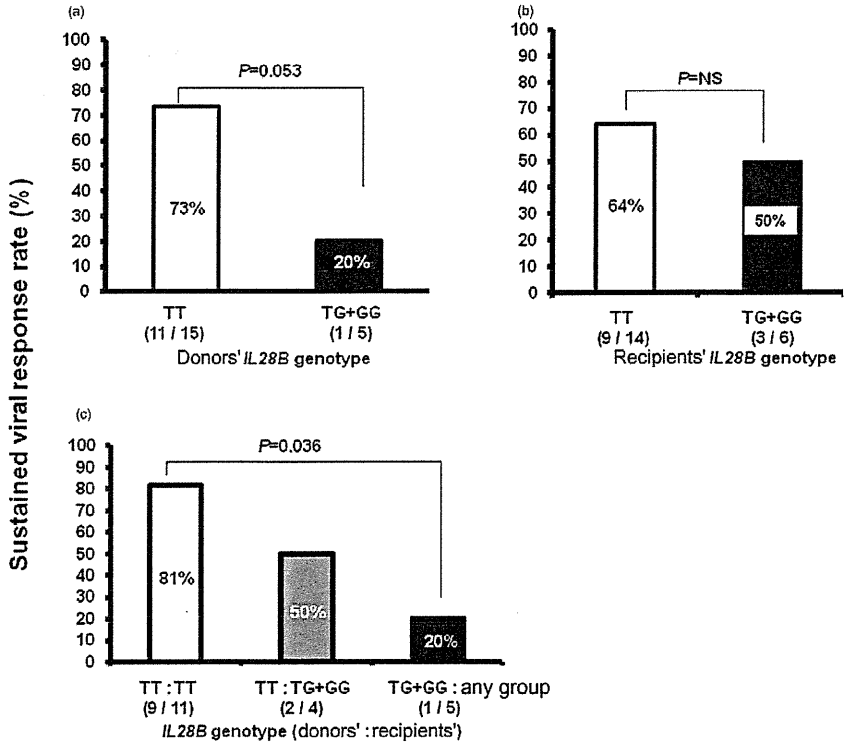

Figure 2 Sustained viral response rates according to (a) donors' interleukin-28B (*IL28B*), (b) recipients' *IL28B*, and (c) donors' and recipients' *IL28B* in patients infected with hepatitis C virus genotype 1. TT : TT group (donors' *IL28B* TT: recipients' *IL28B* TT), TT : TG + GG group (donors' *IL28B* TT: recipients' *IL28B* TG + GG), TG + GG : any group (donors' *IL28B* TG + GG: recipients' *IL28B* either TT or TG + GG). NS, not significant.

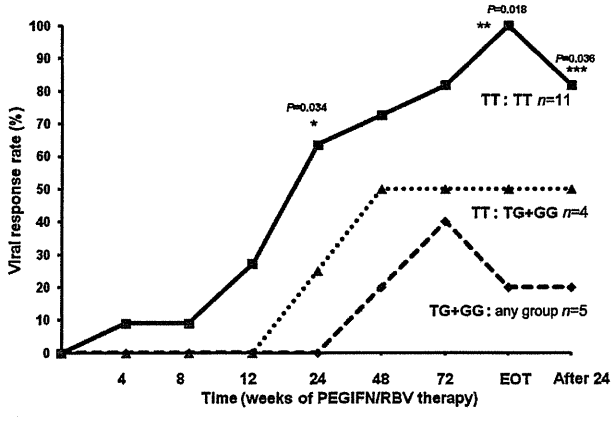

Figure 3 Viral response rates according to donors' and recipients' interleukin-28B (*IL28B*) genotyping. TT : TT group (donors' *IL28B* TT: recipients' *IL28B* TT), TT : TG + GG group (donors' *IL28B* TT: recipients' *IL28B* TG + GG), TG + GG : any group (donors' *IL28B* TG + GG: recipients' *IL28B* either TT or TG + GG). *Viral rate (VR) of the TT : TT group was 63.6% ($n = 7/11$), which was higher than the VR rate of the TG + GG : any group (0%, $n = 0/5$) at 24 weeks. **VR rate of the TT : TT group was 100% ($n = 11/11$), which was higher than the VR rate of the TG + GG : any group (20%, $n = 1/5$) at the end of treatment (EOT). ***Sustained VR (SVR) rate of the TT : TT group was 100% ($n = 11/11$), which was higher than the SVR rate of the TG + GG : any group (20%, $n = 1/5$) at 24 weeks at the EOT. PEGIFN, pegylated interferon; RBV, ribavirin.

Discussion

The SVR rate has improved since the introduction of PEGIFN/RBV for patients who undergo LT for HCV-related end-stage liver disease. The current estimated SVR rate for LT patients with a history of HCV-1 infection is 30–50%.[21–24,26,27] These results are much better than those reported in the 1990s and early 2000s; however, more than half of recipients still suffer from recurrent chronic hepatitis C.

Although many studies have determined the predictive factors of the viral response for PEGIFN/RBV among patients with chronic hepatitis C, recent molecular biological analyses and genome-wide analyses of the human genome have identified genetic variations of *IL28B* and amino-acid substitution of HCV core 70 as the most significant predictive factors for IFN response.[3–5,32,33] *IL28B* encodes a cytokine distantly related to type I IFN and the IL-10 family. It has been reported that the expression level of the *IL28* gene in peripheral blood mononuclear cells is significantly lower in individuals with minor alleles than in individuals with major alleles.[5]

Several studies have determined the predictive factors for the viral response to PEGIFN/RBV in patients with recurrent post-LT hepatitis C viral infection, and recent molecular and genome wide analyses of the human genome have demonstrated that genetic variation of *IL28B* is the most significant predictive factor of the response to IFN.[8,34–37] In the present study, we examined whether the same factors can also predict the response to PEGIFN/RBV in LT recipients. Several groups have reported that recipients' and donors' *IL28B* influenced the SVR to PEGIFN/RBV in patients with recurrent hepatitis C after LT.[8,36,37] Furthermore, others

Table 2 Univariate analysis of factors associated with sustained viralresponse (SVR) during interferon therapy in genotype 1 patients with recurrent hepatitis C

	SVR ($n = 12$)	Non-SVR ($n = 8$)	*P*-value
Age (years)†	60 (44–69)	57 (47–65)	0.48
Sex (male/female)	10/2	5/3	0.3
Body mass index (kg/m²)†	24.1 (21.4–26.5)	24.2 (18.9–42.2)	0.4
Viral load at therapy (LogIU/mL)†	6.3 (5.8–6.6)	6.6 (5.9–7.2)	0.52
Time from transplantation to therapy (months)†	4 (1–41)	3 (1–6)	1.7
No. mutations in the ISDR (0–1/2–5)	7/5	5/3	1.0
HCV core70 region (mutant/wild)	7/5	5/3	1.0
HCV core 91 region (mutant/wild)	7/5	3/5	0.6
Donors' *IL28B* genotype TT/TG + GG	11/1	4/4	0.053
Recipients' *IL28B* genotype TT/TG + GG	9/3	5/3	0.6
Donors' and recipients' *IL28B* genotype TT : TT/others	9/3	2/6	0.037
Immunosuppression (tacrolimus/cyclosporine)	9/3	7/1	1.0
Adherence to PEGIFN ≥ 70/< 70 (%)†	8/4	3/5	0.3
Adherence to RBV ≥ 50/< 50 (%)†	7/5	1/7	0.076

†Values are median (range). HCV, hepatitis C virus; *IL28B*, interleukin-28B; ISDR, interferon sensitivity-determining region; PEGIFN, pegylated interferon; RBV, ribavirin.

reported that donors' *IL28B* influenced the SVR in patients treated with PEGIFN/RBV for recurrent hepatitis C after LT,[34] and that recipients' *IL28B* influenced the SVR to PEGIFN/RBV in patients with recurrent post-LT hepatitis C.[35,36]

The results of the present study indicate that both donors' and recipients' *IL28B* influence the SVR to PEGIFN/RBV in patients with recurrent post-LT hepatitis C. Both recipients' and donors' *IL28B* influenced the SVR to PEGIFN/RBV in recurrent hepatitis C after LT; however it is not clear whether the recipients' or donors' *IL28B* influenced the SVR to PEGIFN/RBV.

However, the donors' *IL28B* might have influenced the SVR to PEGIFN/RBV in patients with recurrent post-LT hepatitis C more than the recipients' *IL28B*. This conclusion is based on the following results: although the SVR rate of the TT group (64.2%) was similar to that of the TG + GG group (50%), according to the recipients' *IL28B*, the SVR rate of the TT group (73.3%) was higher than that of the TG + GG group (20%), according to the donors' *IL28B*. Furthermore, the VR rates of TT : TT, TT : TG + GG, TG + GG : any group at 12 weeks were 28%, 0%, and 0%; those at 48 weeks were 70%, 50%, and 20%; and those at the end of treatment were 100%, 50%, and 20%, respectively. That is, the time to VR of the TG + GG : any group was the latest among the three groups. Lange *et al.* reported that donors' *IL28B* influenced the SVR in patients treated with PEGIFN/RBV for recurrent hepatitis C after LT.[34] In this regard, Hiraga *et al.*[38] reported that IFN-stimulated gene expression levels in mice livers measured at 2 weeks after IFN treatment were significantly higher in mice transplanted with donor human hepatocytes (*IL28B*; TT) than from donor (*IL28B*; TG + GG) mice. Furthermore, previous studies reported that the expression level of IFN- λ-3, coded for the *IL28B* gene, was higher in hepatocytes than hematopoietic cells.[39]

However, we demonstrated the feasibility of treatment of LT recipients with PEGIFN/RBV until HCV–RNA reached undetectable levels, followed by the continuation of treatment for at least 48 weeks (i.e. long-term IFN therapy). In fact, the SVR rate (50%) of the recipients' *IL28B* TG + GG group was higher than that reported by others[8] (SVR rate: 11%). Furthermore, the SVR rate (81%) of the combination of donors' and recipients' *IL28B* (TT : TT) group was higher than that reported by Fukuhara *et al.*[8] (SVR rate: 56%). However, the SVR rate of the donors' *IL28B* TG + GG group (SVR rate: 20%) was similar to that reported by Fukuhara *et al.*[8] (SVR rate: 9%). We believe that the treatment of LT recipients with PEGIFN/RBV until HCV–RNA reaches undetectable levels, followed by the continuation of treatment for at least 48 weeks, is not useful for donors with *IL28B* TG + GG.

In Japan, LDLT is more common than orthotopic LT. In finding a suitable donor, it is better to select a donor with TT of the *IL28B* gene than a TG or GG donor. In conclusion, our results demonstrated the suitability of donors with the TT *IL28B* genotype, and that long-term PEGIFN/RBV therapy seems useful for recipients of LDLT who develop recurrent hepatitis C after transplantation.

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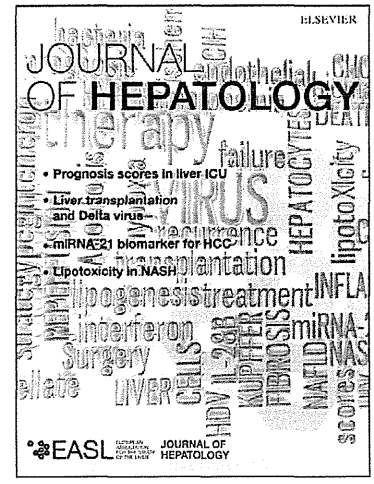

Characterization of virologic escape in hepatitis C virus genotype 1b patients treated with the direct-acting antivirals daclatasvir and asunaprevir

Yoshiyasu Karino, Joji Toyota, Kenji Ikeda, Fumitaka Suzuki, Kazuaki Chayama, Yoshiiku Kawakami, Hiroki Ishikawa, Hideaki Watanabe, Dennis Hernandez, Fei Yu, Fiona McPhee, Hiromitsu Kumada

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Title: Characterization of virologic escape in hepatitis C virus genotype 1b patients treated with the direct-acting antivirals daclatasvir and asunaprevir

Authors: Yoshiyasu Karino[1], Joji Toyota[1], Kenji Ikeda[2], Fumitaka Suzuki[2], Kazuaki Chayama[3], Yoshiiku Kawakami[3], Hiroki Ishikawa[4], Hideaki Watanabe[4], Dennis Hernandez[5], Fei Yu[5], Fiona McPhee[5,*], Hiromitsu Kumada[2]

Affiliations: [1]Sapporo Kosei General Hospital, Sapporo, Japan; [2]Toranomon Hospital, Tokyo, Japan; [3]Hiroshima University, Hiroshima, Japan; [4]Bristol-Myers KK, Tokyo, Japan; [5]Bristol-Myers Squibb Research and Development, Wallingford, CT, USA

*Corresponding author:

Fiona McPhee

Bristol-Myers Squibb Research and Development

Wallingford, CT 06492, USA

Tel: 203-677-7573

Fax: 203-677-0688

E-mail: fiona.mcphee@bms.com

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Abbreviations: DAA, direct-acting antiviral; HCV, hepatitis C virus; SVR, sustained virologic response; GT, genotype; alfa/RBV, peginterferon alfa and ribavirin; DCV, daclatasvir; ASV, asunaprevir; LLOQ, lower limit of quantitation; PCR, polymerase chain reaction; FU, follow-up; RAV, resistance-associated variant; BL, baseline; VBT, viral breakthrough; SD, standard deviation.

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Abstract

Background and Aims: Daclatasvir and asunaprevir are NS5A- and NS3 protease-targeted antivirals currently under development for treatment of chronic hepatitis C virus infection. Clinical data on baseline and on-treatment correlates of drug resistance and response to these agents are currently limited.

Methods: Hepatitis C virus genotype 1b Japanese patients (prior null-responders to peginterferon-alfa/ribavirin [n=21] or peginterferon-alfa/ribavirin ineligible or intolerant [n=22]) were administered daclatasvir/asunaprevir for 24 weeks during a phase 2a open-label study. Genotypic and phenotypic analyses of NS3 and NS5A substitutions were performed at baseline, after virologic failure, and post-treatment through follow-up Week 36.

Results: There were three viral breakthroughs and four relapsers. Baseline NS3 polymorphisms (T54S, Q80L, V170M) at amino acid positions previously associated with low-level resistance (<9-fold) to select NS3 protease inhibitors were detected in four null-responders and three ineligibles but were not associated with virologic failure. Baseline NS5A polymorphisms (L28M, L31M, Y93H) associated with daclatasvir resistance (<25-fold) were detected in five null-responders and six ineligibles. All three viral breakthroughs and 2/4 relapsers carried a baseline NS5A-Y93H polymorphism. NS3 and NS5A resistance-associated variants were detected together (NS3-D168A/V,NS5A-L31M/V-Y93H) after virologic failure. Generally, daclatasvir-resistant substitutions persisted through 48 weeks

post-treatment whereas asunaprevir-resistant substitutions were no longer detectable. Overall, 5/10 patients with baseline NS5A-Y93H experienced virologic failure while 5/10 achieved a sustained virologic response.

Conclusions: The potential association of a pre-existing NS5A-Y93H polymorphism with virologic failure on daclatasvir/asunaprevir combination treatment will be examined in larger studies. The persistence of treatment-emergent daclatasvir- and asunaprevir-resistant substitutions will require assessment in longer-term follow-up studies.

Abstract word count: 250 words

Keywords: asunaprevir, daclatasvir, drug resistance, direct-acting antivirals, hepatitis C, peginterferon-sparing.

Introduction

The introduction of direct-acting antivirals (DAA) targeting hepatitis C virus (HCV) NS3 protease activity has substantially increased sustained virologic response (SVR) in chronic HCV genotype 1 (GT1) infection. In combination with peginterferon-alfa and ribavirin (alfa/RBV), treatment with the recently approved protease inhibitors boceprevir or telaprevir results in SVR rates of around 70–75% in treatment-naïve patients [1, 2]. Despite these improvements, SVR rates vary by genotype and remain suboptimal in some patients, such as null-responders to alfa/RBV [3], and patients for whom alfa/RBV is poorly tolerated or medically contraindicated. Furthermore, alfa/RBV is associated with frequent side effects [3], and the addition of these DAAs results in elevated rates of anemia and additional events such as dysgeusia (boceprevir), or rash, pruritis, and nausea (telaprevir) [4, 5].

Daclatasvir (DCV) and asunaprevir (ASV) are currently undergoing clinical development for HCV infection. DCV (BMS-790052) is a first-in-class, highly selective NS5A replication complex inhibitor with picomolar potency and broad HCV genotypic coverage [6] that has demonstrated antiviral efficacy and good tolerability in combination with alfa/RBV [7]. ASV (BMS-650032) is a selective inhibitor of NS3 protease with antiviral activity *in vitro* against GT1 and GT4 [8]; it has also been shown to be efficacious and generally well tolerated in combination with alfa/RBV [9]. Clinical interest is increasingly focusing on exploring DAA-only regimens without alfa/RBV, whose potential benefits might include better tolerability and compliance, and a reduced duration of therapy. One recent alfa/RBV-sparing study of DCV plus ASV (AI447017) has examined the efficacy and safety of this combination for 24 weeks in a small cohort of ten GT1b null-responders, in which an SVR rate of 90% was

observed [10]. The study was then expanded to include an additional cohort of null-responders and a group of patients ineligible to receive, or intolerant of, alfa/RBV [11].

As with other antiviral agents, the efficacy of DCV and ASV can be compromised by the development of drug resistance. *In vitro* data suggest that DCV and ASV should provide additive or synergistic activity that enhances the genetic barrier to resistance [8]. Here we characterize virologic escape observed on DCV plus ASV treatment in the expanded AI447017 study [11], its associations with baseline characteristics including *IL28B* genotype and HCV polymorphisms, and an assessment of on- and off-treatment genotypic changes in NS5A and NS3 protease and their phenotypic consequences.

Patients and methods

Study design and patients

This was an open-label, Phase 2a study (AI447017; clinicaltrials.gov identifier NCT01051414) evaluating the antiviral activity and safety of DCV plus ASV in 43 patients with HCV GT1 infection. Patients comprised (a) 21 alfa/RBV null-responders (<2 log_{10} decline in plasma HCV-RNA after 12 weeks); and (b) 22 patients who discontinued previous alfa/RBV within 12 weeks for intolerance or were considered medically poor candidates for alfa/RBV for reasons such as advanced age, complications of depression, anemia, myelosuppression, diabetes, or cardiovascular or renal dysfunction. Patients enrolled in four cohorts; two each of null-responders and ineligible/intolerant patients. The initial sentinel cohort of null-responders has been described previously [10]. All enrolled patients were infected with GT1b.

Patients received DCV 60mg once-daily with ASV 200mg twice-daily for 24 weeks, with a further 48 weeks' post-treatment follow-up. ASV dosing in the expanded study was reduced from the 600mg twice-daily administration used in the sentinel cohort following reports of hepatic enzyme elevations at this dose in another clinical study [12].

The full study design, including inclusion/exclusion criteria, and safety/efficacy endpoints, is described elsewhere [11]. Briefly, eligible patients were men and women aged 20–75 years with HCV genotype 1 infection ≥6 months and HCV RNA $\geq 10^5$ IU/mL. Patients were excluded if they had evidence of liver cirrhosis within 24 months of screening; a history of

hepatocellular carcinoma, other chronic liver disease, variceal bleeding, hepatic encephalopathy, or ascites requiring diuretics or paracentesis; coinfection with hepatitis B virus or HIV; or other clinically significant medical conditions.

Laboratory assessments

Plasma samples for resistance testing were collected at baseline and study Weeks 1, 2, 4, 6, 8, 10, 12, 16, 20, and 24 and post-treatment weeks 4, 8, 12, 24, 36, and 48. HCV-RNA was determined at a central laboratory using the Roche COBAS® TaqMan® HCV Auto assay, (Roche Diagnostics KK, Tokyo, Japan) with a lower limit of quantitation (LLOQ) of 15 IU/mL. HCV genotype and subtype, and *IL28B* genotype (rs12979860 single-nucleotide polymorphism) were determined by polymerase chain reaction (PCR) amplification and sequencing.

Genotypic and phenotypic analysis of clinical samples

Testing was performed on all baseline samples and on samples indicative of slow virologic response at Week 1 or virologic failure with HCV-RNA levels ≥1000 IU/mL. Virologic failure, for the purpose of the study, was defined as an HCV-RNA level (a) ≥LLOQ at Week 4 (futility rule), (b) >1 $\log_{10}$ IU/mL above nadir or ≥LLOQ after confirmed undetectable (virologic breakthrough), or (c) ≥LLOQ at any follow-up visit after being undetectable at end of treatment (relapse).

Population sequencing of PCR amplicons was performed using methods described elsewhere [13-15]. For clonal analysis, amplicons were cloned into the TOPO vector and transformed into TOP10 *Escherichia coli* using a commercially available kit (TOPO® TA-cloning® kit, Invitrogen, Carlsbad, CA) according to manufacturer's instructions, with ≥20 individual colonies expanded and sequenced for each analysis.

Phenotypic analyses of resistance-associated substitutions were performed by employing *in vitro* HCV replicon systems according to previously published methodologies [15-17].

Results

Viral response to DCV and ASV

Overall, plasma HCV-RNA was undetectable in 77% (33/43) of patients at 24 weeks post-treatment. SVR was higher among the null-responders than in the alfa/RBV ineligible population; all viral breakthroughs (n=3) and relapses (n=4) occurred in the ineligible/intolerant subpopulation. Three patients discontinued the study without subsequent SVR or virologic failure (Tables 1 and 2) [11].

Null-responders

Virologic response

Rapid and similar decreases in plasma HCV-RNA levels were observed among patients who initiated treatment with ASV 600mg (Fig. 1A) or ASV 200mg (Fig. 1B). Mean reduction in HCV-RNA at Week 1 was comparable for both groups (–4.4 versus –4.3 $\log_{10}$ IU/mL,

respectively). Of the patients still receiving treatment (P-6 discontinued at Day 16 due to an AE), all but one patient (P-13) had HCV-RNA <15 IU/mL at Week 4 and 52% had undetectable HCV-RNA at this time.

Baseline analysis

Baseline *IL28B* genotype and naturally occurring polymorphisms associated with ASV or DCV resistance (resistance-associated variants [RAVs]) are shown in Table 1. As anticipated for this prior null-responder population, the majority (18/21) were non-CC *IL28B*. The NS5A polymorphism Y93H (24-fold DCV resistance [13]) was observed in three patients. Other polymorphisms conferring minimal (2- to 3-fold) DCV resistance were detected in two patients (NS5A-L28M-R30Q and NS5A-L31M). Polymorphisms associated with minimal to low-level resistance to select NS3 protease inhibitors (one patient, NS3-T54S-Q80L; one patient, NS3-Q80L-V170I/M; two patients, NS3-Q80L) [4, 5, 18] were also observed.

Baseline polymorphisms and *IL28B* genotype did not appear to influence either the Week 1 response or SVR rate (Fig. 2A). Five patients had RNA levels ≥1000 IU/mL after 1 week, of whom one (P-21) had significantly slower initial HCV-RNA declines when compared with mean reductions (standard deviation [SD]) in HCV-RNA for null-responders on the study (–3.4 versus –4.35 ± 0.49 $\log_{10}$ IU/mL). This patient had a CC *IL28B* genotype and an NS5A polymorphism (Q54L; no fold-change in DCV resistance). The other four patients had polymorphisms that have been associated with DCV and NS3 protease inhibitor low-level resistance [13, 19]—specifically NS5A-Q54H/Q-Q62Q/E-Y93H/Y with NS3-T54S-Q80L (P-1, no fold-change to DCV/ASV), NS3-Q80L-V170I/M (P-2, no fold-change to ASV), NS5A-R30Q

with NS3-S122G (P-20, no fold-change to either DCV/ASV), or NS5A-Q54H (P-13, no fold-change to DCV). P-13 was the only patient with HCV-RNA <15 IU/mL (target detectable) at Week 6 and was, therefore, considered a treatment failure. Treatment-emergent resistance at Week 1 in the five patients could not be determined because of PCR failure. A comparison of initial virologic response versus dose and polymorphisms associated with resistance revealed no differences. Among null-responders who received ASV 600mg, mean HCV-RNA declines at Week 1 for those with versus without RAVs were –4.6 versus –4.3 $\log_{10}$ IU/mL, which were similar to the Week 1 declines among those who received ASV 200mg (–4.5 $\log_{10}$ IU/mL with RAVs (one patient) versus –4.3 $\log_{10}$).

Baseline HCV-RNA levels did not impact response to treatment; patients with high baseline viral load still experienced rapid and robust responses to therapy (Fig. 1; Table 1).

Ineligible/intolerant patients

Virologic response

Virologic response at Week 4 was greater in alfa/RBV ineligible patients than null-responders. Undetectable HCV-RNA at Week 4 was observed in 86% of the ineligible group versus 52% of null-responders. However, by Week 12, undetectable HCV-RNA was similar in both groups. Early HCV-RNA declines appeared unaffected by *IL28B* genotype, the presence of baseline polymorphisms associated with resistance, or virologic outcome (Fig. 3). Adherence to therapy, assessed through pill counts, was found to be high in six of the seven patients experiencing virologic failure. However, DCV/ASV exposures were high in the one non-compliant patient (P-31) who subsequently experienced relapse.

Baseline analysis

Baseline *IL28B* genotype, polymorphisms associated with resistance, and virologic outcome are shown in Table 2 and Fig. 2B. Three patients presented with DCV resistance at baseline: one (P-25) with an NS5A-L31M-Y93H combination (7,105-fold DCV resistance [13]), and two with an NS5A-Q54Y-Y93H (58-fold resistance). All three subsequently experienced viral breakthrough at Week 10 or 16.

Other patients had baseline polymorphisms conferring minimal or low-level resistance to DCV and/or protease inhibitors; NS5A-Y93H (n=4), NS5A-L28M-R30L (n=1), NS3-T54S (n=1), and NS3-Q80L (n=5). Variable responses were observed among these patients (Fig. 2B); the majority responded, but two patients with baseline NS5A-Y93H experienced post-treatment relapse. One patient (P-24) with baseline NS5A-L28M-R30L-Q54H-A92T and NS3-Q80L-S122G had a slower response to treatment at Week 1 when compared with mean HCV-RNA reductions (SD) for ineligible/intolerant patients on the study (–3.4 versus –4.74 [0.58] $\log_{10}$ IU/mL) but subsequently achieved SVR with only 16 weeks' treatment. Neither NS3-Q80L-S122G nor NS5A-L28M-R30L-Q54H-A92T conferred resistance to ASV or DCV, respectively.

Baseline viral load did not appear to affect response; mean HCV-RNA levels (SD) were 6.4 (0.7) $\log_{10}$ IU/mL among patients achieving SVR compared with 6.8 (0.3) $\log_{10}$ IU/mL among patients experiencing virologic failure. However, four of six patients with the *IL28B* CT allele subsequently failed treatment (three breakthroughs, one relapse) versus only three of 16 patients with *IL28B* CC (all relapsed).

Genotypic analysis of patients with viral breakthrough

Treatment-emergent RAVs were assessed through post-treatment Week 48 in the three patients with virologic breakthrough (Table 3).

Patient P-25: This patient was *IL28B* CT genotype with baseline HCV-RNA 6.8 $\log_{10}$ IU/mL and a linked baseline NS5A-L31M-Y93H/Y polymorphism. Despite undetectable HCV-RNA by Week 4 (Fig. 4A), viral breakthrough occurred at Week 16, associated with high-level resistance to both DCV (NS5A-L31M-P58A-Y93H; 65,000-fold) and ASV (D168A; ~120-fold in GT1b). Other minor variants detected at baseline by clonal analysis (NS5A-Q62R, -A92T) were not present at breakthrough. NS5A variants present at end of therapy persisted through follow-up Week 48, and although P58A had largely changed to P58G (73% of 33 clones, Fig. 5A) by Week 36, a similar ratio of P58G to A was detected at follow-up Week 48. By contrast, NS3-D168A had mostly been replaced by wild-type at Week 48 (83% of 64 clones).

Patient P-29: This patient was *IL28B* CT genotype, with baseline HCV-RNA 6.7 $\log_{10}$ IU/mL and a pre-existing linked NS5A-Q54Y-Y93H/Y (Fig. 5B) and NS3-Q80L. Undetectable HCV-RNA by Week 3 was followed by viral breakthrough at Week 16 (Fig. 4A) associated with NS5A-L31M-Q54Y-Y93H (6,467-fold DCV resistance) and NS3-Q80L-D168V (~280-fold ASV resistance). These RAVs remained stable through 48 weeks post-treatment.

Patient P-43: This patient was *IL28B* CT genotype with baseline HCV-RNA 7.0 $\log_{10}$ IU/mL, and a pre-existing NS5A-Q54Y-Y93H variant (Fig. 5C). HCV-RNA was undetectable at Week 2 and breakthrough occurred at Week 10 (Fig. 4A), associated with a linked NS5A-L31M-Q54Y-Y93H variant (Fig. 5C; 6,467-fold DCV resistance) and a NS3-D168V variant (~270-fold ASV

resistance). Again, NS5A variants remained stable through Week 48 post-treatment while NS3-D168V was replaced by wild-type (100% of 60 clones).

For the three patients experiencing viral breakthrough, DCV and ASV trough exposures were less than drug levels required to achieve a 90% effective concentration (EC_{90}) value against emergent RAVs (Table 3).

Genotypic analysis of patients experiencing post-treatment relapse

Four ineligible patients with undetectable HCV-RNA at end of treatment experienced relapse (Fig. 4B). Resistance polymorphisms through Week 48 off-treatment are shown in Table 3. Baseline polymorphisms associated with resistance were not detected in two patients (P-32 and P-36), but both displayed post-relapse resistance by follow-up Weeks 8 and 4, respectively. Patient P-32 relapsed with NS5A-L31M-P58L-Y93H (8,300-fold DCV resistance) and NS3-D168V (270-fold ASV resistance). Patient P-36 relapsed with a NS5A-L31V/M-Y93H genotype (L31V-Y93H: 14,789-fold DCV resistance versus L31M-Y93H: 7,105-fold) [13] and NS3-D168V. The remaining two patients had detectable NS5A-Y93H at baseline (24-fold DCV resistance) and additional substitutions at NS5A-L31 and NS3-D168 were detected after relapse. Patient P-31 displayed NS5A-L31M-Y93H (7,105-fold DCV resistance) [13] and NS3-D168A (~120-fold ASV resistance); patient P-37 relapsed with the same NS5A-L31V/M-Y93H and NS3-D168V, as described for patient P-36.

Baseline HCV-RNA and *IL28B* genotype did not appear to influence relapse; three of four relapse patients were *IL28B* CC genotype and baseline HCV-RNA was not appreciably higher

than for those with SVR (mean HCV-RNA [SD]: 6.8 [0.4] vs. 6.4 [0.7] log_{10} IU/mL, respectively).

Changes in the DCV resistance pattern present at relapse through follow-up Week 48 were seen in three of four relapsers; comprising Y93H changing to wild-type (100% of 68 clones) in patient P-32. Clonal analysis of the baseline sequence revealed the presence of Y93H as a minor species (~2%; 1/61 clones). Genotypic changes resulting in a lower level of phenotypic resistance (L31V-Y93H to L31M-Y93H) were detected in patients P-36 and P-37. NS3 substitutions observed at relapse were not detectable by population sequencing by follow-up Week 36. The D168V substitution detected in patient P-37 was replaced by D168E (78-fold ASV resistance [19]) at follow-up Weeks 36 and 48. As with the patients who experienced virologic breakthrough, ASV and DCV trough values in the three drug-compliant patients who relapsed were less than the observed EC_{90} values for the respective RAVs.

Discussion

This study assessed resistance and virologic failure in a difficult-to-treat population of null-responders and alfa/RBV ineligible/intolerant patients treated with the dual oral combination of DCV and ASV. Overall, 77% achieved an SVR [11], with all viral breakthroughs and post-treatment relapses occurring in the ineligible/intolerant subpopulation. It is possible that pharmacokinetics may have played a role in these failures, since patients experiencing failure had DCV and/or ASV trough values below median or documented non-compliance [11]. However, since most patients with troughs below the median achieved SVR, the influence of drug exposure is hard to assess.

NS5A-Y93H was identified as the predominant polymorphism at baseline in all three patients with viral breakthrough and in two of the four patients with relapse. However, three null-responders and two ineligible/intolerant patients also had a pre-existing NS5A-Y93H polymorphism and all achieved SVR, making the significance of Y93H alone for response in the broader patient population difficult to assess. Furthermore, where Y93H polymorphisms existed at baseline, their effects on DCV inhibition were minimal (Y93H EC_{50} = 49 pM [6] compared with C_{trough} values that ranged from 75 to 620 nM. The global prevalence of NS5A-Y93H is approximately 4%, based on data from the Los Alamos database [20] and unpublished data from nine DCV studies, and approximately 11% in other recent Japanese DCV studies [21], which is considerably lower than the 23% (10/43) prevalence observed in this study. Further analysis of DCV study data indicates that Y93H pre-exists at higher levels in patients infected with GT1b (10%) than GT1a (1%); however, the link with *IL28B* is not so clear given that most failures to date with DCV have been observed in GT1a patients with no baseline Y93H. Other polymorphisms observed at a higher frequency among this GT1b population included NS3-Q80L (~19%, 8/43), versus Q80K which has been observed more frequently in GT1a populations [18, 19].

Baseline HCV-RNA did not appear to influence virologic response in either population, and response was too rapid to allow successful genomic sequencing after 1 week of treatment. ASV dose (600mg or 200mg twice-daily) did not impact the initial decline in HCV-RNA in null-responders, and the *IL28B* CT allele, present in 86% (18/21) of null-responders, did not prevent patients achieving a very high (90%) SVR. By contrast, although only 27% (6/22) of ineligible/intolerant patients were *IL28B* CT, this genotype was present in all three viral breakthroughs and one of four relapses. While *IL28B* genotype is known to influence