects with $<1 \log_{10}$ decrease in HCV RNA from BL at or after 4 weeks, or $<2 \log_{10}$ decrease from BL in HCV RNA at or after Week 12 of IFN-based therapy [null responder] or had achieved $>2 \log_{10}$ decrease in HCV RNA from BL by Week 12 of IFN based therapy, but detectable HCV RNA when therapy was discontinued [partial responder]). In this double-blind study, prior partial responder (PR) were randomized 4:4:1 to receive once daily DCV 20 mg or 60 mg or placebo in combination with PegIFN α /RBV. Prior null responder (NR) were randomized 1:1 to either 20 mg or 60 mg DCV QD in combination with PegIFN α /RBV.

All subjects received triple therapy with DCV (DCV/PegIFN α /RBV) or placebo plus PegIFN α /RBV (placebo/PegIFN α /RBV) through Week 24. A second randomization occurred at Week 24 for subjects initially

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assigned to 20 mg or 60 mg DCV who achieved a Protocol Defined Response (PDR), defined as HCV RNA <LLOQ at Week 4 and undetectable HCV RNA at Week 12. Subjects who achieved PDR were randomized (1:1) to complete therapy at Week 24 and enter follow-up (24 triple regimen), or continue therapy with PegIFN α /RBV alone for an additional 24 weeks before entering follow-up (24 + 24 PegIFN α /RBV regimen).

At Week 24, subjects who were randomized to DCV who did not achieve PDR and subjects randomized to placebo (regardless of PDR status) received an additional 24 weeks of Peg IFN α /RBV alone for a total of 48 weeks of therapy. Following completion of the follow-up period of AI444011, subjects were asked to enroll into a separate observational study for an additional 3-year follow-up to assess long-term sustained virologic response (SVR), resistance, and HCV related complications.

Resistance testing was performed on plasma samples from 402 subjects (4 GT-1, 261 GT-1a; 136 GT-1b, and 1 undetermined GT) receiving a DCV-containing regimen. BL sequence data were obtained for 391/402 subjects examined; the NS5A region could not be amplified from 7 GT-1a subjects and 4 GT-1b subjects.

III. Resistance analysis of variants from subjects infected with HCV GT-1a and GT 1b

IV (a). GT-1a NS5A resistance-associated polymorphisms at baseline

BL NS5A sequences were obtained from 254/261 (130 in the 20 mg and 124 in the 60 mg DCV cohort) GT-1a infected subjects. A further 7/254 BL subject samples (3 receiving 20-mg and 4 receiving 60-mg DCV) were not included in the analysis since 4/7 subjects (all prior NR) were mis-genotyped and 3/7 subjects did not have appropriate visit identifier. Pre-existing NS5A polymorphisms at amino acid positions associated with DCV resistance (amino acid positions 28, 30, 31, 54, 58, 62, and 93) were detected by population sequencing in samples from 15% (36/247) of GT-1a infected subjects included in the DCV analysis (NR, 14% [22/162]; PR, 17% [14/85], (NDA 206843, BMS Study Report 930071295, Page 18, Table 4). The BL GT-1a NS5A polymorphisms included M28L/V/T, Q30H, L31M, H54Y, H58C/D/N/P/Q, E62D, and Y93C substitutions. The NS5A substitutions M28T, Q30H, L31M, H58D, and Y93C each conferred a significant decrease in susceptibility to DCV (250-to-1367-fold) compared with the reference GT-1a (H77c) [NDA 206843, BMS Study Report 930071295, Page 19, Table 5]. Of those 36 subjects with NS5A resistance-associated polymorphisms, 86% (31/36) did not achieve SVR24.

Assessment of the BL NS5A polymorphism versus virologic outcome showed the following:

- L/T/V28 polymorphisms were detected in the viruses from 25% (9/36) of the subjects whose viruses had NS5A resistance-associated polymorphisms. Of those 9 subjects, 8/9 experienced on-treatment failure and 1/9 achieved SVR24.
- A H30 polymorphisms was detected in the viruses from 6% (2/36) of the subjects whose viruses had NS5A resistance-associated polymorphisms. Of those 2 subjects, 1/2 experienced on-treatment failure while 1/2 achieved SVR24
- An M31 polymorphisms was detected in the viruses from 25% (9/36) of the subjects whose viruses had NS5A resistance-associated polymorphisms. Of those 9 subjects, 8/9 experienced on-treatment failure and 1/9 achieved SVR24 but experienced a late relapse.

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- An Y54 polymorphisms was detected in the viruses from 8.3% (3/36) of the subjects whose viruses had NS5A resistance-associated polymorphisms. Of those 3 subjects, 3/3 experienced on-treatment failure.
- C/D/N/P/Q58 polymorphisms were detected in the viruses from 28% (10/36) of the subjects whose viruses had NS5A resistance-associated polymorphisms. Of those 10 subjects, 8/10 experienced on-treatment failure and 2/10 achieved SVR24.
- An D62 polymorphisms was detected in the viruses from 17% (6/36) of the subjects whose viruses had NS5A resistance-associated polymorphisms. Of those 6 subjects, 4/6 experienced on-treatment failure, 1/6 relapsed, and 1/6 achieved SVR24.
- A C93 polymorphisms was detected in the viruses from 6% (2/36) of the subjects whose viruses had NS5A resistance-associated polymorphisms. Of those 2 subjects, 1/2 experienced on-treatment failure and 1/2 relapsed.

There were 85% (211/247) subjects without detectable BL NS5A resistance-associated polymorphisms. Of those 211 subjects, 83% (175/211) failed treatment (NDA 206843, BMS Study Report 930071295, Page 17).

IV (b). GT-1a NS5A resistance variants at virologic failure

There were ~84% (218/261 subjects; 150 NR and 68 PR) of subjects who were classified as not achieving SVR24. However it should be noted that one PR subject (AI444011-71-512) experienced a relapse at FUWK48 making a total of 219 virologic failures. Of those 219 subjects, 6/219 failures were not included in the analysis due to misgenotyping or incorrect sample identifiers.

Of the 213 GT-1a failures, 181/213 (122 NR and 59 PR) of the subjects failed while on treatment and 32/213 (22 NR and 10 PR) relapsed. GT-1a resistance data were analyzed at or close to the time of virologic failure where possible and examined for 197 of the 213 subjects since an additional 16 subjects (9 NR and 7 PR) were not included either due to BL sequence not being available to compare with emergent NS5A variants or HCV RNA was <1,000 IU/mL.

Of the 197 GT-1a failures whose viruses were analyzed for NS5A resistance-associated substitutions 167/197 (113 NR and 54 PR) subjects failed while on treatment and 30/197 (22 NR and 8 PR) subjects relapsed [NDA 206843, BMS Study Report 930071295, Page 23, Table 6]. GT-1a NS5A resistance-associated substitutions detected at or close to virologic failure included M28A/G/S//T/V, Q30D/E/G/H/K/N/R/T, L31I/M/V, H54R/Y, H58D/N/P/Q/V, A92P, and Y93C/H/N/R/S; Q30 variants were detected most frequently (91%; 180/197 failures) and were detected as the only variant in 25% (50/197) of GT-1a failures. The most prevalent Q30 variants contained Q30E substitution which was detected in the viruses from 27% (53/197) of GT-1a failures and Q30R substitution which was detected in the viruses from 24% (48/197) of GT-1a failures. The Q30R substitution was associated with a second NS5A resistance-associated substitution (M28T, L31M, H58D, and/or Y93N). Other NS5A variants to be detected included Y93 variants (Y93C/H/N/R/S detected in 36% [71/197] failures), L31 variants (L31M/V) detected in 58% [114/197] failures). M28A/G/S//T/V and H58D/N/P/Q/V were detected in the viruses from 29% (58/197) and 25% (49/197) virologic failure subjects, respectively.

Assessment of the susceptibility of the detected NS5A resistance-associated substitutions in the viruses from 197 failures showed that the DCV EC₉₀ values against these NS5A variants were higher than the C_{trough} exposure level of DCV for these subjects (NDA 206843, BMS Study Report 930071295, Pages 24-45, Table 7).

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However, there were 16 subjects who failed treatment although DCV C_{trough} exposure levels were higher than the EC_{90} values in cell culture replicon for their respective substitutions.

IV (c) GT-1b NS5A resistance-associated polymorphisms at baseline

BL NS5A sequences were obtained from 131/136 GT-1b subjects' viruses. An additional 5/136 BL subject samples were not included in the analysis due to either mis-genotyping (2/136) or not having the appropriate visit identifier (3/136).

Of the 131 confirmed GT-1b subjects treated with DCV (82 NR and 49 PR), BL NS5A sequences were available from samples from 127/131 subjects. Pre-existing NS5A polymorphisms at amino acid positions associated with DCV resistance (amino acid positions 28, 30, 31, 54, 58, 62, 92, and 93) were detected by population sequencing in samples from 65% (82/127) of subjects (NR, 73% [58/80]; PR, 51% [24/47], (NDA 206843, BMS Study Report 930071295 Page 53, Table 9). The GT-1b BL NS5A resistance-associated polymorphisms included M/V28, H/Q30, M31, H/N/Y54, A/Q/S58, E/K/N/R/S62, T/V92, and F/H93. The NS5A substitution Y93H conferred 30-fold decrease in susceptibility to DCV (EC_{50} value = 90 pM), however combination of Y93H with R30H, L31M, or L31M Q54H each conferred a significant decrease in susceptibility (2,666- to-16,000-fold) to DCV (EC_{50} value range of 8 to 48 nM) compared with the reference GT-1b (Con1) (DCV EC_{50} value = 3 pM). Of those 82 subjects whose viruses had NS5A resistance-associated polymorphisms, 65% (53/82) did not achieve SVR24.

Assessment of the BL NS5A polymorphism versus virologic outcome showed the following:

- H/Q30 polymorphisms were detected in the viruses from 11/11 subjects who experienced virologic failure; 7/11 subjects experienced on-treatment failure (4 virologic breakthrough, 3 WK4 stopping rule) and 4/11 relapsed.
- M31 polymorphism alone or in combination with H54 or H93 polymorphisms was detected in the viruses from 11 subjects; 9/11 of these subjects experienced virologic failure; 6/9 subjects experienced on-treatment failure and 3/9 relapsed. Two of the 11 subjects with M31, H54 polymorphisms achieved SVR 24.
- H/Y/N54 polymorphisms were detected in the viruses from 55 subjects; H54 polymorphisms accounted for 85.5% (47/55) of these subjects, N54 polymorphism was detected in the viruses from 9.1% (5/55), Y54 polymorphism was detected in the viruses from 3.6% (2/55), and H/N54 polymorphism was detected in the viruses from 1.8% (1/55) of subjects. Thirty-eight of 55 (69%) subjects were virologic failure; 27/38 subjects experienced on-treatment failure, 8/38 relapsed, and 3/38 were undefined. Seventeen of 47 subjects whose viruses H54 polymorphisms achieved SVR24; H54 was detected in the viruses from all 17 subjects.
- F/H93 polymorphisms were detected in the viruses from 7/9 subjects who experienced virologic failure; 4/7 subjects experienced on-treatment failure and 3/7 relapsed.

IV(d). GT-1b NS5A resistance-associated substitutions at virologic failure

There were 59% (80/136 of subjects; 57 NR and 23 PR) of GT-1b subjects who were classified as virologic failures. Of those 80 subjects, 3/80 failures were not included in resistance analysis (NDA 206843, BMS Study Report 930071295, Page 58, Table 11) due to mis-genotyping or incorrect sample identifiers. These 3 subjects were all prior NR:

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Genotypes for baseline matched treatment failure isolates were available only for 50 subjects although the sponsor stated that resistance data were available for 70 of the 77 subjects (NDA 206843, BMS Study Report 930071295, Page 58, Table 11). Resistance data for baseline matched treatment failure isolates were not available for 16 NR and 11 PR subjects (NDA 206843, BMS Study Report 930071295, Pages 59-69, Table 12).

GT-1b NS5A resistance-associated substitutions detected at or close to virologic failure included L28M, P29X, R30H/K/L/P/Q/S, L31F/I/M/V, P32X, Q54H/Y, P58S, A92E/K/T, and Y93H. Variants with L31I/M/V 96% (48/50), and Y93H 90% (45/50) substitutions emerged in isolates from most of the virologic failure subjects. In addition, Q54H 54 % (27/50) and R30Q 22% (11/50) substitutions also emerged in isolates from virologic failure subjects.

Assessment of the susceptibility of the detected NS5A variants from 70 virologic failures (NDA 206843, BMS Study Report 930071295, Pages 59-69, Table 12) showed that the plasma DCV C_{trough} drug concentrations were lower than the DCV EC_{90} value against these NS5A substitutions, as determined in a GT-1b NS5A replicon assay. There were 16 subjects who failed treatment although DCV C_{trough} exposure levels were 2-to-4 fold higher than EC_{90} values.

Study AI444014

Title: Genotypic and Phenotypic Analysis of Viral Variants in HCV Genotype 1-Infected Subjects Treated with Daclatasvir (BMS 790052) and PegInterferon Alfa-2a (Pegasys[®]) plus Ribavirin (Copegus[®])

Subjects and Study Design

Subjects eligible for the study included those with chronic HCV GT-1 infection who were treatment-naive or who had less than 4 weeks exposure to RBV or PegIFN α based therapy. Subjects were required to have HCV RNA levels $\geq 10^5$ IU/mL and to be 18-70 years of age. In this double-blind, parallel-group study, 48 subjects from 14 centers in the United States and France were randomized 1:1:1:1 to placebo or once daily DCV 3 mg, 10 mg, or 60 mg in combination with PegIFN α -2a, 180 μ g/week, and RBV, 1.0 to 1.2 gm/day, for 48 weeks.

All baseline (BL; Day 1) samples were analyzed by population sequencing. Resistance testing was performed by population sequencing on stored samples at Weeks 4, 12, 24, and 48 on-treatment, and post-treatment samples were collected at Week 4, 12, and 24 if HCV RNA was detected at ≥ 1000 IU/mL.

V. NS5A resistance-associated polymorphisms at baseline

Pre-existing NS5A polymorphisms at amino acid positions associated with DCV resistance were detected by population sequencing in samples from 39% (14/36) of subjects (8 GT-1a, 6 GT-1b) from the 3 DCV dosing cohorts (3 mg, 42% [5/12]; 10 mg, 33% [4/12]; 60 mg, 42% [5/12]) (NDA, 206843, BMS Study Report 030068888, Pages 24-27, Table 3). In subjects infected with HCV GT-1a, NS5A polymorphisms included V28, P58, and D62; while for GT-1b NS5A polymorphisms included Q30, H/N/Y54, T/A/S58, E62, E/T/V92 and C/H93 (NDA, 206843, BMS Study Report 030068888, Pages 24-27, Table 3). Of those 14 subjects with these

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NS5A polymorphisms, 29% (4/14) experienced virologic failure although 1 was due to being lost to follow-up. There were 22 subjects with no detectable NS5A resistance-associated polymorphisms; 32% (7/22) were virologic failures although 2 of these 7 subjects did not complete the study.

DCV 3 mg Cohort

V(a). NS5A resistance-associated polymorphisms at baseline

- GT-1a NS5A resistance-associated polymorphisms detected in 2 GT-1a subjects (AI444014-5-26 and AI444014-7-55) included V28 and P58. In the GT-1a H77c replicon, V28 and P58 conferred no change in susceptibility to DCV when compared to the wild-type replicon sequences (Source: NDA 206843, BMS Study Report 030068888, Pages 28-29, Table 4).
- GT-1b NS5A resistance-associated polymorphisms detected in the 3 GT-1b subjects (AI444014-7-58, -12-49, -12-66) included Q30, H/N54, T/A/S58, and E62 substitutions. When assessed in GT-1b Con1 replicons, none of these polymorphisms conferred a change in susceptibility to DCV (Source: NDA 206843, BMS Study Report 030068888, Pages 29-30, Table 5). Two of the subjects achieved a SVR24 while one experienced virologic breakthrough.

V(b). NS5A resistance-associated substitutions in virologic failures

In the 3 mg cohort, 7/12 subjects (4 GT-1a, 3 GT-1b) were classified as treatment failures; 2 experienced virologic breakthrough, 2 relapsed, 1 discontinued due to an AE, 1 was lost to follow-up and 1 no longer met the study criteria (NDA 206843, BMS Study Report 030068888, Pages 24-27, Table 3).

Two subjects (1 GT-1a and 1 GT-1b) experienced virologic breakthrough

- AI444014-8-19 (GT-1a) experienced virologic breakthrough at Week 4 of treatment. At Week 4 and Week 24, Q30H and Q30G substitutions emerged, respectively. In the GT-1a H77c replicon transient HCV replication assay, Q30H and Q30G substitutions exhibited 1,083- and 6,333-fold reduced susceptibility to DCV, respectively (Source: NDA 206843, BMS Study Report 030068888, Pages 28-29, Table 4).
- AI444014-7-58 (GT-1b) experienced virologic breakthrough at Week 12. At Week 12, the Y93H substitution emerged. This subject also had NS5A H54, A58, E62 polymorphisms at baseline (NDA 206843, BMS Study Report 030068888, Pages 24-27, Table 3). In the GT-1b Con1 replicon, Q54H, P58A, Q62E, Y93H substitutions exhibited an EC₅₀ value of 2 nM (667-fold resistance to DCV [NDA 206843, BMS Study Report 030068888, Page 30, Table 5

Two subjects (1 GT-1a and 1 GT-1b) experienced relapse after 48 weeks of treatment (NDA 206843, BMS Study Report 030068888, Pages 24-27, Table 3).

Three GT-1a failures discontinued treatment after either receiving ≤ 2 weeks or 12 weeks of therapy (Source: NDA 206843, BMS Study Report 030068888, Pages 24-27, Table 3).

DCV 10 mg Cohort

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V(c). NS5A resistance-associated polymorphisms at baseline

Pre-existing NS5A resistance-associated polymorphisms were detected in the viruses from 33% (4/12) of the subjects (2 GT-1a, 2 GT-1b) in the DCV 10 mg cohort (NDA 206843, BMS Study Report 030068888, Pages 24-27, Table 3). Of the subjects whose viruses had NS5A resistance-associated polymorphisms, 1/4 experienced relapse and 3/4 achieved SVR24.

- GT-1a NS5A resistance-associated polymorphisms detected in the viruses from 2 GT-1a subjects (AI444014-8-50 and AI444014-12-15) included V28 substitution. In the GT-1a H77c replicon, M28V conferred no decrease in susceptibility to DCV (Source: NDA 206843, BMS Study Report 030068888, Page 28, Table 4). Both subjects achieved SVR24.
- GT-1b NS5A resistance-associated polymorphisms detected in the viruses from 2 GT-1b subjects (AI444014-1-54 and AI444014-17-37) included H/Y54, E/V92 and H93 substitutions. When assessed in GT-1b Con1 replicons, only Y93H conferred 31-fold decrease in DCV susceptibility in cell culture replicon. Both subjects had H93 polymorphisms at BL, although it was only present as a minor species in AI44401-1-54. Interestingly, AI444014-1-54 experienced relapse while AI444014-17-37 achieved SVR24.

V(b). NS5A resistance-associated substitutions in virologic failures

In the 10 mg cohort, 2/12 subjects (1 GT-1a, 1 GT-1b) were classified as treatment failures; 1 was a partial responder (AI444014-6-61) and 1 relapsed (NDA 206843, BMS Study Report 030068888, Pages 24-27, Table 3).

- AI444014-6-61 (GT-1a) was a partial responder. Although BL viral load was 443,000 IU/mL, HCV RNA remained detectable from Week 2 through Week 20 (HCV RNA of 132 to 249 IU/mL) before declining to <25 IU/mL at Week 40 through Week 48 of treatment. HCV RNA rebounded at FUWK4; Q30E substitution was detected at FUWK4 through FUWK24 but not at BL, as determined by clonal analysis. NS5A Q30E exhibited 18,500-fold decrease in susceptibility to DCV in the GT-1a H77c transient HCV replication assay (Source: NDA 206843, BMS Study Report 030068888, Pages 28- 29, Table 4).
- AI444014-1-54 (GT-1b) experienced relapse at FUWK24 after discontinuing 28 weeks of therapy. The DCV C_{trough} level in this subject was ~24 nM (18 ng/mL) which was significantly greater than the observed EC_{50} values for the resistance-associated polymorphisms detected at BL (A92E/V conferred no resistance to DCV whereas Y93H conferred 31-fold decrease in DCV susceptibility in GT-1b Con replication assay (NDA 206843, BMS Study Report 030068888 Pages 29-30, Table 5]. NS5A L28M, Y93H substitutions were detected at FUWK24 and resulted in 933-fold decrease in susceptibility to DCV in the transient GT-1b Con replication assay.

DCV 60 mg Cohort

V(d). NS5A resistance-associated polymorphisms at baseline

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Pre-existing NS5A resistance-associated polymorphisms were detected in the viruses from 42% (5/12) of subjects (3 GT-1a, 2 GT-1b) in the DCV 60 mg cohort. Of the subjects whose viruses had NS5A resistance-associated polymorphisms, 1/5 experienced relapse and 4/5 achieved SVR24.

- GT-1a NS5A resistance-associated polymorphisms detected in the viruses from 3 GT-1a infected subjects (AI444014-1-53, -5-25, -17-36) included V28, P58, and D62.. In the GT-1a H77c replicon, these substitutions conferred no change in susceptibility to DCV when compared to the wild-type replicon sequence (NDA 206843, BMS Study Report 030068888, Pages 28-29, Table 4). AI444014-5-25 experienced relapse while the other two achieved SVR24.
- GT-1b NS5A resistance-associated polymorphisms detected in the viruses from 2 GT-1b infected subjects (AI444014-1-52, -2-7) included H54, T92, and C93. Only the Y93C substitution conferred a 2-fold decrease in susceptibility to DCV. Both subjects achieved SVR24 (NDA 206843, BMS Study Report 030068888, Pages 30-31, Table 5).

V (e). NS5A resistance-associated substitutions in virologic failures

In the 60 mg cohort, 2/12 subjects (2 GT-1a) were classified as treatment failures; 1 experienced virologic breakthrough (AI444014-5-40) and 1 relapsed (AI444014-5-25; [NDA 206843, BMS Study Report 030068888; Pages 24-27, Table 3]).

- AI444014-5-40 (GT-1a) experienced a transient virologic breakthrough at Week 24, had undetectable HCV RNA from Week 32 to the end of treatment (Week 48); and then relapsed (FUWK12); Q30R, H58D substitutions were emerged at FUWK12. In the transient GT1a-H77c replication assay, Q30R, H58D substitutions exhibited DCV EC₅₀ value of 1,867 nM (311,167-fold decrease in susceptibility to DCV [NDA 206843, BMS Study Report 030068888, Pages 28- 29, Table 4]).
- AI444014-5-25 (GT-1a) experienced relapse after discontinuing treatment at Week 8 due to an AE; M28M/V substitution was detected at BL while Q30E was detected at FUWK4 and M28A, Q30R substitutions were detected at FUWK24. In transient GT-1a H77c replication assays, Q30E and M28A/Q30R substitutions had DCV EC₅₀ values of 111 nM and 1,262 nM, respectively, or 18,500- and 210,333-fold decrease in susceptibility to DCV, respectively (NDA 206843, BMS Study Report 030068888, Pages 28- 29, Table 4).

AI444021

Title: Genotypic and Phenotypic Analysis of Viral Variants in HCV Genotype 1-Infected Japanese Subjects Treated (BMS-790052) and Peginterferon Alfa-2b (PegIntron[®]) plus Ribavirin (Rebetol[®])

Subjects and Study Design

Japanese subjects eligible for the study included those with chronic HCV GT-1b infection who were NV (defined as those who had never been exposed to any HCV therapy with interferon containing regimens, including PegIFN α -2b/RBV, or those containing DAA against HCV), or who were NR to previous therapy (defined as subjects who failed to achieve ≥ 2 log₁₀ reduction of HCV RNA at Week 12 [null responder] or had

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achieved a $\geq 2 \log_{10}$ reduction but never attained undetectable HCV RNA levels after at least 12 weeks [partial responder] of the current standard of care, PegIFN α /RBV or PegIFN α -2b/RBV). Subjects were non-cirrhotic adults (20-70 years old) with HCV RNA levels $\geq 10^5$ IU/mL. In this double-blind study, 45 subjects were randomized equally to receive once daily DCV 10 mg, or 60 mg or placebo (only NV received placebo) in combination with PegIFN α -2b/RBV. Subjects receiving DCV + PegIFN α -2b/RBV who achieved a protocol-defined response (PDR) were treated for 24 weeks. PDR was defined as HCV RNA <LLOQ (15 IU/mL) at Week 4 and undetectable at Week 12. Subjects not achieving PDR received DCV + PegIFN α -2b/RBV for 48 weeks. Subjects treated with placebo (NV only) received PegIFN α -2b/RBV for 48 weeks. The primary efficacy endpoint was the proportion of subjects with extended rapid viral response (eRVR), defined as undetectable HCV RNA at both Week 4 and Week 12. Secondary efficacy assessments included the proportion of subjects with rapid virologic response (RVR), defined as undetectable HCV RNA at Week 4; the proportion of subjects with complete early virologic response (cEVR), defined as undetectable HCV RNA at Week 12; the proportion of subjects with sustained virologic response (defined as undetectable HCV RNA) at week 12 (SVR12) and week 24 (SVR24) post-treatment. Serum HCV RNA levels were determined at a central laboratory ((b) (4)) using the Roche COBAS[®] TaqMan[®] HCV Auto assay, (Roche (b) (4)), with LLOQ of 15 IU/mL.

Resistance testing was performed by BMS using population sequencing on (1) all BL samples, and (2) samples in subjects with HCV RNA $\geq 1,000$ IU/mL at or close to the time of virologic failure, defined by the following criteria:

- Virologic breakthrough (virologic breakthrough), defined as confirmed $>1 \log_{10}$ increase in HCV RNA over nadir or confirmed HCV RNA \geq LLOQ after confirmed undetectable HCV RNA while on treatment.
- Measurements were confirmed at the next scheduled assessment $<1 \log_{10}$ decrease in HCV RNA from BL at Week 4 of treatment.
- Failure to achieve EVR, defined as $<2 \log_{10}$ decrease in HCV RNA from BL at Week 12 of Treatment.
- Detectable HCV RNA at Week 12 and HCV RNA \geq LLOQ at Week 24 of treatment.
- Detectable HCV RNA at EOT (including early discontinuation).
- Relapse, defined as detectable HCV RNA during follow-up after undetectable HCV RNA at EOT

VI. Resistance analysis of variants from subjects infected with GT-1a and GT-1b

Treatment-Naive Subjects

DCV 10 mg Cohort

VI (a). NS5A resistance-associated polymorphisms at baseline

Pre-existing NS5A resistance-associated polymorphisms were detected in the viruses from 67% (6/9) of subjects in the DCV 10 mg cohort (NDA 206843, BMS Study Report 930069253, Pages 21-22, Table 1). Only 2/6 subjects whose viruses had baseline polymorphisms experienced virologic failure while 4/6 achieved SVR24. BL polymorphisms were not detected in the viruses from 3/9 subjects; 1/3 relapsed and 2/3 achieved SVR24.

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- GT-1b NS5A resistance-associated polymorphisms detected in the viruses from 6 GT-1b infected subjects (AI444021-1-21107, -1-21119, -2-21122, -2-21125, -3-21123, and -3-21134) included H/Y54 and E62 substitutions. When assessed in GT-1b Con1 replicons, these variants conferred no change in susceptibility to DCV (NDA 206843, BMS Study Report 930069253, Pages 23-25, Table 2).

VI (b). NS5A resistance-associated substitutions in virologic failures

In the 10 mg cohort, 3/9 subjects were treatment failures.

- 1/3 subjects (AI444021-2-21122) experienced virologic breakthrough at Week 2 of treatment. At Week 2, L31V, Y93H substitutions emerged and were still detected at FUWK4. L31I/V, Q54H, and Y93H substitutions were detected at Week 12. In the GT-1b Con1 replicon transient HCV replication assay, L31V, Q54H, Y93H substitutions exhibited 8,667-fold reduced susceptibility to DCV (NDA 206843, BMS Study Report 930069253, Pages 23-25, Table 2).
- 1/3 subjects (AI444021-3-21123) was a partial responder with HCV RNA <LLOQ (TD) at Week 24 of treatment. At FUWK4, L31M/V, Y93H substitutions emerged. In the GT-1b Con1 replicon transient HCV replication assay, L31V, Y93H substitutions conferred 33,667-fold decrease in susceptibility to DCV (NDA 206843, BMS Study Report 930069253, Pages 23-25, Table 2).
- 1/3 subjects (AI444021-3-21117) experienced relapse at FUWK4. NS5A L31V, Y93H substitutions emerged at this time and were still detected at FUWK24. In the GT-1b Con1 replicon transient HCV replication assay, L31V, Y93H substitutions conferred 33,667-fold decrease in susceptibility to DCV (NDA 206843, BMS Study Report 930069253, Pages 23-25, Table 2).

DCV 60 mg Cohort

VI (c). NS5A resistance-associated polymorphisms at baseline

Pre-existing NS5A resistance-associated polymorphisms were detected in the viruses from 60% (6/10) of subjects in the DCV 60 mg cohort (NDA 206843, BMS Study Report 930069253, Pages 21-22, Table 1); 1/6 subjects relapsed and 5/6 subjects achieved SVR24. The one failure subject had the Y93H polymorphism at baseline.

- NS5A resistance-associated polymorphisms detected in 6 subjects (AI444021-1-21101, -2-21121, -3-21115, -4-21102, 4-21111, and -4-21128) included V28, Q30, H/Y54, H62, and H93 substitutions. When assessed in GT-1b Con1 replicons, only the Y93H substitution conferred 31-fold decrease in susceptibility to DCV whereas the other substitutions conferred no change in susceptibility to DCV when compared to a wild-type replicon (NDA 206843, BMS Study Report 930069253, Pages 23-25, Table 2).
- 1/6 subjects relapsed; AI444021-1-21101 had a H93 polymorphism at baseline.

Baseline polymorphisms were not detected in 4/10 subjects; 4/4 achieved SVR24.

VI (d). NS5A resistance-associated substitutions in virologic failures

In the 60-mg cohort, 1/10 subjects was treatment failure.

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- AI444021-1-21101 experienced relapse at FUWK4. At FUWK4, L31V, Y93H substitutions emerged and were still detected at FUWK12. In the GT-1b Con1 replicon transient HCV replication assay, L31V, Y93H conferred 33,667-fold reduced susceptibility to DCV when compared to a reference replicon (NDA 206843, BMS Study Report 930069253, Pages 23-25, Table 2).

Prior Non-responders

DCV 10 mg Cohort

VI (e). NS5A resistance-associated polymorphisms at baseline

Pre-existing NS5A resistance-associated polymorphisms were detected in the viruses from 56% (5/9) of subjects in the DCV 10 mg cohort of prior non-responders (NDA 206843, BMS Study Report 930069253, Pages 25-27, Table 3). Of the subjects whose viruses had NS5A resistance-associated polymorphisms, 3/5 subjects failed treatment and 2/5 achieved SVR24.

- 3/5 subjects whose viruses NS5A resistance-associated polymorphisms experienced virologic breakthrough (AI444021-1-21219, -2-21206 and -4-21204); H54 and H93 polymorphisms were detected at BL. When assessed in GT-1b Con1 replicons, only Y93H substitution conferred 31-fold decrease in susceptibility to DCV when compared to a wild-type replicon (NDA 206843, BMS Study Report 930069253, Pages 28-31, Table 4).
- 2/5 subjects whose viruses NS5A resistance-associated polymorphisms achieved SVR24 (AI444021-3-21211, and -4-21203); an H54 polymorphism was detected at BL.

BL polymorphisms were not detected in 4/9 subjects; 4/4 subjects (AI444021-1-21217, -2-21210, -2-21216, and -6-21215) experienced virologic failure (1 virologic breakthrough, 3 relapsers).

VI (f). NS5A resistance-associated substitutions in virologic failures

In the 10 mg cohort, 7/9 subjects were treatment failures; 4 experienced virologic breakthrough and 3 relapsed (NDA 206843, BMS Study Report 930069253, Pages 25-27, Table 3). Of the 7 treatment failures, 4/7 were prior null responders and 3/7 were prior partial responders whereas 2/2 subjects achieving SVR24 were prior partial responders to IFN-based treatment.

- 4/7 subjects experienced virologic breakthrough.
 - AI444021-1-21219 rebounded at Week 28. NS5A L31M, Y93H substitutions emerged at Week 4 whereas R30Q, L31M, Q54H, P58S, Y93H were detected at FUWK4. In the transient GT1b-Con1 replication assay, L31M, Y93H conferred 16,033-fold decrease in susceptibility to DCV whereas R30Q, L31M, Q54H, P58S, Y93H conferred 16,333-fold decrease in susceptibility to DCV when compared to a reference replicon (NDA 206843, BMS Study Report 930069253, Pages 28-31, Table 4).
 - AI444021-2-21206 rebounded at Week 6. NS5A L31M, Y93H substitutions emerged at Week 24 and was detected at FUWK24. In the transient GT-1b Con1 replication assay, L31M, Y93H in combination with a BL polymorphism H54 exhibited a 4,233-fold decrease in susceptibility to DCV in cell culture replicon system (NDA 206843, BMS Study Report 930069253, Pages 28-31, Table 4).
 - AI444021-4-21204 rebounded at Week 4. NS5A- Δ P32 was detected at Week 8 of treatment whereas L31M, Y93H substitutions emerged at Week 12 and were still detected at FUWK4. In the transient GT-

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1b Con1 replication assay, Δ P32 conferred >1,666,667-fold decrease in susceptibility to DCV in cell culture replicon (NDA 206843, BMS Study Report 930069253, Pages 28-31, Table 4). Susceptibility analysis of the Week 12 subject full-length NS5A population sequence, when introduced into the GT-1b Con1 replicon backbone, resulted in a significant loss in DCV activity; EC_{50} value = 19 nM, and EC_{90} value = 127 nM; (NDA 206843, BMS Study Report 930069253, Pages 31-32, Table 5).

- AI444021-6-21215 rebounded at Week 8. NS5A L31I/V, Y93H substitutions were detected at FUWK4. In the transient GT1b-Con1 replication assay, L31V, Y93H variants had DCV EC_{50} value of 101 nM (33,667-fold decrease in susceptibility to DCV in cell culture replicon system (NDA 206843, BMS Study Report 930069253, Pages 28-31, Table 4).
- 3/7 subjects relapsed:
 - AI444021-1-21217 relapsed at FUWK12. NS5A L31I, Y93H were detected at FUWK12. In the transient GT-1b Con1 replication assay, L31I, Y93H variants exhibited a 1,733-fold decrease in susceptibility to DCV when compared to a reference replicon (NDA 206843, BMS Study Report 930069253, Pages 28-31, Table 4). Susceptibility analysis of the FUWK12 subject full-length NS5A population sequence, when introduced into the GT-1b Con1 replicon backbone, resulted in a significant loss in DCV activity sufficient to explain virologic failure (EC_{90} value = 39 nM versus C_{trough} = 37 nM; NDA 206843, BMS Study Report 930069253, Pages 31-32, Table 5).
 - AI444021-2-21210 relapsed at FUWK4. NS5A L31V, Y93H substitutions were detected at FUWK4 through FUWK24. In the transient GT1b-Con1 replication assay, L31V, Y93H substitutions conferred 33,667-fold decrease in susceptibility to DCV in cell culture (NDA 206843, BMS Study Report 930069253, Pages 28-31, Table 4).
 - AI444021-2-21216 relapsed at FUWK4. NS5A L31V, Y93H substitutions were detected at FUWK4 through FUWK24. In the transient GT1b-Con1 replication assay, L31V, Y93H substitutions conferred 33,667-fold decrease in susceptibility to DCV in cell culture (NDA 206843, BMS Study Report 930069253, Pages 28-31, Table 4).

DCV 60 mg Cohort

VI (g). NS5A resistance-associated polymorphisms at baseline

Pre-existing NS5A resistance-associated polymorphisms were detected in the viruses from 100% (9/9) of subjects in the DCV 60 mg cohort (NDA 206843, BMS Study Report 930069253, Pages 25-27, Table 3). Of the subjects with NS5A resistance-associated polymorphisms, 6/9 subjects failed treatment and 3/9 achieved SVR24.

- 4/9 subjects whose viruses had NS5A resistance-associated polymorphisms experienced virologic breakthrough (AI444021-1-21214, -2-21212, -2-21213, and -4-21205); M28, Q30, M/V31, H54, R62, and/or T92 polymorphisms were detected. When assessed in a GT-1b Con1 replicon, baseline polymorphisms did not reduce susceptibility to DCV in cell culture (NDA 206843, BMS Study Report 930069253, Pages 28-31, Table 4).
- 2/9 subjects whose viruses had NS5A resistance-associated polymorphisms relapsed (AI444021-2-21208 and 5-21201); V31 and H54 polymorphisms were detected. Only the L31V substitution conferred 34-fold decrease in susceptibility to DCV cell culture replicon system (NDA 206843, BMS Study Report 930069253, Pages 28-31, Table 4).

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- 3/9 subjects whose viruses had NS5A resistance-associated polymorphisms achieved SVR24 (AI444021-3-21207, -3-21209, and -6-21218); M28, Q30, M31, H54 polymorphisms were detected at baseline.

VI (h). NS5A resistance-associated substitutions in virologic failures

In the 60 mg cohort, 6/9 subjects were treatment failures; 4 experienced virologic breakthrough and 2 relapsed (NDA 206843, BMS Study Report 930069253, Pages 25-27, Table 3); 4/4 virologic breakthroughs were prior null responders and 2/2 relapsers were prior partial responders to pegIFN α treatment.

- 4/6 subjects experienced virologic breakthrough
 - AI444021-1-21214 had L31V, Y93H substitutions emerge at Week 8 of treatment through to FUWK4. When assessed in a GT-1b Con1 replicon, L31V, Y93H substitutions conferred 33,667-fold decrease in susceptibility to DCV, respectively, when compared to a reference replicon (NDA 206843, BMS Study Report 930069253, Pages 28-31, Table 4). Susceptibility analysis of the Week 8 subject full-length NS5A population sequence resulted in a significant loss in DCV activity (EC₉₀ value = 224 nM (NDA 206843, BMS Study Report 930069253, Pages 31-32, Table 5).
 - AI444021-2-21212 had L31F, P32 Δ substitutions emerge at Week 24 through to FUWK4. NS5A L31F, P32 Δ conferred >1,666,667-fold decrease in susceptibility to DCV in cell culture replicon system (NDA 206843, BMS Study Report 930069253, Pages 28-31, Table 4).
 - AI444021-2-21213 had L31M, Y93H substitutions emerge at Week 1 through FUWK4. L31M, Y93H in combination with the BL polymorphism H54 conferred 4,233-fold decrease in susceptibility to DCV in cell culture replicon system (NDA 206843, BMS Study Report 930069253, Pages 28-31, Table 4). A similar loss in activity was also observed when assessing the subject full-length NS5A population sequence (NDA 206843, BMS Study Report 930069253, Pages 31-32, Table 5).
 - AI444021-4-21205 had L31V, Y93H substitutions emerge at Week 8 of treatment through to FUWK4. When assessed in a GT-1b Con1 replicon, L31V, Y93H in combination with the BL polymorphisms M28, Q30 conferred 557,333-fold decrease in susceptibility to DCV in cell culture replicon system (NDA 206843, BMS Study Report 930069253, Pages 28-31, Table 4). It should be noted that amplification of NS5A from samples collected at Week 12 and FUWK4 revealed a complicated mixture of NS5A resistance variants.
 - Clonal analysis of the Week 12 sample revealed L28M, R30Q, A92K predominated (6/19 clones) although L28M, R30H, L31V, A92K (4/19 clones) and R30Q or R30H with L28M, L31V, Y93H were also prevalent (5/19 clones [NDA 206843, BMS Study Report 930069253, Page 33, Table 6).
- 2/6 subjects relapsed
 - AI444021-2-21208 relapsed at FUWK4. NS5A, L31V-Y93H were detected at FUWK4 through to FUWK24. This linked variant was also detected after 1 week of DCV treatment.
 - Clonal analysis of the BL sample revealed minor NS5A polymorphisms at P29 (3/107 clones with P29S) and Y93 (1/107 clones with Y93H), each linked with V31; V31 polymorphism was detected in 100% (107/107) of clones (NDA 206843, BMS Study Report 930069253, Page 32, Table 6).
 - AI444021-5-21201 relapsed at FUWK4. NS5A, R30Q, Q54H, A92K were detected at FUWK4 through to FUWK24. This variant in combination with the BL polymorphism H54 exhibited a 8,667-fold decrease in susceptibility to DCV (NDA 206843, BMS Study Report 930069253, Pages 28-31, Table 4). Susceptibility analysis of the FUWK4 subject full-length NS5A population sequence, when introduced into the GT-1b

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Con1 replicon backbone, resulted in EC₉₀ value of 739 nM, similar to the plasma C_{trough} value of 804 nM (NDA 206843, BMS Study Report 930069253, Page 32, Table 5).

AI444022 Study

Title: Genotypic and Phenotypic Analysis of Viral Variants in HCV Genotype 1-Infected Japanese Subjects Treated with Daclatasvir (BMS-790052) and PegInterferon Alfa-2a (Pegasys[®]) plus Ribavirin (Rebetol[®])

Subjects and Study Design

Japanese subjects eligible for the study included those with chronic HCV GT-1 infection who were NV (defined as those who had never been exposed to any HCV therapy with interferon containing regimens, including PegIFN α -2a/RBV, or those containing direct-acting agents against HCV, or who were non-responders (NR) to previous therapy (defined as subjects who failed to achieve ≥ 2 log₁₀ reduction of HCV RNA at Week 12 [null responder] or had achieved a ≥ 2 log₁₀ reduction but never attained undetectable HCV RNA levels after at least 12 weeks [partial responder] of the current standard of care, PegIFN α -2a/RBV or PegIFN α -2b/RBV). Subjects were non-cirrhotic adults (20-70 years old) with HCV RNA levels $\geq 10^5$ IU/mL. In this double-blind study, 42 subjects (2 GT-1a, 40 GT-1b) were randomized equally to receive once daily DCV 10 mg, or 60 mg or placebo (only NV received placebo) in combination with PegIFN α -2a, and RBV. Subjects receiving DCV + PegIFN α -2a/RBV who achieved a protocol-defined response (PDR) were treated for 24 weeks. PDR was defined as HCV RNA <LLOQ (15 IU/mL) at Week 4 and undetectable at Week 12. Subjects not achieving PDR received DCV + PegIFN α -2a/RBV for 48 weeks. Subjects treated with placebo (NV only) received PegIFN α /RBV for 48 weeks. The primary efficacy endpoint was the proportion of subjects with extended rapid viral response (eRVR), defined as undetectable HCV RNA at both Week 4 and Week 12. Secondary efficacy assessments included the proportion of subjects with rapid virologic response (RVR), defined as undetectable HCV RNA at Week 4; the proportion of subjects with complete early virologic response (cEVR), defined as undetectable HCV RNA at Week 12; the proportion of subjects with SVR12 and SVR24. Serum HCV RNA levels were determined at a central laboratory ((b)(4)) using Roche COBAS[®] TaqMan[®] HCV Auto assay (Roche (b)(4)), with LLOQ of 15 IU/mL.

VII. Resistance analysis of variants from subjects infected with GT-1a and GT-1b

Treatment-Naive Subjects

DCV 10 mg Cohort

VII (a). NS5A resistance-associated polymorphisms at baseline

Pre-existing NS5A resistance-associated polymorphisms were detected in the viruses from 67% (6/9) of subjects (1 GT-1a, 5 GT-1b) in the DCV 10 mg cohort (NDA 206843, BMS Study Report 930069119, Pages 19-20, Table 1). Only 1/6 subjects whose viruses had baseline polymorphisms experienced virologic breakthrough while 5/6 achieved SVR24. Of the 5 subjects who achieved SVR24, 3 subjects had viruses with the H93 polymorphism at baseline.

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- GT-1a NS5A resistance-associated polymorphisms detected in the GT-1a subject AI444022-2-22134 included L30 and H93 polymorphisms. In the GT-1a H77c replicon, Q30L and Y93H substitutions conferred 583-fold decrease in susceptibility to DCV (NDA 206843, BMS Study Report 930069119, Pages 21-22, Table 2). Despite these baseline polymorphisms, AI444022-2-134 achieved SVR24.
- GT-1b NS5A resistance-associated polymorphisms detected in the viruses from 5 GT-1b subjects (AI444022-2-22105, -3-22108, -3-22121, -4-22131, and -6-22119) included Q30, C/H/N54, S58, R62, and H93 substitutions. When assessed in GT-1b Con1 replicons, Q54H, Y93H and R30Q, Y93H substitutions conferred 6.7-fold and 184-fold decrease in susceptibility to DCV potency whereas Q54H/N, P58S substitutions did not confer any change in susceptibility to DCV when compared to a reference replicon (NDA 206843, BMS Study Report 930069119, Pages 21-22, Table 2).
 - 1/5 subjects with baseline polymorphisms experienced virologic breakthrough; AI444022-4-22131 had H/N54 and S58 substitutions. In the GT-1b Con1 replicon, Q54H/N and P58S substitutions were equally susceptible to DCV as the parent replicon (EC_{50} value = 0.002 to 0.003 nM (NDA 206843, BMS Study Report 930069119, Pages 21-22, Table 2).
 - 4/5 subjects achieved SVR24; 2/5 (AI444022-3-22108, -6-22119) had the H93 polymorphism.

BL polymorphisms were not detected in 3/9 subjects (1 GT-1a, 2 GT-1b); 3/3 achieved SVR24.

VII (b). NS5A resistance-associated substitutions in virologic failures

In the 10 mg cohort, 1/9 subjects (1 GT-1b) was a treatment failure.

- AI444022-4-22131 experienced viral breakthrough at Week 24 of treatment. At Week 28 L31V and Y93H substitutions emerged. In the GT-1b Con1 replicon transient HCV replication assay, L31V, Y93H substitutions conferred 33,667-fold decrease in susceptibility to DCV (NDA 206843, BMS Study Report 930069119, Pages 21-22, Table 2). At FUWK4, a mixture of L31I/M substitutions were detected along with Q54H, P58S and Y93H substitutions. The EC_{50} values of variants at FUWK 4 ranged from 42 to 53 nM resulting in 13,836 to 17,667-fold decrease in susceptibility to DCV compared to 1 b Con1 replicon (NDA 206843, BMS Study Report 930069119, Pages 21-22, Table 2).

DCV 60 mg Cohort

VII (c). NS5A resistance-associated polymorphisms at baseline

Pre-existing NS5A resistance-associated polymorphisms were detected in the viruses from 62.5% (5/8) subjects (all GT-1b) in the DCV 60-mg cohort (NDA 206843, BMS Study Report 930069119, Pages 19-20, Table 1). All 5 subjects whose viruses had baseline polymorphism achieved SVR24. One of these 5 subjects who achieved SVR24 had virus with the Y93H polymorphism.

- NS5A resistance-associated polymorphisms detected in the viruses from 5 subjects (AI444022-1-22109, -2-22133, -3-22101, -6-22117 and -6-22124) included Q30, H/Y54, L58, G62, E92, and H93. When assessed in GT-1b Con1 replicons, Q54H, Y93H substitutions conferred 6.7-fold decrease in susceptibility to DCV whereas the other substitutions (Q54H/Y, P58L, R30Q, A92E individually) conferred no change in

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susceptibility to DCV when compared to a reference replicon (NDA 206843, BMS Study Report 930069119, Pages 21-22, Table 2).

BL polymorphisms were not detected in the viruses from 3/8 subjects (3 GT-1b); 3/3 achieved SVR24 (NDA 206843, BMS Study Report 930069119, Pages 19-20, Table 1).

Prior Non-Responders

DCV 10 mg Cohort

VII (d). NS5A resistance-associated polymorphisms at baseline

Pre-existing NS5A resistance-associated polymorphisms were detected in the viruses from 87.5% (7/8) of subjects (all GT-1b) in the DCV 10 mg cohort (NDA 206843, BMS Study Report 930069119, Pages 23-24, Table 3). Of the subjects with baseline NS5A resistance-associated polymorphisms, 3/7 subjects failed treatment and 4/7 subjects achieved SVR24.

- 1/7 subjects whose viruses had NS5A resistance-associated polymorphisms experienced virologic breakthrough (AI444022-1-22209); M28, Q30, H54, T92 were detected at BL. When assessed in a GT-1b Con1 replicon, these substitutions conferred no change in susceptibility to DCV compared to a reference replicon (NDA 206843, BMS Study Report 930069119, Page 25, Table 4).
 - AI444022-1-22209 was a prior null responder to IFN-based treatment.
- 2/7 subjects whose viruses had NS5A resistance-associated polymorphisms relapsed (AI444022-4-22202, -6-22215); H54, E62 were detected at BL in these two subjects. These polymorphisms conferred no changes in susceptibility to DCV in cell culture replicon (NDA 206843, BMS Study Report 930069119, Pages 25-26, Table 4).
 - These 2 subjects were prior null responders to IFN-based treatment.
- 4/7 subjects whose viruses had NS5A resistance-associated polymorphisms achieved SVR24 (AI444022-1-22210, -2-22218, -6-22205, -6-22213); M28, Q30, H54, S58, H62 were detected in these subjects at baseline. These polymorphisms conferred no changes in susceptibility to DCV in cell culture replicon.
 - 3/4 subjects were prior partial responders and 1/4 subjects was a prior null responder.

BL polymorphisms were not detected in 1/8 subjects (AI444022-1-22221).

VII (e). NS5A resistance-associated substitutions in virologic failures

In the 10 mg cohort, 4/8 subjects were treatment failures; 1 experienced virologic breakthrough and 3 relapsed (NDA 206843, BMS Study Report 930069119, Pages 23-24, Table 3). All 4 treatment failures were prior null responders compared with only 1/4 responders being prior null responders.

- 1/4 subjects experienced virologic breakthrough. AI444022-1-22209 rebounded at Week 12. In addition to baseline polymorphisms, L31V substitution emerged at Week 24 and FUWK4. In the transient GT1b-Con1 replication assay, WK 24 isolates with L31V substitutions in combination with the BL polymorphisms had DCV EC₅₀ value of 41 nM (13,800-fold decrease in susceptibility to DCV when compared to a reference replicon (NDA 206843, BMS Study Report 930069119, Pages 25-27, Table 4).

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- 3/4 subjects relapsed:
 - AI444022-1-22221 had L28M, Y93H substitutions at FUWK12 and L31I/V, Y93H substitutions at FUWK24; these variants exhibited 933- and 33,667-fold reduced susceptibility to DCV in cell culture replicon, respectively.
 - AI444022-4-22202 had L31M, Q54H, Q62E, Y93H substitutions at FUWK4/12 and L31I/M, Q54H, Q62E, Y93H substitutions at FUWK24; these variants exhibited 9,333-fold decrease in susceptibility to DCV in cell culture replicon at FUWK4 and FUWK24 (NDA 206843, BMS Study Report 930069119, Pages 25-27, Table 4).
 - AI444022-6-22215 had L31V, Q54H, Y93H substitutions at FUWK4 through to FUWK24; these variants exhibited 8,667-fold decrease in susceptibility to DCV in cell culture replicon (NDA 206843, BMS Study Report 930069119, Pages 25-27, Table 4).

DCV 60 mg Cohort

VII (f). NS5A resistance-associated polymorphisms at baseline

Pre-existing NS5A resistance-associated polymorphisms were detected in the viruses from 78% (7/9) of subjects (all GT-1b) in the DCV 60 mg Cohort (NDA 206843, BMS Study Report 930069119, Pages 23-24, Table 3). Of the subjects with NS5A resistance-associated polymorphisms, 2/7 subjects failed treatment and 5/7 achieved SVR24.

- 1/7 subjects whose viruses had NS5A resistance-associated polymorphisms experienced virologic breakthrough (AI444022-5-22217); H/Y54 polymorphisms were detected at baseline. When assessed in a GT-1b Con1 replicon, these substitutions conferred no change in susceptibility to DCV in cell culture replicon when compared to a reference replicon (NDA 206843, BMS Study Report 930069119, Pages 25-27, Table 4).
- 1/7 subjects whose viruses had NS5A resistance-associated polymorphisms relapsed (AI444022-4-22206); H54, E62 polymorphisms were detected at baseline. These polymorphisms conferred no changes in susceptibility to DCV in cell culture replicon system (NDA 206843, BMS Study Report 930069119, Pages 25-27, Table 4).
- 5/7 subjects whose viruses had NS5A resistance-associated polymorphisms achieved SVR24 (AI444022-1-22203, -2-22211, -3-22201, -3-22214, and -4-22208); H/Y54, S58, E62, H93 polymorphisms were detected at baseline. Other than the one subject with H93, detected polymorphisms conferred no changes in susceptibility to DCV in cell culture replicon. Q54Y, Y93H substitutions conferred 59-fold decrease in susceptibility to DCV in cell culture replicon system (NDA 206843, BMS Study Report 930069119, Pages 25-27, Table 4).

BL polymorphisms were not detected in the viruses from 2/9 subjects; 2/2 subjects (AI444022-1-22220, AI444022-5-22216) achieved SVR24.

VII (g). NS5A resistance-associated substitutions in virologic failures

In the 60 mg cohort, 2/9 subjects were treatment failures; 1 experienced virologic breakthrough and 1 relapsed (NDA 206843, BMS Study Report 930069119, Pages 23-24, Table 3). Both treatment failures were prior null responders compared with 6/7 responders being prior null responders.

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- 1/2 subjects experienced virologic breakthrough (AI444022-5-22217); L31V, Q54H, Y93H substitutions were detected at FUWK4. When assessed in a GT-1b Con1 replicon, these substitutions conferred 8,667-fold decrease in susceptibility to DCV compared to a reference replicon (NDA 206843, BMS Study Report 930069119, Pages 25-27, Table 4).
- 1/2 subjects relapsed (AI444022-4-22206); L31M, Y93H substitutions in addition to BL polymorphisms H54, E62 were detected at FUWK12 and FUWK 24). These substitutions in combination conferred 9,333-fold decrease in susceptibility to DCV in cell culture replicon. When the DCV susceptibility of the full-length NS5A variant from the subject was assessed in a hybrid replicon assay, DCV EC₅₀ and EC₉₀ values of 69 nM and 383 nM were obtained. The sponsor stated that plasma C_{trough} concentration of DCV (341 nM) was approximately half of the protein-adjusted EC₅₀ value of 690 nM for DCV (NDA 206843, BMS Study Report 930069119, Page 27, Table 5). Thus, C_{trough} concentration of DCV (341 nM) for this subject was not enough to suppress variants detected at FUWK12 and FUWK 24.

Study AI444031

Title: Genotypic and Phenotypic Analysis of Viral Variants in Treatment Naive Subjects Infected with HCV Genotype 2 and Genotype 3 and Treated with Daclatasvir (BMS-790052) plus PegInterferon Alfa-2a (Pegasys®) and Ribavirin (Copegus®) in the Phase 2 Study AI444031

Comment

This study is not reviewed here because the sponsor is not seeking an indication against HCV genotype 2 and 3. However, this study (BMS Report 930073558) was previously reviewed (see Virology Review of IND 79599 SDN 655 dated 02/06/14).

Study AI444042

Title: A Phase 3 Evaluation of Daclatasvir (DCV) in Combination with Peg-Interferon Alfa-2a and Ribavirin in Treatment-Naive Subjects with Chronic Hepatitis C Genotype 4.

This clinical study report (BMS Study Report 930078446) presents the safety and efficacy results for all subjects through follow-up Week 12.

Comment

This study is still on-going. Limited resistance data were available for virologic failure subjects (see Virology Review of IND 79599 SDN748 dated 07/14/14).. It is expected that the sponsor will submit additional resistance data at the completion of the study.

METHODOLOGY

Methodology for transient protein expression and immunoblotting assays are described by [Fridell et al., 2011](#)).

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Cell lines and viral constructs, cell culture assays, HCV replicon luciferase and FRET assays, transient replicon replication assays, quantification of HCV RNA, assays for cytotoxicity and cell culture inhibitor combination effect are described in BMS study report 930023288 and previously reviewed (Microbiology review of IND 79599 SDN 000 dated 11/08/07).

Construction of HCV hybrid replicons, chimeric replicons, HCV replicon luciferase assay, transient replicon replication assays, and infectious HCV assays were performed as described in Addendum 1 to Scientific Report 930023288 (NDA 206843),

Methods for the selection of BMS-790052 resistant HCV genotype 1a and 1b replicon cells are described in BMS Scientific Report 930023286 and previously reviewed (Microbiology Review of IND 79599 SDN 000 dated 11/08/07).

Methodology for the selection of DCV resistant GT 2a, 3a, 4a, 5a and 6a hybrid replicons are described in addendum 1 ,2 and 3 to BMS Scientific Report 930023288, (NDA 206843) and and previously reviewed (Virology Review of IND 79599 SDN 557 dated 04/23/13, SDN 647 dated 12/19/13 and SDN 655 dated 02/06/14).

Please refer to Virology Review of IND 79559 SDN 605 dated 11/20/13 for description of methodologies for genotypic and phenotypic analyses of plasma samples from subjects from studies AI444010, AI444011, AI444014, AI444021, AI444022.

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Label

We propose to delete sentences marked red font strikethrough and insert sentences shown in blue font.

CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

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DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
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Daclatasvir is a direct-acting antiviral agent (DAA) against the hepatitis C virus [see *Microbiology (12.4)*].

12.4 Microbiology

Mechanism of Action

Daclatasvir is an inhibitor of NS5A, a non-structural protein encoded by HCV. Daclatasvir binds to the N-terminus of NS5A and thereby (b) (4) inhibits both viral RNA replication and virion assembly. (b) (4) -Characterization of daclatasvir resistant viruses, biochemical studies and computer modeling data indicate that daclatasvir interacts with the N-terminus within Domain 1 of the protein, which may cause structural distortions that interfere with NS5A functions.

Antiviral Activity

(b) (4)

(b) (4)

In cell based HCV replicon assays, daclatasvir inhibited HCV genotypes 1a (H77 strain) and 1b (Con1 strain) with effective concentration (50% reduction EC_{50} values) of 20 pM and 4 pM, respectively. The median EC_{50} values of daclatasvir against HCV replicons containing NS5A gene from a panel of genotype 1a and 1b isolates from treatment naive subjects were 9 pM (n=11; range 3-19 pM) and 2 pM (n=5; range 1 to 3 pM), respectively. The median EC_{50} value of daclatasvir for hybrid replicons encoding genotype 4A NS5A was 7 pM (n=3, range 7-13 pM). For genotypes 2a, 3a, and 5a clinical isolates in a hybrid JFH -1 replicon, the median EC_{50} values of daclatasvir were 18,000 pM (n=3; range 8,800 to 19,000 pM), 530 pM (n=3, range 140 to 1,250 pM) and 3 pM (n=3; range 3 to 7.2 pM, respectively.). Daclatasvir was not antagonistic with interferon alfa, HCV NS3/4A protease inhibitors, HCV NS5B nucleoside analog inhibitors, and HCV NS5B non-nucleoside inhibitors in cell culture combination antiviral activity studies using the cell-based HCV replicon system.

Resistance

In Cell Culture

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Substitutions conferring daclatasvir resistance in HCV genotypes 1 a and 1b were selected in the cell based replicon system and (b) (4) occurred in the N-terminal 100 amino acid region of NS5A. L31F/V and Y93H/N were frequently observed (b) (4) substitutions in genotype 1b, while M28T, L31V/M, Q30E/H/R, and Y93C/H/N were frequently observed (b) (4) -substitutions in genotype 1a- (b) (4)

(b) (4) Substitutions L31F/V alone reduced 4 -to 24-fold susceptibility to daclatasvir, Y93H 19-fold and L31V/Y93H combination >4000-fold in cell culture replicon system. Amino acid substitutions M28T, Q30H, Q30R, L31V, Y93C and M28T/Y93C conferred 43 to 1130-fold reduced susceptibility to DCV in HCV 1a replicon, Amino acid substitutions R30G/H/S or L30H/R/V, M31I, P32I, Y93H/R identified in genotype 4-a replicons conferred 130-to1700-fold, or 21 to >9000-fold reduced susceptibility to daclatasvir depending on the polymorphism at amino acid position 28 (L or M) in the replicon system used.

Resistance patterns observed in the clinical isolates are very similar to patterns generated in cell (b) (4) based replicon system except that linked substitutions are more complex in clinical specimens.

The majority of wild-type HCV genotype 2a contain a pre-existing resistance substitution (L31M) with EC₅₀ values of 9 to 19 nM. Amino acid substitutions F28S, L38M, C92R, and Y93H were selected in genotype 2a replicons and these substitutions conferred 440- to 56,300-fold reduced susceptibility to daclatasvir.

(b) (4)

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/s/

LALJI MISHRA
08/26/2014

JULIAN J O REAR
08/26/2014

**DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
CLINICAL VIROLOGY REVIEW**

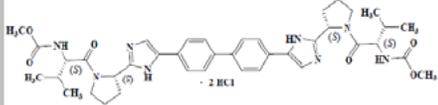
NDA: 206844 SDN: 000 (Original NDA) REVIEW COMPLETED: 08/07/2014

Clinical Virology Reviewer: Patrick R. Harrington, Ph.D.

NDA#: 206844 **SDN:** 000 (Original NDA)
Reviewer's Name(s): Patrick R. Harrington, Ph.D.

Sponsor's Name and Address: Bristol-Myers Squibb Company
5 Research Parkway
Wallingford, CT 06492
Charles D. Wolleben, Ph.D.
Group Director, Global Regulatory Sciences

Initial Complete Submission Dates:
Correspondence Date: 3/31/2014
CDER Receipt Date: 3/31/2014
Assigned Date: 3/31/2014
Review Complete Date: 08/07/2014
PDUFA Date: 11/30/2014 (Internal 11/21/2014)

NDA #	<u>206844</u>	<u>206843</u>
Proprietary Names	(b) (4)	DAKLINZA™
Drug Name	(b) (4)	daclatasvir (DCV, BMS-790052)
Drug Class	(b) (4)	NS5A inhibitor
Associated IND #s	(b) (4)	<u>79599</u> , <u>101977</u>
Chemical Name	(b) (4)	Methyl((1S)-1-(((2S)-2-(5-(4'-(2-((2S)-1-((2S)-2-((methoxycarbonyl)amino)-3-methylbutanoyl)-2-pyrrolidinyl)-1H-imidazol-5-yl)-4-biphenyl)-1H-imidazol-2-yl)-1-pyrrolidinyl)carbonyl)-2-methylpropyl)carbamate dihydrochloride
Structure	(b) (4)	 <p align="center">daclatasvir</p>
Molecular Formula	(b) (4)	C ₄₀ H ₅₀ N ₈ O ₆ • 2HCl
Molecular Weight	(b) (4)	811.80 (dihydrochloride), 738.88 (free base)

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/s/

PATRICK R HARRINGTON
08/20/2014

JULIAN J O REAR
08/20/2014

MEMORANDUM



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

DATE: 29 May 2014

TO: NDA 206843

FROM: Bryan S. Riley, Ph.D.
Team Leader (Acting)
OPS/New Drug Microbiology Staff

THROUGH: John W. Metcalfe, Ph.D.
Senior Review Microbiologist
OPS/New Drug Microbiology Staff

cc: Sohail Mosaddegh, PharmD
Regulatory Project Manager
OND/DAP

SUBJECT: Product Quality Microbiology assessment of Microbial Limits for
DAKLINZA (Daclatasvir 30mg and 60 mg Tablet) [Submission Date:
31 March 2014]

The Microbial Limits specification for DAKLINZA is acceptable from a Product Quality Microbiology perspective. Therefore, this submission is recommended for approval from the standpoint of product quality microbiology.

DAKLINZA is a Tablet for oral administration.

The drug product is tested for Microbial Limits at release using a method consistent with USP Chapter <61> (Microbiological Examination of Non-sterile Products: Microbial Enumeration Tests) and <62> (Microbiological Examination of Non-sterile Products: Tests for Specified Microorganisms). The Microbial Limits acceptance criteria are consistent with USP Chapter <1111> (Microbiological Examination of Non-sterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use).

MEMORANDUM

Table 1 – Microbial Limits Specifications

Test	Acceptance Criteria
Total Aerobic Microbial Count (USP <61>)	NMT (b) (4)
Total Yeast and Mold Count (USP <61>)	NMT (b) (4)
<i>E. coli</i> (USP <62>)	Absent in (b) (4)

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

The drug product will not be tested for Microbial Limits as part of the post-approval stability protocol. Microbial Limits (b) (4) of the drug product were monitored on the initial stability batches. Microbial Limits acceptance criteria were met at all time-point (b) (4) of the drug product remained low throughout stability.

ADEQUATE

Reviewer Comments – The microbiological quality of the drug product is controlled via a suitable release testing protocol. Microbial quality of the drug product on stability is assured (b) (4) of the drug product (which prevents microbial proliferation).

END

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/s/

BRYAN S RILEY
05/29/2014

JOHN W METCALFE
05/29/2014
I concur.