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Original Article

Effectiveness and safety of reduced-dose telaprevir-based triple therapy in chronic hepatitis C patients

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Aim: To compare the early virological effectiveness, sustained virological response and safety of telaprevir 1500 mg/day with telaprevir 2250 mg/day, when combined in triple therapy with pegylated interferon and ribavirin in Japanese patients with high viral loads of genotype 1 hepatitis C virus.

Methods: The telaprevir 2250 mg/day and 1500 mg/day groups each contained 60 patients matched by age, sex and history of previous interferon-based treatment. Serum levels of genotype 1 hepatitis C virus RNA, hemoglobin levels, drug adherence and drug discontinuation rates were monitored during and after triple therapy.

Results: Patients receiving telaprevir 1500 mg/day had significantly lower telaprevir adherence and lower initial ribavirin dose but similar or superior pegylated interferon and ribavirin adherence and a lower rate of telaprevir discontinuation than did those receiving telaprevir 2250 mg/day. The early virological responses and sustained virological response rates were similar in both groups. Hemoglobin levels decreased to a greater extent in patients treated with telaprevir 2250 mg/day.

Conclusion: Compared to triple therapy including telaprevir 2250 mg/day, that including telaprevir at a reduced dose of 1500 mg/day was associated with lower rates of anemia and similar antiviral efficacy. Such a regimen may meaningfully improve sustained virological response rates, especially among female and elderly Japanese patients.

Key words: chronic hepatitis, hepatitis C virus, pegylated interferon, ribavirin, telaprevir

INTRODUCTION

APPROXIMATELY 170 MILLION people are chronically infected with hepatitis C virus (HCV) worldwide,[1] and approximately 30% develop serious liver disease such as decompensated cirrhosis and hepatocellular carcinoma (HCC).[2,3] Currently, interferon (IFN) is the only antiviral drug capable of eliminating HCV infection. The present standard of care (SOC) for patients infected with HCV genotype 1, the most prevalent global genotype, is pegylated interferon (PEG IFN) combined with ribavirin (RBV) for 48 weeks.[4] However, sustained virological response (SVR), defined as the reduction of serum HCV RNA to undetectable levels 24 weeks after the completion of therapy, is achieved in only 42–52% of patients.[5–7] Moreover, response rates are influenced by patient factors such as sex, age and ethnicity,[8–10] as well as virological factors such as genotype and viral load.[11] SVR rates remain unsatisfactorily low (22%) in women aged 50 years or more who are infected with HCV genotype 1 in Japan.[12] Hence, there is a pressing need to improve the efficacy of antiviral treatment in such patients.

Recently, a new class of drugs, with a mechanism based on inhibition of the NS3/NS4 protease of the HCV polyprotein, has been investigated for the treatment of chronic hepatitis C. Of the drugs in this class, telaprevir has been selected as a clinical candidate for further development.[13] Telaprevir combined with PEG IFN and RBV has shown potent antiviral activity in phase II[14,15] and III clinical trials;[16,17] SVR rates of

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approximately 70% have been reported in patients infected with HCV-1. Similarly, in Japan, a phase III study was conducted in patients with HCV-1 to compare the efficacy and safety of the telaprevir regimen with those of the current SOC in treatment-naïve patients,[18] and to assess the efficacy and safety of the telaprevir regimen in relapsers and non-responders after previous IFN-based therapy.[19] However, the high efficacy was offset by treatment-induced anemia: early hemoglobin levels during triple therapy decreased by up to 4 g/dL, whereas decreases with SOC were not higher than 3.0 g/dL.[14,15] Additionally, we have previously reported that the factors associated with decreases in hemoglobin levels during triple therapy included female sex and age of more than 50 years.[20] Japanese patients infected with HCV genotype 1b with high viral loads are, on average, much older than Western patients infected with the same genotype, owing to a widespread HCV infection that occurred in Japan approximately 20 years ago.[21] Therefore, we considered that triple therapy would be highly effective when combined with careful monitoring of hemoglobin levels and prompt modification of RBV dose.

Consequently, in this study, we evaluated the effectiveness and safety of telaprevir-based triple therapy, administrated at an initial telaprevir dose of 2250 or 1500 mg/day, in the retrospective matched control study of 120 Japanese patients with chronic HCV-1 infection with high viral loads.

METHODS

Patients

FROM DECEMBER 2008 to August 2012, 204 patients with chronic hepatitis C were recruited to receive triple therapy with telaprevir, PEG IFN and RBV for 24 weeks at the Department of Hepatology in the Toranomon Hospital in Metropolitan Tokyo. All patients had the following characteristics: (i) positive for HCV RNA genotype 1 and antibody to HCV (anti-HCV), absence of co-infection with HCV of other genotypes; (ii) negative for hepatitis B surface antigen; (iii) HCV RNA levels of 5.0 log IU/mL or more as determined with the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan); (iv) platelet counts of more than $80 \times 10^3/mm^3$ without cirrhosis diagnosed by ultrasonography; (v) not pregnant or lactating; (vi) total previous alcohol intake of less than 500 kg; (vii) absence of HCC, hemochromatosis, Wilson's disease, primary biliary cirrhosis, alcoholic hepatitis or autoimmune hepatitis; and (viii) absence of antiviral or immunosuppressive treatment during the previous 3 months.

Patients were followed for liver function and virological markers at least monthly during treatment and until 24 weeks after completion of the triple therapy. Informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in the a priori approval of the institution's human research committee.

Study design

Telaprevir (Telavic; Mitsubishi Tanabe Pharma, Osaka, Japan) was administrated at the dose of 2250 (750 mg three times daily) or 1500 mg/day (750 mg twice daily). We selected 60 patients per group who were matched by age, sex and history of previous IFN-based treatment from the telaprevir 2250 and 1500 mg/day groups (Table 1), because 204 patients had many differences in baseline characteristics in both groups. PEG IFN-α-2b (PEG-Intron; Schering Plough, Kenilworth, NJ, USA) was injected s.c. at a median dose of 1.5 μg/kg (range, 1.1–1.8) once a week. RBV (Rebetol; Schering Plough) was administrated at 200–1000 mg/day; RBV dose of 600 mg/day (for bodyweight ≤60 kg), 800 mg/day (for bodyweight >60 to ≤80 kg) or 1000 mg/day (for bodyweight >80 kg) in principle. Since November 2011, the initial dose of RBV was reduced by 200 mg in cases of female sex, aged 66 years or older, hemoglobin level of less than 13 g/dL, bodyweight of less than 45 kg or platelet counts of less than $150 \times 10^3/mm^3$ at baseline by the judgment of the physician. All participating patients received these three drugs for the initial 12 weeks, followed by PEG IFN and RBV for an additional 12 weeks. All patients were followed up for at least 24 weeks after the last dose of study drugs to assess SVR.

Doses of telaprevir, PEG IFN and RBV were reduced or their administration discontinued as required, based on the reduction of hemoglobin levels; reduction of white blood cell, neutrophil or platelet counts; or the development of adverse events. Thus, the total dose of each drug administrated during the 12–24 weeks was calculated as the ratio of the actual administrated total dose to the anticipated total dose of each drug; these ratios provided adherence measures for telaprevir, PEG IFN and RBV.

HCV RNA measurements

Blood samples were obtained at weeks 1, 2, 4, 6, 8, 12, 16, 20 and 24 after initiation of treatment and at week 24 after completion of treatment, and routine biochemical

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Table 1 Baseline characteristics of the patients infected with genotype 1 HCV who received triple therapy with pegylated interferon, ribavirin and TVR

	TVR 2250 mg/day	TVR 1500 mg/day	*P*-value
n	60	60	
Sex (male/female)	30/30	30/30	Matched
Age (years)	60 (53–63)	62 (56–64)	Matched
Body mass index (kg/m^2)	22.1 (20.4–24.0)	22.7 (20.1–24.8)	0.278
IL28B genotype (rs8099917) TT/TG + GG	40/20	54/6	0.003
ITPA genotype (rs12979860) CC/CA + AA	44/16	36/23	0.175
Hemoglobin (g/dL)	14.3 (13.5–15.2)	14.2 (13.0–14.8)	0.223
Platelets (×104/μL)	17.6 (14.9–21.0)	16.9 (13.8–19.9)	0.227
Albumin (g/dL)	3.8 (3.7–4.0)	3.8 (3.7–4.1)	0.404
Alanine aminotransferase (IU/L)	35 (25–49)	37 (25–58)	0.437
γ-Glutamyltransferase (IU/L)	29 (18–49)	22 (17–39)	0.230
Creatinine (mg/dL)	0.7 (0.6–0.8)	0.6 (0.6–0.7)	0.333
Uric acid (mg/dL)	5.6 (4.9–6.5)	5.5 (4.7–6.3)	0.487
α-Fetoprotein (μg/L)	4 (3–7)	5 (3–8)	0.740
HCV RNA (log10 IU/mL)	6.8 (6.4–7.0)	6.7 (6.3–7.0)	0.551
Core a.a. 70 (wild/mutant)	38/22	45/15	0.235
Core a.a. 91 (wild/mutant)	28/32	36/24	0.200
Previous IFN-based treatment			
Naïve/relapsed/null response	23/25/12	23/25/12	Matched

Values are number with percentage in parentheses or median with interquartile range in parentheses.
a.a., amino acid; HCV, hepatitis C virus; IFN, interferon; TVR, telaprevir.

and hematological tests were performed. The antiviral effects were assessed by measuring plasma HCV RNA levels using the COBAS TaqMan HCV test. The linear dynamic range of the assay was 1.2–7.8 $\log_{10}$ IU/mL; undetectable samples were defined as negative.

Detection of amino acid substitutions in the core of HCV-1b

Amino acid (a.a.) substitutions in the HCV core region were determined using direct sequencing of polymerase chain reaction products after extraction and reverse transcription of HCV RNA. Core a.a. substitutions at positions 70 and 91 (core 70 and 91, respectively) were determined according to the methods of our previous reports.[22,23]

Determination of IL28B and ITPA genotypes

ITPA (rs1127354) and *IL28B* (rs8099917 and rs12979860) were genotyped using the Invader assay, TaqMan assay or direct sequencing, as described.[24,25]

Statistical analyses

Non-parametric tests, including the χ^2-test, Fisher's exact test, Mann–Whitney *U*-test and Kruskal–Wallis tests, were used to analyze differences in the baseline clinical profiles of patients. Kaplan–Meier analysis and the log-rank test were applied to estimate and compare serum HCV RNA elimination rates between the groups. $P < 0.05$ by two-tailed test was considered statistically significant. All analyses were performed using SPSS software version 10.1 (SPSS, Chicago, IL, USA).

RESULTS

Baseline characteristics

THE BASELINE CHARACTERISTICS of the 120 patients are listed in Table 1. There were no significant differences in the baseline characteristics between the telaprevir 2250 mg/day group and 1500 mg/day group, except for *IL28B* genotypes. Patients receiving telaprevir 1500 mg/day had a significantly higher incidence of TT in *IL28B* genotypes than did those receiving 2250 mg/day.

Initial drug doses, drug adherence and discontinuation rate up to 12 weeks

Patients receiving telaprevir 1500 mg/day had a significantly lower initial telaprevir dose and initial RBV dose than those receiving 2250 mg/day (Table 2). Telaprevir adherence was significantly lower in the 1500 mg/day

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Table 2 Initial drug doses, drug adherence up to 24 weeks and discontinuation rates up to 12 weeks

	TVR 2250 mg/day	TVR 1500 mg/day	*P*-value
n	60	60	
Initial TVR dose (mg/kg per day)	38.1 (33.6–45.1)	25.6 (22.5–29.6)	<0.001
TVR adherence up to 12 weeks (%)	100 (75–100)	67 (65–67)	<0.001
Discontinuation of TVR	15 (25.0%)	6 (10.0%)	0.053
Discontinuation of TVR due to anemia	12 (20%)	3 (5%)	0.025
Initial PEG IFN dose (μg/kg per week)	1.5 (1.4–1.6)	1.5 (1.4–1.6)	0.706
PEG IFN adherence up to 24 weeks (%)	100 (85–100)	100 (89–100)	0.062
Initial RBV dose (mg/kg per day)	11.6 (10.6–12.8)	9.9 (7.9–11.3)	<0.001
RBV adherence up to 24 weeks (%)	51 (41–61)	59 (46–68)	0.090
Discontinuation of all drugs up to 12 weeks	5 (8.3%)	1 (1.7%)	0.207

Values are number with percentage in parentheses or median with interquartile range in parentheses.
PEG IFN, pegylated interferon; RBV, ribavirin; TVR, telaprevir.

group than in the 2250 mg/day group, while there were no differences in adherence for the other two drugs. Although there were no significant differences between the groups in the rates of discontinuation of telaprevir or all drugs up to 12 weeks, the rates of discontinuation of telaprevir due to anemia in the 1500 mg/day group were significantly lower than in 2250 mg/day group.

Loss of serum HCV RNA according to *IL28B* genotypes

Figure 1 compares the on-treatment virological response over the first 12 weeks for the telaprevir 2250 and 1500 mg/day groups according to *IL28B* genotypes, respectively, because there were significant differences in distribution of *IL28B* genotypes between both groups.

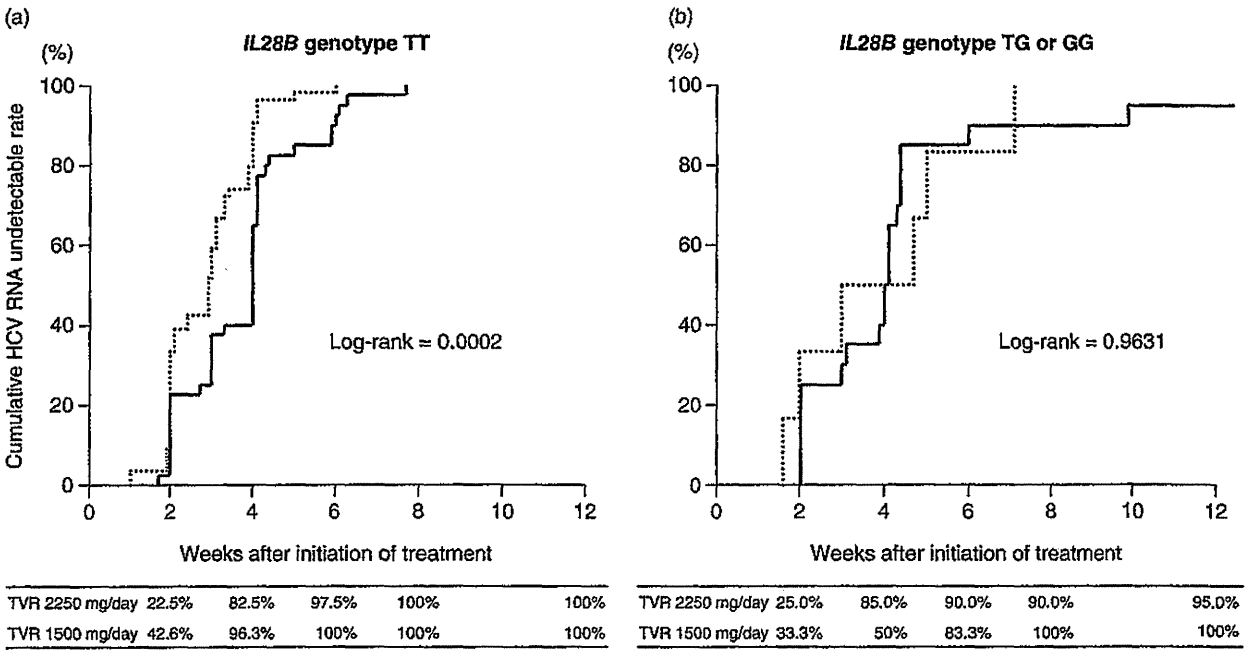

Figure 1 Cumulative rate of undetectable hepatitis C virus (HCV) RNA during triple therapy with pegylated interferon, ribavirin and telaprevir (TVR) at either 2250 mg/day or 1500 mg/day. (a) *IL28B* genotype TT, (b) *IL28B* genotype TG or GG. (———) TVR 2250 mg/day, (·····) TVR 1500 mg/day.

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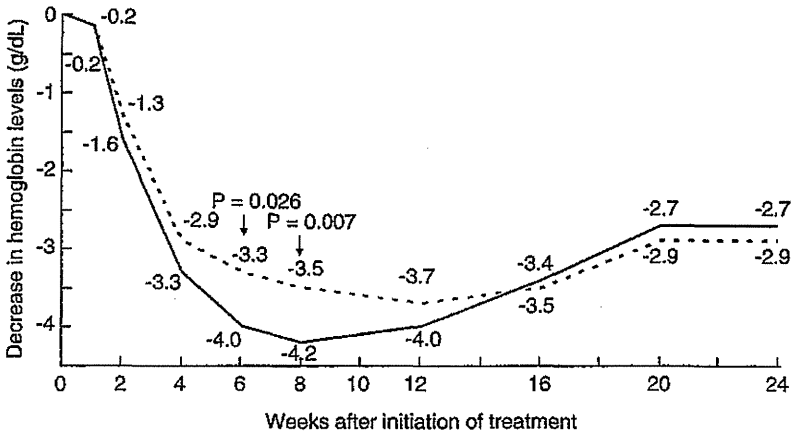

Figure 2 Decreases in hemoglobin levels during triple therapy with pegylated interferon (PEG IFN), ribavirin (RBV) and telaprevir (TVR) at either 2250 mg/day or 1500 mg/day. Each time point in this figure corresponds to median values. Patients evaluated at each time point are indicated below, with the number of patients who discontinued TVR (continued PEG IFN and RBV) in parentheses. (———) TVR 2250 mg/day, (·····) TVR 1500 mg/day.

Number of patients (TVR withdrawn)

2250 mg/day	60	60	60 (1)	59 (4)	55 (10)	55	55	55
1500 mg/day	60	60	60 (1)	59 (2)	59 (3)	59	59	59

Triple therapy suppressed HCV RNA levels quickly and effectively in both groups. In the 2250 and 1500 mg/day groups of *IL28B* genotype TT, HCV RNA became undetectable in 22.5% and 42.6% of patients at 2 weeks, 82.5% and 96.3% at 4 weeks, and 100% and 100% at 8 weeks, respectively (Fig. 1a). The early virological response of the telaprevir 1500 mg/day group was significantly higher than that of the 2250 mg/day group in *IL28B* genotype TT (log-rank test = 0.0002).

In the subgroups of *IL28B* genotype non-TT patients receiving telaprevir 2250 and 1500 mg/day, HCV RNA became undetectable in 25.0% and 33.3% of patients at 2 weeks, 85.0% and 50% at 4 weeks, 90.0% and 100% at 8 weeks, and 95.0% and 100% at 12 weeks, respectively. The virological responses during the first 12 weeks in this subgroup of patients did not significantly differ between the telaprevir 2250 and 1500 mg/day groups (log-rank test = 0.9631, Fig. 1b).

Safety

Figure 2 shows the decreases in hemoglobin levels in telaprevir 2250 and 1500 mg/day recipients. Data from six patients were omitted (five receiving telaprevir 2250 mg/day and one receiving 1500 mg/day) because treatment was withdrawn between 8 and 12 weeks after initiation. Telaprevir was discontinued in 15 of the 60 (25.0%) patients receiving telaprevir 2250 mg/day (one at week 6, four at week 8 and 10 at week 12) and six of the 60 (10.0%) receiving 1500 mg/day (one at week 6, two at week 8 and three at week 12). Hemoglobin decreased to a greater extent in patients receiving telaprevir 2250 mg/day than in those receiving 1500 mg/day at week 6 (−4.0 [−6.7 to −1.2] vs −3.3 [−5.2 to 0.2] g/dL, $P = 0.026$) and week 8 (−4.2 [−7.7 to −1.3] vs −3.5 [−6.9 to −1.3] g/dL, $P = 0.007$).

Skin disorder frequency was comparable between the telaprevir 2250 mg/day group and 1500 mg/day group (81.7% and 75%, respectively). However, skin disorders of grades 2–3 occurred more frequently in the telaprevir 2250 mg/day group than in the 1500 mg/day group (55% vs 35%, $P = 0.043$).

With respect to renal dysfunction, increases in serum creatinine (sCR) levels during therapy were not significantly different between both groups. However, blood uric acid levels increased to a greater extent in patients receiving telaprevir 2250 mg/day than in those receiving 1500 mg/day at week 1 (1.3 [−1.6 to 4.8] vs 0.9 [−2.1 to 4.3] g/dL, $P = 0.015$), week 2 (1.2 [−2.3 to 4.1] vs 0.5 [−2.3 to 2.7] g/dL, $P = 0.004$), week 4 (1.6 [−1.1 to 5.5] vs 0.7 [−2.4 to 3.8] g/dL, $P < 0.001$), week 6 (1.6 [−1.7 to 4.8] vs 0.5 [−3.5 to 3.6] g/dL, $P < 0.001$) and week 8 (1.1 [−3.6 to −4.9] vs 0.7 [−1.6 to 3.7] g/dL, $P = 0.029$).

Predictive factors associated with SVR

The overall SVR rate was 83% (169/204) in our hospital. SVR was accomplished in 106 (88%) of 120 patients selected for this study, including 50 of 60 (83%) patients in the telaprevir 2250 mg/day and 56 of 60 (93%) patients in telaprevir 1500 mg/day groups (Fig. 3).

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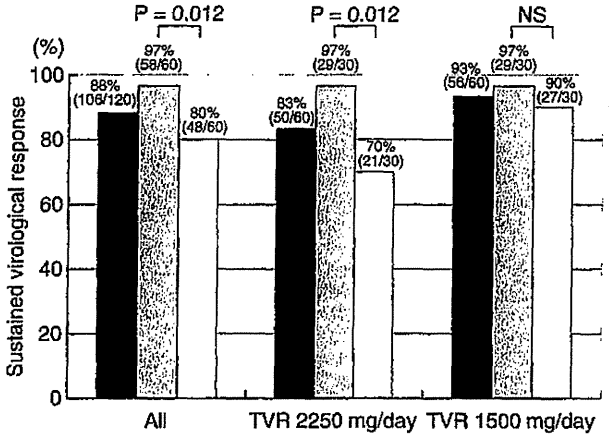

Figure 3 Sustained virological response in patients with chronic hepatitis C to triple therapy with telaprevir (TVR), pegylated interferon and ribavirin for 24 weeks. Sustained virological response was compared among all patients (men and women), TVR 2250 mg/day patients and TVR 1500 mg/day patients, respectively. (■) Total, (□) male, (▨) female.

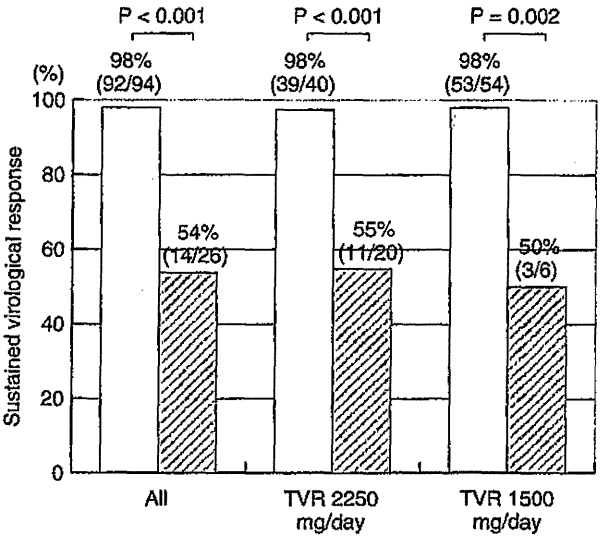

Figure 4 Sustained virological response in patients with chronic hepatitis C to triple therapy with telaprevir (TVR), pegylated interferon and ribavirin for 24 weeks. Sustained virological response was compared between *IL28B* (rs8099917) genotype TT and TG/GG in all patients, TVR 2250 mg/day patients and TVR 1500 mg/day patients, respectively. (□) TT, (▨) TG or GG.

Significant univariate predictors for SVR included male sex, *IL28B* genotype TT, and HCV core a.a. 70 wild type, except for null response to prior treatment, initial telaprevir dose of 37.5 mg/kg per day or more, telaprevir dosing period of 10 weeks or more, 100% PEG IFN adherence up to 24 weeks, PEG IFN adherence up to 12 weeks of 80% or more, RBV adherence up to 12 weeks of 50% of more, γ-glutamyltransferase of 35 IU/mL or less, and sCr of 0.6 mg/dL or more ($P < 0.05$). Of these, male sex (odds ratio [OR] = 13.7; $P = 0.028$) and *IL28B* genotype TT (OR = 44.4; $P = 4.47 \times 10^{-5}$) were identified as significant independent predictors for SVR (Table 3).

Therefore, we assessed the SVR rate of triple therapy according to sex and *IL28B* genotype. SVR was much less frequent in women than in men (48/60 [80%] vs 58/60 [97%], $P = 0.0012$, Fig. 3). Especially, in the telaprevir 2250 mg/day group, there were significant differences between men and women (29/30 [97%] vs 21/30 [70%], $P = 0.0012$). However, there were no differences between men and women in the telaprevir 1500 mg/day group (29/30 [97%] and 27/30 [90%], respectively).

Patients with *IL28B* genotype TT were significantly more likely to achieve SVR (92/94 [98%] vs 14/26 [54%], $P < 0.001$, Fig. 4), compared with patients with TG or GG genotypes. There were significant differences between *IL28B* genotype TT and non-TT in both the telaprevir 2250 and 1500 mg/day groups (39/40 [98%] vs 11/20 [55%], $P < 0.001$ and 53/54 [98%] vs 3/6 [50%], $P = 0.002$, respectively).

Table 3 Multivariate analysis of factors associated with sustained virological response of TVR, pegylated interferon and ribavirin triple therapy in Japanese patients infected with HCV

Factor	Category	Odds ratio (95% CI)	*P*-value
Sex	1; female	1	
	2; male	13.7 (1.33–141.2)	0.028
IL28B genotype (rs8099917)	1; TG or GG	1	
	2; TT	44.4 (7.18–274.2)	4.47×10^{-5}

CI, confidence interval; HCV, hepatitis C virus; TVR, telaprevir.

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DISCUSSION

IN JAPANESE PATIENTS, virological response to triple therapy with telaprevir, PEG IFN and RBV was excellent. We have previously reported that in 20 patients with chronic HCV-1b infection with high viral load who received triple therapy for 12 weeks, HCV RNA became undetectable in 50% at 2 weeks, 79% at 4 weeks, 88% at 6 weeks, 94% at 8 weeks and 100% at 12 weeks.[26] This previous study was a randomized open-label study in which telaprevir was administrated at doses of 2250 or 1500 mg/day. Early virological response at 7 and 14 days was similar for both telaprevir doses, suggesting that virological response to triple therapy is not affected by lowering the telaprevir dose. Therefore, to expand the dataset, we retrospectively evaluated HCV RNA response and safety during 12 weeks of triple therapy including the two different telaprevir doses followed by PEG IFN and RBV for an additional 12 weeks: we analyzed 204 cases in total. However, because of the non-random nature of treatment allocation, there was a preponderance of women, elderly and anemic patients in the group receiving telaprevir 1500 mg/day. Because there were many differences in baseline characteristics between telaprevir 2250 and 1500 mg/day groups, we selected 60 patients per group who were matched by age, sex and history of previous IFN-based treatment. Therefore, there were no differences in baseline characteristics between both groups in this analysis, except for *IL28B* genotype. Although we tried to match the distribution of *IL28B* genotypes between both groups, this was not possible because of the small number of cases. Therefore, we matched the groups by the history of previous IFN-based treatment, which we considered a similarly strong predictive factor of triple therapy. Moreover, there was a significant difference in the initial dose of RBV between both groups. A significant number of patients underwent RBV dose reductions at the beginning of treatment in the telaprevir 1500 mg/day group because we considered that such patients were likely to experience hemoglobin decrements during triple therapy, but before November 2011, we could not reduce the initial dose of telaprevir and RBV. Nine patients (15.0%) receiving telaprevir 2250 mg/day and 32 cases (53.3%) receiving 1500 mg/day underwent RBV dose reduction at the beginning of treatment. In other words, the group receiving telaprevir 1500 mg/day had a significantly lower initial dose of telaprevir and RBV dose than did the group receiving 2250 mg/day (Table 2).

However, in the present study, HCV RNA became undetectable during the 12 weeks of treatment at similar or higher rates in the telaprevir 1500 mg/day group than in the 2250 mg/day group (Fig. 1). In the *IL28B* TT genotype, the early virological response of the telaprevir 1500 mg/day group was significantly higher than that of the 2250 mg/day group. Although we assessed baseline factors, drug adherence and drug discontinuation rates only in the *IL28B* TT genotype, there were no significant differences between both groups, except for lower telaprevir adherence up to 12 weeks and a greater number of cases of PEG IFN and RBV dose reductions at the beginning of treatment in the telaprevir 1500 mg/day group. Therefore, the reason for significant differences in the early virological response between both groups is unclear. However, we considered that these results did not affect the SVR rate because HCV RNA became undetectable in all patients in both groups at 8 weeks after the start of triple therapy. In all cases, *IL28B* TT cases and non-TT cases, there were no significant differences in SVR rates after triple therapy between those receiving telaprevir 2250 and 1500 mg/day (Figs 3,4). By examining the detailed course of drug administration from 12–24 weeks (Table 2), we found that the group receiving telaprevir 1500 mg/day had a lower discontinuation rate of telaprevir and higher adherence to RBV and PEG IFN up to 24 weeks in spite of the low initial RBV dose. Furthermore, hemoglobin levels showed greater reductions during triple therapy with telaprevir 2250 mg/day than with telaprevir 1500 mg/day, and the group receiving telaprevir 2250 mg/day had a significantly higher discontinuation rate of telaprevir due to anemia than did the group receiving telaprevir 1500 mg/day (Fig. 2). Therefore, telaprevir 1500 mg/day may be a safe option as part of triple therapy, while maintaining PEG IFN and RBV adherence.

Viral breakthrough or relapse can occur during telaprevir monotherapy or telaprevir plus PEG IFN dual therapy (without RBV) because of the development of mutations that confer resistance to telaprevir.[14,27–29] Furthermore, in a Japanese phase III trial of triple therapy in relapsers and non-responders who had not achieved SVR to a previously administrated IFN-based regimen, SVR rates increased as RBV adherence increased, particularly in previous non-responders.[19] In triple therapy with telaprevir, PEG IFN and RBV, we consider that telaprevir could be important for early virological response, but it could also be important for maintaining high adherence to PEG IFN and RBV, which is a key factor for achieving SVR. We speculate that triple therapy including telaprevir at the reduced dose of 1500 mg/day could maintain high levels of adherence

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to PEG IFN and RBV, and consequently achieve high SVR rates.

In this study, we investigated the independent predictors for SVR in the multivariate analysis (Table 3). As reported in previous studies, *IL28B* genotype remained the strongest predictor of SVR.[30,31] The next strongest predictive factor was sex: women had significantly lower SVR rates than did men (Fig. 3). However, when we investigated the SVR rates of the telaprevir 2250 mg/day group and 1500 mg/day group, we found that there were significant differences in SVR rates between men and women in the telaprevir 2250 mg/day group but no differences in the telaprevir 1500 mg/day group. In the previous study, we reported that female sex was one of the factors influencing decreases in hemoglobin levels during triple therapy administrated 2250 mg/day of initial telaprevir dose.[20] In the present study, the discontinuation rates of telaprevir due to anemia were significantly higher in women in the telaprevir 2250 mg/day group as compared with men (36.7% vs 3.3%, $P = 0.002$, data not shown), but there were no differences in the discontinuation rates of telaprevir due to anemia between men and women in the telaprevir 1500 mg/day group (0% vs 10%, $P = 0.237$, data not shown). Therefore, we speculate that there were significant differences in SVR rates between men and women because of high telaprevir discontinuation rates owing to anemia in women.

In conclusion, after the completion of 24 weeks of therapy, triple therapy including telaprevir at a reduced dose of 1500 mg/day was as effective as triple therapy including telaprevir 2250 mg/day at suppressing HCV RNA to undetectable levels and achieving SVR. Of note, we found that telaprevir 1500 mg/day was associated with lower levels of anemia and discontinuation of telaprevir owing to anemia, and higher PEG IFN and RBV adherence during triple therapy. These results suggest that the telaprevir 1500 mg/day regimen is an effective and safe alternative for the treatment of elderly and female Japanese patients. This study is a retrospective study. Prospective randomized controlled studies with longer follow-up periods are required to fully assess the efficacy and safety of an initial telaprevir dose of 1500 mg/day.

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Research Article

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Dual oral therapy with daclatasvir and asunaprevir for patients with HCV genotype 1b infection and limited treatment options

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See Editorial, pages 643–645

Background & Aims: Improved therapeutic options for chronic hepatitis C virus (HCV) infection are needed for patients who are poor candidates for treatment with current regimens due to anticipated intolerability or low likelihood of response.

Methods: In this open-label, phase 2a study of Japanese patients with chronic HCV genotype 1b infection, 21 null responders (<2 $\log_{10}$ HCV RNA reduction after 12 weeks of peginterferon/ribavirin) and 22 patients intolerant to or medically ineligible for peginterferon/ribavirin therapy received dual oral treatment for 24 weeks with the NS5A replication complex inhibitor daclatasvir (DCV) and the NS3 protease inhibitor asunaprevir (ASV). The primary efficacy end point was sustained virologic response at 12 weeks post-treatment (SVR_{12}).

Results: Thirty-six of 43 enrolled patients completed 24 weeks of therapy. Serum HCV RNA levels declined rapidly, becoming undetectable in all patients on therapy by week 8. Overall, 76.7% of patients achieved SVR_{12} and SVR_{24}, including 90.5% of null responders and 63.6% of ineligible/intolerant patients. There were no virologic failures among null responders. Three ineligible/intolerant patients experienced viral breakthrough and four relapsed post-treatment. Diarrhea, nasopharyngitis, headache, and ALT/AST increases, generally mild, were the most common adverse events; three discontinuations before week 24 were due to adverse events that included hyperbilirubinemia and transaminase elevations (two patients).

Conclusions: Dual therapy with daclatasvir and asunaprevir, without peginterferon/ribavirin, was well tolerated and achieved high SVR rates in two groups of difficult-to-treat patients with hepatitis C virus genotype 1b infection.

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Introduction

Therapies for chronic hepatitis C virus (HCV) infection have improved markedly over the past decade. The recent approval of the first direct-acting antivirals (DAAs) was an important milestone in the evolution of HCV therapy, establishing that DAAs can enhance regimen efficacy and provide durable viral clearance. These new agents in combination with peginterferon and ribavirin (PegIFN-α/RBV) achieve overall sustained virologic response (SVR) rates of approximately 70% in treatment-naïve patients with HCV genotype 1 infection [1,2].

Despite these advances, current treatment options remain inadequate for some patients. Patients with prior null response to PegIFN-α/RBV (<2 $\log_{10}$ decline in HCV RNA after 12 weeks) have a particularly acute need for further therapeutic improvements. Null responders generally respond poorly to retreatment with PegIFN-α/RBV; fewer than 10% achieve SVR [3]. Retreatment of null responders with PegIFN-α/RBV combined with telaprevir or boceprevir increases SVR rates to approximately 30–38%, suggesting that addition of a DAA to PegIFN-α/RBV increases efficacy, but that more potent regimens are still urgently needed [4,5]. There are also many patients who cannot be treated with current therapies; this group includes patients with prior intolerance to PegIFN-α/RBV and patients who are ineligible for PegIFN-α/RBV -containing therapy for medical reasons.

There is precedence for use of combination antiviral regimens to treat human immunodeficiency virus (HIV) infections;

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Abbreviations: HCV, hepatitis C virus; DAA, direct-acting antiviral; PegIFN-α/RBV, peginterferon alfa and ribavirin; SVR, sustained virologic response; HIV, human immunodeficiency virus; NS5A, non-structural protein 5A; NS3, non-structural protein 3; ALT, alanine aminotransferase; ULN, upper limit of the normal reference range; INR, international normalized ratio; CYP3A4, cytochrome P450 3A4.

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evidence is mounting that DAA regimens can also provide durable clearance of HCV infections. Thus, there is a strong rationale for exploration of dual DAA regimens, without Peg-IFN-α/RBV. In combination, DAAs with different molecular targets can increase regimen potency and raise the barrier to resistance, potentially eliminating the need for PegIFN-α/RBV and providing a viable therapy for patients who are anticipated to be poorly responsive or intolerant to current PegIFN-α/RBV-containing regimens. The improved tolerability and convenience that can be anticipated with dual DAA regimens suggests that they may also benefit treatment-naïve patients and other groups. Previous studies of DAA-only regimens, or DAAs combined with RBV, have demonstrated marked antiviral effects in treatment-naïve and experienced patients, including null responders, supporting the further evaluation of dual DAA therapy reported here [6–10].

Daclatasvir (DCV; BMS-790052) is a first-in-class, highly selective NS5A replication complex inhibitor with picomolar potency and broad genotypic coverage; asunaprevir (ASV; BMS-650032) is a potent NS3 protease inhibitor active against genotypes 1 and 4. Daclatasvir and asunaprevir have different modes of action and resistance-associated variants, and in combination show increased antiviral potency *in vitro* and a high genetic barrier to resistance [11,12]. Daclatasvir and asunaprevir had no clinically meaningful pharmacokinetic interaction in healthy volunteers [13]. Initial efficacy evaluations of daclatasvir and asunaprevir (DUAL therapy) showed potent antiviral effects and SVR rates ≥90% in Japanese and US/European null responders with HCV genotype 1b infection [7,8].

We present final results of an open-label trial evaluating DUAL oral therapy with daclatasvir and asunaprevir in Japanese patients with chronic HCV genotype 1b infection. Initial results from a sentinel cohort of 10 patients with prior null response to PegIFN-α/RBV have been reported [7]. The present report combines these data with results for 11 additional null responders, together with results for 22 patients with prior intolerance to PegIFN-α/RBV or who were medically ineligible for PegIFN-α/RBV-containing therapy.

Materials and methods

Study design

This open label, phase 2a study (AI447-017; clinicaltrials.gov identifier NCT01051414) was conducted in two populations of patients with HCV genotype 1 infection, including null responders (<2 $\log_{10}$ decline of serum HCV RNA levels after 12 weeks of prior PegIFN-α/RBV), and PegIFN-α/RBV ineligible/intolerant patients. The latter group discontinued prior therapy with PegIFN-α/RBV due to intolerance after <12 weeks, or patients were treatment-naïve but poor candidates for PegIFN-α/RBV for medical reasons such as advanced age or complications of depression, anemia, myelosuppression, diabetes, or cardiovascular or renal dysfunction.

Patients were enrolled in two cohorts of null responders and two cohorts of PegIFN-α/RBV ineligible/intolerant patients. One cohort of each population included intensive sampling for pharmacokinetic analyses; both cohorts of each population were combined for efficacy and safety assessments. The sentinel cohort of null responders, reported previously, provided 4-week safety data for review by the study Safety Committee, prior to initiation of the other cohorts [7]. The primary efficacy end point was the proportion of patients with undetectable HCV RNA at 12 weeks post-treatment (SVR_{12}). Key secondary end points included the proportions of patients with HCV RNA undetectable at week 4, week 12, the end of treatment, and post-treatment week 24 (SVR_{24}).

Written informed consent was obtained from all patients. The study was approved by institutional review boards at each site and was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice Guidelines, and local regulatory requirements.

Patients

Eligible patients were men and women aged 20–75 years with HCV genotype 1 infection ≥6 months and HCV RNA ≥10^5 IU/ml. Women of childbearing potential were using adequate contraception. Patients were excluded if they had evidence of liver cirrhosis within 24 months of screening by laparoscopy, imaging studies, or liver biopsy; a history of hepatocellular carcinoma, other chronic liver disease, variceal bleeding, hepatic encephalopathy, or ascites requiring diuretics or paracentesis; co-infection with hepatitis B virus or HIV; other clinically significant medical conditions; exposure to any investigational drug or placebo within 4 weeks, or any previous exposure to NS5A or NS3 protease inhibitors.

Exclusionary laboratory findings included alanine aminotransferase (ALT) >5× upper limit of normal (ULN), total bilirubin ≥2 mg/dl, direct bilirubin >1.5× ULN, international normalized ratio (INR) ≥1.7, albumin ≤3.5 g/dl, hemoglobin <9.0 g/dl, white blood cells <1500/mm^3, absolute neutrophils <750/mm^3, platelets <50,000/mm^3, and creatinine >1.8× ULN. Prohibited concomitant medications included CYP3A4 inducers or moderate/strong CYP3A4 inhibitors, non-study medications with anti-HCV activity, prescription or herbal products not prescribed for treatment of a specific condition, proton pump inhibitors, and erythropoiesis-stimulating agents. Prescribed H2 receptor antagonists were administered ≥2 h after and ≥10 h prior to daclatasvir; other acid modifying agents were administered ≥2 h prior and ≥2 h after daclatasvir.

Study drug dosing

Patients received 24 weeks of treatment with daclatasvir 60 mg once daily (two 30 mg tablets), combined with asunaprevir 200 mg twice daily, with 24 weeks of post-treatment follow-up. In the sentinel cohort of null responders, asunaprevir was initially administered as three 200 mg tablets twice daily (600 mg BID), subsequently reduced to 200 mg BID during treatment following reports from another study of greater and more frequent aminotransferase elevations with the higher dose [14].

Patients with HCV RNA <15 IU/ml on or after week 4 continued treatment to week 24; patients discontinued treatment if HCV RNA decreased <2 $\log_{10}$ IU/ml from baseline on or after week 2. Patients with viral breakthrough on or after week 2, or quantifiable HCV RNA (≥15 IU/ml) on or after week 4, either discontinued treatment or weight-based PegIFN-α/RBV was added (null responders only), for up to 48 additional weeks, at the discretion of the investigator based on anticipated tolerability. Viral breakthrough was defined as confirmed ≥1 $\log_{10}$ IU/ml increase from nadir of HCV RNA, or HCV RNA ≥15 IU/ml after confirmed undetectable. Post-treatment relapse was defined as confirmed HCV RNA ≥15 IU/ml during follow-up in patients with undetectable HCV RNA at the end of treatment.

Safety and efficacy assessments

HCV RNA, physical examinations, adverse events, laboratory parameters, and concomitant medications were assessed at screening, study days 1 (baseline), weeks 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, and 24, and post-treatment weeks 4, 8, 12, and 24. Twelve-lead electrocardiograms were recorded at all visits except weeks 3 and 6.

Serum HCV RNA levels were determined at a central laboratory using the Roche COBAS® TaqMan® HCV Auto assay, (Roche Diagnostics KK, Tokyo, Japan), lower limit of quantitation 15 IU/ml. HCV genotype and subtype and *IL28B* genotype (rs12979860) were determined by PCR amplification and sequencing. Baseline liver fibrosis was assessed by serum blood markers (APRI; AST and Platelet Ratio Index) [15]. HCV resistance-associated polymorphisms were analyzed in stored baseline samples from all patients and post-failure samples from patients with viral breakthrough or post-treatment relapse. Polymorphisms were analyzed by PCR amplification and population sequencing of the HCV NS3 protease and NS5A domains.

Statistical analysis

Categorical variables were summarized using counts and percents; continuous variables were summarized with univariate statistics.

Table 1. Baseline demographic and disease characteristics.

Parameter		Null responders n = 21	Ineligible/intolerant n = 22
Age, median yr (range)		61 (31-70)	68 (47-75)
Male, n (%)		8 (38.1)	6 (27.3)
HCV genotype 1b, n (%)		21 (100)	22 (100)
IL28B genotype, n (%)			
(rs12979860)	CT	18 (85.7)	6 (27.3)
	CC	3 (14.3)	16 (72.7)
HCV RNA, mean log_{10} IU/ml (SD)		6.8 (0.47)	6.6 (0.64)
ALT, mean U/L (SD)		57.9 (24.86)	45.7 (25.79)
APRI score			
	Score >2, n (%)	3 (14.3)	1 (4.5)
	Median (range)	0.96 (0.24-3.41)	0.57 (0.40-2.79)
PegIFN-α/RBV ineligible, n (%)		n.a.	18 (81.8)
PegIFN-α/RBV intolerant, n (%)		n.a.	4 (18.2)

n.a., Not available.

Results

Patient characteristics and disposition

Forty-nine patients were screened of which six failed to meet entry criteria; 21 null responders and 22 ineligible/intolerant patients were enrolled and treated (Table 1). The enrolled population was generally older (median 62 years), consistent with HCV epidemiology in Japan, and primarily female (67%); all patients were Japanese. No patient had prior exposure to HCV DAAs. Although any HCV genotype 1 subtype was permitted, all enrolled patients had genotype 1b infection, reflecting the high proportion of this subtype in Japan [16]. Null responders were primarily *IL28B* genotype CT (rs12979860) as expected [17]; ineligible/intolerant patients were primarily genotype CC, consistent with the distribution of *IL28B* genotypes in Japan [18]. Eighteen ineligible/intolerant patients were treatment-naïve and considered ineligible for PegIFN-α/RBV due to anticipated difficulty in completing therapy due to advanced age (≥70 years) (seven patients), cytopenia (two), depression (two), hypertension (one), or other reasons (six), consistent with common clinical practice in Japan. Four patients had prior PegIFN-α/RBV intolerance due to cytopenia (two patients), depression (one), or other reasons (one). Baseline HCV RNA and ALT levels were similar across patient groups. Although patients with cirrhosis by imaging criteria were excluded, four enrolled patients had APRI scores >2 at baseline, indicating probable cirrhosis [15].

Thirty-six of 43 enrolled patients completed 24 weeks of therapy (Fig. 1). Two null responders discontinued study medication due to hyperbilirubinemia (week 2) and aminotransferase elevation (week 12), respectively. One null responder achieved very low HCV RNA (50 IU/ml) at week 4; however, stringent protocol-defined rules required discontinuation from DAA-only therapy and addition of PegIFN-α/RBV to the dual DAA regimen at week 6. Study drugs were discontinued in four ineligible/intolerant patients due to aminotransferase elevation (week 16), viral breakthrough (week 16), or patient request (weeks 8 and 16); all four patients remained on study for assessment of SVR.

Virologic response

High rates of virologic response were seen at all time points in both study populations (Table 2). Overall, 77% of patients achieved SVR_{12} and SVR_{24}. HCV RNA was undetectable in more ineligible/intolerant patients than null responders at week 4, suggesting a more rapid initial antiviral effect, but HCV RNA was undetectable in similar proportions of both populations at week 12 and the end of treatment. Rates of SVR_{24} were higher in null responders (91%) than in ineligible/intolerant patients (64%) due to virologic failures in the latter group (3 breakthroughs and 4 relapses). Assessment of virologic response by *IL28B* genotype (rs12979860) showed slightly greater responses at weeks 2, 3, and 4 in patients with genotype CC; however, similar proportions of patients with genotypes CC and CT achieved SVR_{24} (Fig. 2). All four patients with possible cirrhosis based on APRI score achieved SVR_{24}.

HCV RNA declined rapidly after initiation of therapy in all patients (Fig. 3). Mean reductions of HCV RNA from baseline at week 4 were 5.6 and 5.4 log_{10} IU/ml in null responders and ineligible/intolerant patients, respectively; HCV RNA was undetectable by week 8 in all patients on therapy. In the ineligible/intolerant group, initial virologic response in the four intolerant patients was similar to that of the cohort overall; three of these patients subsequently achieved SVR_{24} and one relapsed. The null responder who discontinued at week 2 with hyperbilirubinemia had low-level HCV RNA at discontinuation and undetectable HCV RNA at all post-treatment assessments. The null responder who added PegIFN-α/RBV at week 6 received 46 weeks of quadruple therapy and HCV RNA remained undetectable 24 weeks post-treatment. Among the four ineligible/intolerant patients who discontinued study drugs before week 24, HCV RNA was undetectable at discontinuation (weeks 8 or 16) in three patients and remained undetectable in the two patients who completed post-treatment follow-up.

Viral breakthrough and relapse

No null responders experienced virologic breakthrough or relapse (Table 2). Three ineligible/intolerant patients experienced viral breakthrough at weeks 10 or 16 after ≥4 weeks with undetectable

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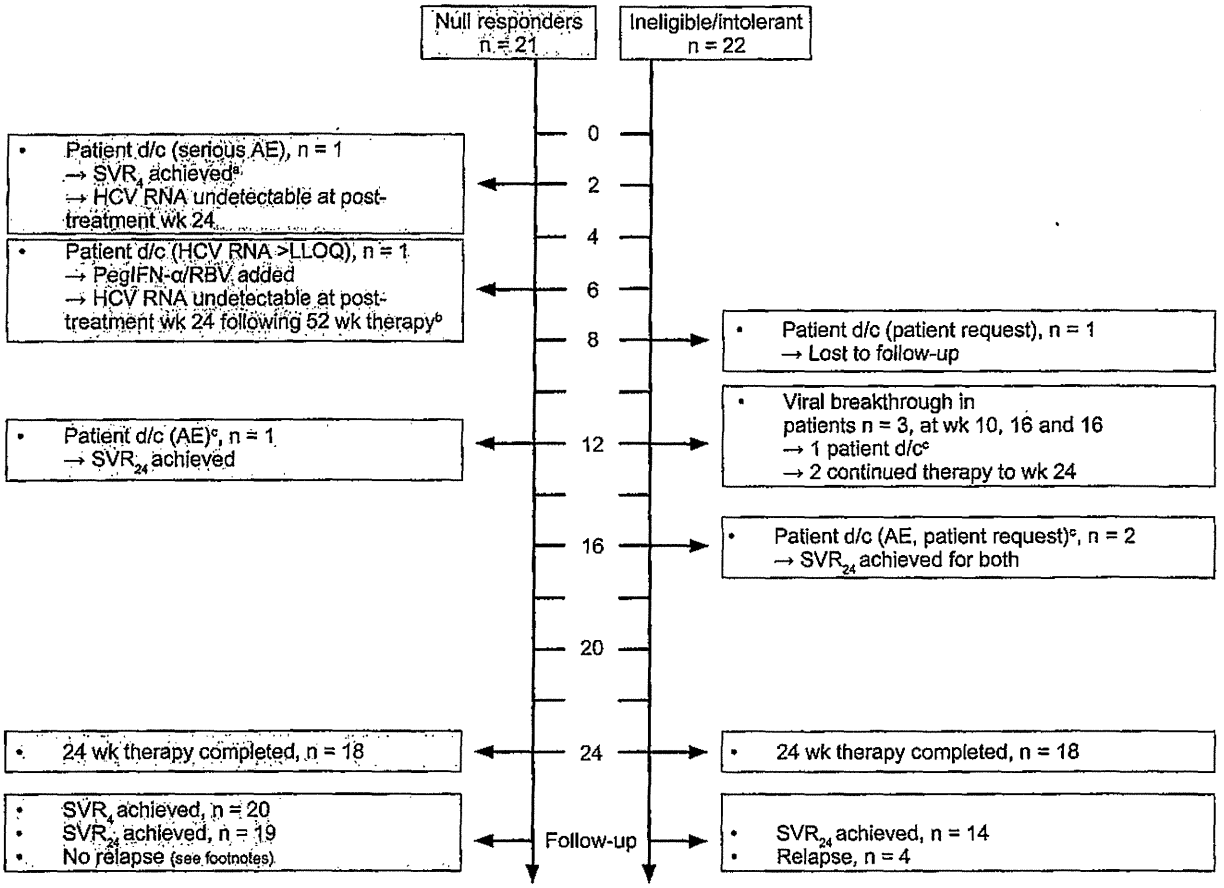

Fig. 1. Patient disposition. Patient flow through treatment and follow-up is shown. d/c, Discontinuation of study medication; SVR_4, SVR_{12} and SVR_{24}, sustained virologic response 4, 12 or 24 weeks post-treatment. [a]On-study follow-up continued to post-treatment week 4; HCV RNA remained undetectable at post-treatment week 24 after study discontinuation, reported as failure for SVR_{24} per statistical protocol requirements; [b]HCV RNA was undetectable at post-treatment week 24 after study discontinuation due to addition of PegIFN-α/RBV, reported as failure for SVR per statistical protocol requirements; [c]on-study follow-up to assess SVR continued after discontinuation of study drugs.

Table 2. Virologic outcomes.

n (%)	Null responders, n = 21	Ineligible/intolerant, n = 22
HCV undetectable		
Wk 4 (RVR)	11 (52.3)	19 (86.4)
Wk 12 (cEVR)	19 (90.5)	20 (90.9)
End of treatment	19 (90.5)	19 (86.4)
SVR_4	20 (95.2)[1]	15 (68.2)[2]
SVR_{12}	19 (90.5)[1]	14 (63.6)[2]
SVR_{24}	19 (90.5)[1]	14 (63.6)[2]
Viral breakthrough	0	3 (13.6)
Post-treatment relapse	0	4 (18.2)

Intention to treat (missing = failure) analysis. End of treatment is week 24 or last on-treatment visit for patients who discontinued early.
RVR, rapid virologic response; cEVR, complete early virologic response; SVR_4, SVR_{12}, and SVR_{24}, sustained virologic response 4, 12 or 24 weeks post-treatment.
[1]Two patients discontinued from the study before completion of follow-up. One patient received added PegIFN-α/RBV per protocol criteria and is counted as failure for SVR_4, SVR_{12}, and SVR_{24} for DAA only therapy; one patient had missing HCV RNA data for follow-up weeks 12 and 24 and is counted as failure for SVR_{12} and SVR_{24} per statistical protocol.
[2]One patient was lost to follow-up for assessment of SVR_{12} and SVR_{24}.

serum HCV RNA, and four patients relapsed at post-treatment week 4 (three patients) or 12 (one patient) after ≥18 weeks with undetectable HCV RNA. All three patients with viral breakthrough were *IL28B* genotype CT (rs12979860), compared with 6/22 ineligible/intolerant patients overall. Three patients who relapsed were *IL28B* genotype CC; one was genotype CT.

Resistance-associated polymorphisms in NS5A and/or NS3 protease were found pretreatment in 33/43 patients overall, most of whom achieved SVR. Daclatasvir and asunaprevir resistance-associated variants were detected post-failure in all seven patients with virologic failure (Table 3). The NS5A-Y93H variant pre-existed in 10/43 study patients, of which five (50%) experienced virologic failure and five (50%) achieved SVR. NS5A-L31 and NS3-D168 substitutions emerged in all failures, but were not detected pretreatment except for NS5A-L31M in one patient.

In general, patients with virologic failure had concurrent asunaprevir and daclatasvir trough concentrations below median values, but within the expected range (Fig. 4). Notably, most patients with trough concentrations below median values achieved SVR. There were no strong associations between virologic failure and pretreatment parameters that included gender, age, baseline HCV RNA level, *IL28B* genotype, reason for PegIFN-

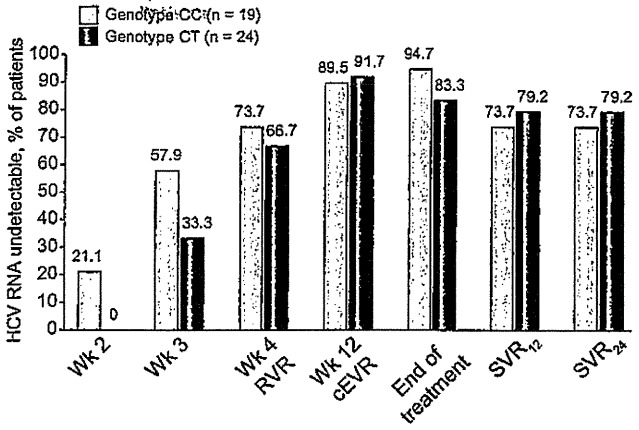

Fig. 2. Outcomes by *IL28B* genotype. Virologic outcomes at milestone time points are shown for the overall population by *IL28B* (rs12979860) genotype. End of treatment is week 24 or the last on-treatment visit for patients who discontinued early. RVR, rapid virologic response; cEVR, complete early virologic response; SVR_{12} and SVR_{24}, sustained virologic response 12 or 24 weeks post-treatment.

α/RBV ineligibility, and fibrosis stage. Adherence to treatment, assessed by pill counts at study visits, was high in six of the seven patients with virologic failure.

Safety

The most frequently reported adverse events were generally mild headache, nasopharyngitis, aminotransferase elevations, and diarrhea (Table 4). The most frequent grade 3 or 4 laboratory abnormalities were serum aminotransferase elevations. There were six serious adverse events in five patients, including grade 2/3 pyrexia (three patients), grade 2 exacerbation of hypochondriasis, and grade 2 gastroenteritis (unrelated to study drugs) with grade 4 hyperbilirubinemia (described in detail previously)

Table 3. Resistance-associated polymorphisms in patients with virologic failure.

Patient			NS5A				NS3	
			L31	Q54	P58	Y93	Q80	D168
Viral breakthrough	1	Baseline	L/M			Y/H		
		Post-VBT	M		A	H		A
	2	Baseline		Y		Y/H	L	
		Post-VBT	M	Y		H		V
	3	Baseline		Y		H		
		Post-VBT	M	Y		H		V
Post-treatment relapse	4	Baseline			P/S	Y/H		
		Post-relapse	M			H		A
	5	Baseline			L			
		Post-relapse	M		L	H		V/D
	6	Baseline						
		Post-relapse	V			H		V
	7	Baseline				H		
		Post-relapse	V/M			H		V

[7]. All three pyrexia events resolved after 4–10 days with continued study treatment; the hypochondriasis persisted for approximately six months and resolved after completion of study treatment. In the patient who discontinued with hyperbilirubinemia, bilirubin normalized four weeks post-treatment [7]. Serum aminotransferases normalized by four weeks post-treatment in the two patients who discontinued for elevations.

Discussion

High rates of SVR_{24} were achieved after 24 weeks of dual oral DAA therapy in null responders and PegIFN-α/RBV ineligible or

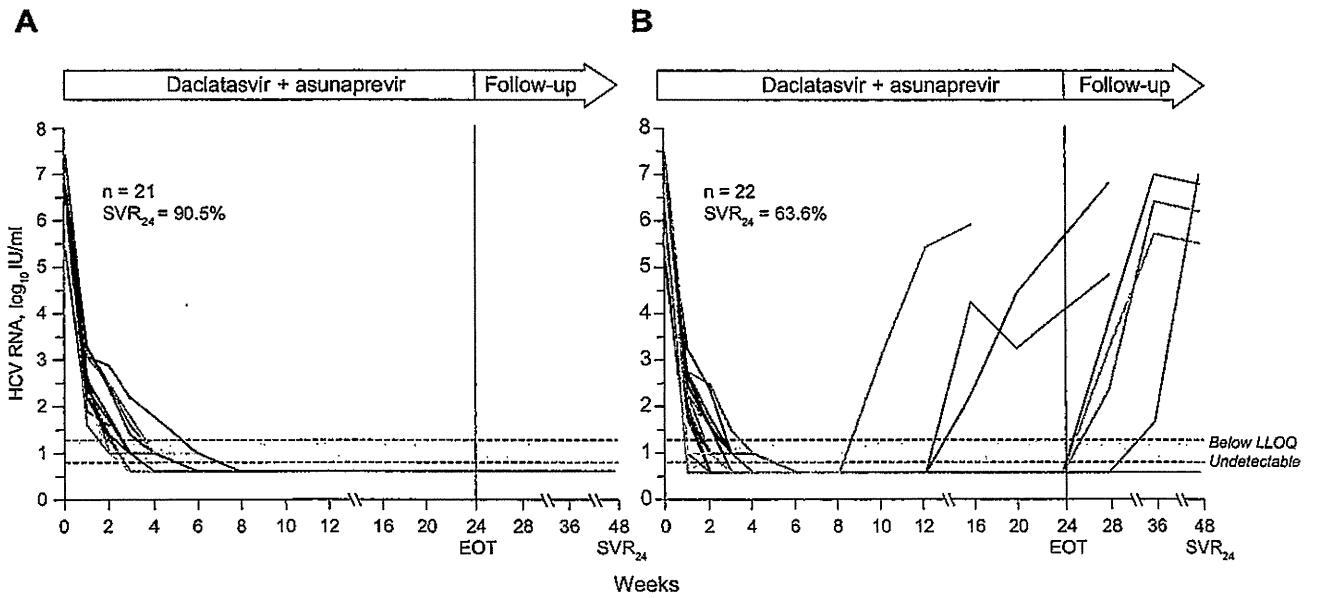

Fig. 3. HCV RNA levels, individual patients. Serum HCV RNA levels over time are shown for each patient. (A) Null responders; (B) ineligible/intolerant patients. EOT, end of treatment; SVR_{24}, sustained virologic response 24 weeks post-treatment; LLOQ, lower limit of quantitation = 15 IU/ml.

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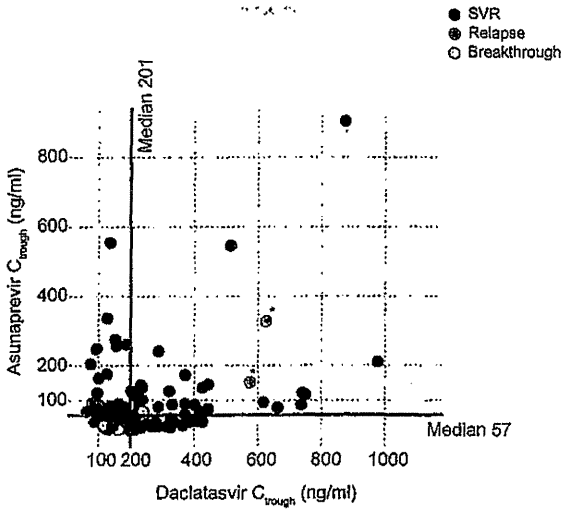

Fig. 4. Daclatasvir and asunaprevir trough plasma concentrations. Available trough plasma concentrations of asunaprevir and daclatasvir for individual patients are plotted and color-coded according to each patient's virologic outcome. Multiple determinations are shown for some patients. *Indicates values from a single patient with documented non-compliance.

Table 4. Most frequent adverse events and laboratory abnormalities.

Event, n (%)		Null responders (n = 21)	Ineligible/ intolerant (n = 22)
Adverse events occurring in ≥3 patients in either group	Headache	8 (38)	6 (27)
	Nasopharyngitis	6 (29)	8 (36)
	ALT increase	6 (29)	6 (27)
	Diarrhea	9 (43)	2 (9)
	AST increase	6 (29)	4 (18)
	Pyrexia	3 (14)	5 (23)
	Eosinophilia	1 (5)	4 (18)
	Abdominal discomfort	3 (14)	2 (9)
	Malaise	2 (10)	3 (14)
	Constipation	2 (10)	3 (14)
	Back pain	3 (14)	1 (5)
	Decreased appetite	0	3 (14)
Grade 3 or 4 lab abnormalities	ALT	2 (10)	2 (9)
	AST	1 (5)	2 (9)
	Lymphocytes	2 (10)	1 (5)
	Phosphorus	1 (5)	1 (5)
	Bilirubin, total	1 (5)	0
	Leukocytes	1 (5)	0

intolerant patients, representing two populations that are particularly difficult to treat due to limited therapeutic options. SVR rates were comparable at post-treatment weeks 4, 12, and 24; only one relapse occurred more than 4 weeks post-treatment. The 90.5% SVR rate in null responders is substantially higher than the generally poor response to PegIFN-α/RBV retreatment and the 37% SVR rate reported for genotype 1b null responders treated with PegIFN-α/RBV and telaprevir [4,19]. Therefore, therapy of this population with daclatasvir and asunaprevir appeared to overcome the poor interferon responsiveness, which may be less relevant to the efficacy of this DAA-only regimen. The SVR rate of 63.6% in ineligible/intolerant patients, although lower than results in null responders, is the first demonstration of a potentially effective treatment for these patients who currently have no therapeutic options. High SVR rates in both populations were achieved despite multiple adverse predictors of response to PegIFN-α/RBV therapy, including older age, high viral load, and a high proportion of *IL28B* genotype CT in the null responders.

Detectable HCV RNA was cleared rapidly; viral suppression was greater at all time points compared to reported results with PegIFN-α/RBV combined with telaprevir or TMC435 in genotype 1 null responders [4,20]. The slightly greater early viral suppression in ineligible/intolerant patients may reflect the higher frequency of *IL28B* CC genotype in this group. In the overall population, early virologic response was greater in patients with CC genotype, although this difference disappeared by week 12. Potentially, CC genotype may increase early viral suppression by increasing responsiveness to endogenous interferons that are released as a result of the rapid antiviral activity of the dual DAA therapy, allowing reversal of HCV-induced immunosuppression [21].

These results in patients with HCV genotype 1b differ from those reported for genotype 1a. In a similar study of US/European null responders, 2/9 patients with genotype 1a achieved SVR with daclatasvir + asunaprevir dual therapy, compared with 10/10 patients with genotype 1a who received quadruple therapy combining daclatasvir and asunaprevir with PegIFN-α/RBV [8]. This difference suggests that viral genotype can influence responses to DAA regimens, and outcomes can be optimized by individualized therapy that considers viral genotype.

The two populations included in this study represent substantial numbers of patients worldwide. Approximately 10% of HCV genotype 1-infected patients receiving PegIFN-α/RBV have a null response [22]. The cumulative prevalence of PegIFN-α/RBV null responders and the frequent failure of retreatment with current regimens, together suggest that a large population of null responders is awaiting improved therapies. The population of PegIFN-α/RBV ineligible or intolerant patients has not been extensively studied but may be substantial. In the IDEAL study, 23.2% of the 4469 patients screened were considered ineligible for PegIFN-α/RBV therapy; of these, 30.3% had hematologic or psychiatric conditions that may not preclude DAA-only regimens [23]. In registration trials, 9.7–14% of patients receiving PegIFN-α/RBV discontinued study treatment due to intolerance [24,25]. Moreover, these clinical trial data are likely to underestimate the true size of the ineligible and intolerant populations in community practice.

Virologic failures occurred relatively late in therapy after extended periods with undetectable HCV RNA. All seven patients with virologic failure had emergent NS5A and NS3 mutations that together confer high-level resistance to both daclatasvir and asunaprevir *in vitro* [11,12]. Pretreatment, NS5A-Y93H was detected in five of the seven patients with virologic failure and in five additional patients who achieved SVR, suggesting that pre-existing Y93H is loosely associated with virologic failure but is not an absolute predictor. Pharmacokinetics may also have contributed; nearly all patients with virologic failure had trough plasma concentrations of daclatasvir and asunaprevir below their respective median values. However, SVR was achieved by most patients with trough drug levels below the median, and by

several patients who discontinued study treatment after 2–16 weeks. Thus, the relationship of drug exposure to virologic outcome remains uncertain; further study is needed to define on-treatment predictors of outcome and the optimal duration of therapy.

Current data do not fully explain the observed differences in rates of virologic failure and SVR, between the two study populations. *IL28B* genotype was the primary difference between the two populations pretreatment. All three breakthroughs occurred in ineligible/intolerant patients with the unfavorable *IL28B* CT genotype; however, null responders had no breakthroughs, despite a much higher frequency of this genotype. Differing proportions of patients with concurrent pre-existing resistance-associated polymorphisms and low plasma drug concentrations may have contributed to differing rates of virologic failure between the two populations. Analysis of baseline parameters failed to identify other factors that may have influenced outcomes. However, these analyses were limited by the relatively small study population and may have been confounded by unreported non-adherence or baseline parameters not quantified absolutely, such as the stage of liver fibrosis. This issue requires further study in larger populations to confirm the apparent difference in outcomes and to identify factors predictive of virologic failure.

The adverse event profile of the study regimen was generally more favorable than that typically observed with PegIFN-α/RBV-containing regimens [26]. There were no significant hematologic or psychiatric abnormalities; the most common adverse events were non-specific in nature and generally mild to moderate in intensity. Mild diarrhea was experienced by 26% of study patients, consistent with previous studies of asunaprevir and other drugs of this class [4,6,14]. The four observed grade 3/4 ALT elevations resolved with continued therapy or after discontinuation and were not associated with significant clinical events. A role for study drugs in the reported serious adverse events cannot be ruled out except for gastroenteritis; however, four of the six events resolved spontaneously with continued treatment. The case of hyperbilirubinemia with gastroenteritis was complicated by multiple confounding factors, and the contribution of study drugs is uncertain [7].

In conclusion, dual oral therapy with daclatasvir and asunaprevir elicited rapid clearance of detectable HCV RNA and achieved high rates of SVR in two difficult-to-treat patient populations. These results confirm initial findings that HCV genotype 1b infections can be cured with daclatasvir combined with asunaprevir, without PegIFN-α/RBV [7,8]. Thus, this regimen has potential to offer effective treatment to null responders who have previously shown little or no response to PegIFN-α/RBV, and to PegIFN-α/RBV ineligible/intolerant patients who have no current treatment options. Further research will assess the benefits of this and other DAA combinations in larger and more diverse patient populations, but the promise of all oral and well-tolerated HCV therapy is on the horizon.

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Conflicts of interest

K Chayama has received research grants and consulting fees from Bristol-Myers Squibb, Dainippon Sumitomo Pharma, Mitsubishi Tanabe Pharma, Daiichi Sankyo, Toray Industries, Otsuka Pharmaceutical Company, and GlaxoSmithKline KK. Hiroki Ishikawa, Hideaki Watanabe, Wenhua Hu, Timothy Eley, Fiona McPhee, and Eric Hughes are employees of Bristol-Myers Squibb. All other authors have no conflicts to report.

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

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See Editorial, pages 643–645

Background & 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 PegIFN-α/RBV [n = 21] or PegIFN-α/RBV 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.

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Keywords: Asunaprevir; Daclatasvir; Drug resistance; Direct-acting antivirals; Hepatitis C; Peginterferon-sparing.

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Abbreviations: DAA, direct-acting antiviral; HCV, hepatitis C virus; SVR, sustained virologic response; GT, genotype; PegIFN-α/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.

Introduction

The introduction of direct-acting antivirals (DAAs) 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 (PegIFN-α/RBV), treatment with the recently approved protease inhibitors boceprevir or telaprevir results in SVR rates of around 70–75% in treatment-naive patients [1,2]. Despite these improvements, SVR rates vary by genotype and remain suboptimal in some patients, such as null responders to PegIFN-α/RBV [3], and patients for whom PegIFN-α/RBV is poorly tolerated or medically contraindicated. Furthermore, PegIFN-α/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, pruritus, 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 PegIFN-α/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 PegIFN-α/RBV [9]. Clinical interest is increasingly focusing on exploring DAA-only regimens without PegIFN-α/RBV, whose potential benefits might include better tolerability and compliance, and a reduced duration of therapy. One recent PegIFN-α/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 whom 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, PegIFN-α/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 PegIFN-α/RBV null responders (<2 $\log_{10}$ decline in plasma HCV RNA after 12 weeks) and (b) 22 patients who discontinued previous PegIFN-α/RBV within 12 weeks for intolerance or were considered medically poor candidates for PegIFN-α/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 60 mg once daily with ASV 200 mg twice daily for 24 weeks, with a further 48 weeks of post-treatment follow-up. ASV dosing in the expanded study was reduced from the 600 mg 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 GT1 infection ≥6 months and HCV RNA ≥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; co-infection 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 the 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 PegIFN-α/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 600 mg (Fig. 1A) or ASV 200 mg (Fig. 1B). Mean reduction in HCV RNA at week 1 was comparable for both groups (−4.4 *vs.* −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 (two- to three-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 *vs.* −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

Table 1. Baseline viral and host characteristics among genotype-1b null responders and their virologic outcome.

Patient	*IL28B* GT	HCV RNA, log_{10} IU/ml	NS5A polymorphism(s)[a]	NS3 polymorphism(s)[a]	Virologic outcome
P-1	CT	7.2	Q54H/Q-Q62Q/E-Y93H/Y	T54S-Q80L	SVR
P-2	CT	7.0		Q80L-V170I/M	SVR
P-3	CT	7.4	Q54H		SVR
P-4	CT	6.7	R30Q		SVR
P-5	CT	7.0	L31L/M-P58P/S		SVR
P-6	CC	5.3	P58P/T-Q62E		D/C at Wk2 due to SAE[b]
P-7	CC	7.2		S122S/G	SVR
P-8	CT	7.0	Q54H	Q80L	SVR
P-9	CT	7.1	Q54H-Y93H/Y	S122N	SVR
P-10	CT	6.4	L28M-R30Q		SVR
P-11	CT	6.8			D/C at Wk12 due to AE; SVR
P-12	CT	6.4	Q54H-P58S-Q62E		SVR
P-13	CT	7.4	Q54H		D/C at Wk6; PDR not achieved[c]
P-14	CT	6.5			SVR
P-15	CT	6.3	R30Q/R-Q62Q/R		SVR
P-16	CT	6.6	Q54H		SVR
P-17	CT	6.6	Q54H-Q62E		SVR
P-18	CT	6.9	Q54Y	Q80L	SVR
P-19	CT	6.6	Q54H-Y93H	N77A	SVR
P-20	CT	7.0	R30Q	S122G	SVR
P-21	CC	6.6	Q54L		SVR

[a]All NS3 and NS5A amino acids were examined with focus on polymorphisms at positions known to be associated with resistance to NS3 protease inhibitors (36, 43, 54, 55, 77, 78, 79, 80, 122, 123, 138, 155, 156, 158, 168, 170, 175) and NS5A inhibitors (21, 23, 24, 28, 30, 31, 32, 54, 58, 62, 92, 93). When a mixture of substitutions is indicated, the most predominant is identified first.
[b]HCV RNA undetectable at post-treatment week 24.
[c]PegIFN-α/RBV added; HCV RNA undetectable at post-treatment week 24 following 52 weeks of therapy.
AE, adverse event; D/C, discontinued; GT, genotype; HCV, hepatitis C virus; PDR, protocol-defined response; SAE, serious adverse event; SVR, sustained virologic response; Wk, week.

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 *vs.* dose and polymorphisms associated with resistance revealed no differences. Among null responders who received ASV 600 mg, mean HCV RNA declines at week 1 for those with *vs.* without RAVs were −4.6 *vs.* −4.3 log_{10} IU/ml, which were similar to the week 1 declines among those who received ASV 200 mg (−4.5 log_{10} IU/ml with RAVs [one patient] *vs.* −4.3 log_{10}).

Baseline HCV RNA levels did not have an impact on 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 PegIFN-α/RBV ineligible patients than in null responders. Undetectable HCV RNA at week 4 was observed in 86% of the ineligible group *vs.* 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 (7105-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 *vs.* −4.74 [0.58] log_{10} IU/ml), but subsequently achieved SVR with only 16 weeks of 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