Japanese patients with CHC [23–25], indicate that the frequency of the rs738409 GG genotype seems to be higher in Japanese patients (21.0–24.0 %) than in European patients (approximately 10 %) with CHC [17, 18]. If so, Japanese patients may be at higher risk for rapid progression of CHC than European patients.

Furthermore, several lines of evidence suggest the association of the rs738409 GG genotype with an increased risk of hepatocellular carcinoma in patients with CHC [17, 23, 26–28], although the association seems to be less pronounced in CHC than in alcoholic liver disease [26, 28].

Alcohol consumption is known to promote the development of steatosis and the progression of fibrosis in CHC. Müller et al. [29] found a distinct effect of the rs738409 genotype on steatosis and fibrosis in German patients with CHC according to the amount of alcohol intake; that is, while the rs738409 GG genotype was associated with steatosis only in abstainers (<30 g alcohol/day), it was associated with liver cirrhosis only in at-risk drinkers (>30 g alcohol/day). Valenti et al. [30] confirmed that the rs738409 GG genotype was associated with steatosis only in abstainers; however, they found that it was associated with cirrhosis in both abstainers and at-risk drinkers. Further studies are needed to examine the interaction between a moderate amount of alcohol intake and the rs738409 variant in CHC. In the present study, we excluded patients who consumed more than 20 g of alcohol per day to eliminate the confounding effect of alcohol on steatosis and fibrosis.

Although the rs738409 GG genotype was associated with severe necroinflammatory activity and advanced fibrosis, it was not related with a higher ALT level or a lower platelet count. The reason for this discrepancy is unknown. However, studies have shown that necroinflammatory activity grade is not well correlated with ALT levels in CHC [31, 32]. We may have failed to show the association of the GG genotype with a lower platelet count in part because the proportion of patients with advanced fibrosis (stage 3 or 4) was relatively low (26 %) and therefore the mean platelet count was not very low ($16.0 \times 10^4/\mu l$) in our patients.

Liver transplantation provides a unique opportunity to assess whether the effect of the rs738409 variant is localized in the liver or in other tissues, because transplantation creates a chimeric individual. A recent study of patients who underwent liver transplantation for hepatitis C in the United States showed that donor, but not recipient, rs738409 GG or CG genotype was associated with increased risk of fibrosis progression, retransplantation, or death after liver transplantation [33]. This finding indicated that the liver is indeed the site where the effect of the variant occurs. However, neither donor nor recipient rs738409 genotype was associated with hepatic steatosis during follow-up biopsies. These observations suggested that the rs738409 variant in the liver is responsible for fibrosis progression but not for steatosis. The rs738409 variant may influence the development of fibrosis and steatosis through different pathways.

PNPLA3 encodes a 481-amino acid protein that contains a highly conserved patatin-like domain at the N-terminal. PNPLA3 is a membrane-bound protein and is most highly expressed in the liver, followed by the skin and adipose tissue in humans [34, 35]. PNPLA3 expression is highly regulated by nutritional stimuli at both the transcriptional and posttranslational levels through the transcription factors SREBP-1c and liver X receptor [35–37]. Studies have found that levels of PNPLA3 were very low in the liver during fasting and were increased with carbohydrate feeding [35, 36].

Despite the strong clinical association of the *PNPLA3* rs738409 variant (I148 M) with liver diseases, the biochemical function of *PNPLA3* and the underlying mechanism by which the I148 M variant affects liver injury remain controversial. Some investigators have proposed that *PNPLA3* shows lipase activity and that the I148 M variant results in a loss of function [34, 38–40], while other authors have suggested that *PNPLA3* plays a role in lipid synthesis and that the I148 M variant exerts a gain-of-function effect [37, 41, 42]. Interestingly, Pirazzi et al. [40] reported that the wild-type *PNPLA3* has retinyl-palmitate lipase activity in human hepatic stellate cells, and that the lipase activity is markedly reduced in the I148 M variant. Because hepatic stellate cells are key players in fibrogenesis in chronic liver disease, *PNPLA3* may possibly be involved in the activation and transformation of hepatic stellate cells in response to hepatic injury and the development of liver fibrosis. Contrary to expectations, *PNPLA3*-deficient mice have not shown any obvious phenotype [43, 44]. However, it must be noted that there are differences in tissue-specific expression of *PNPLA3* between humans and mice [35].

Certain limitations should be considered in the interpretation of our findings. The cross-sectional study design hinders the ability to draw inferences regarding the causality of the rs738409 variant in histological liver damage. Although none of our patients had received any antiviral therapy before the liver biopsy, many of these patients had received ursodeoxycholic acid and herbal medicines. A past history of these treatments for CHC might have slightly influenced our results. A post hoc power analysis was performed using the actual sample size, based on the Chi-square test. Our study had sufficient power (more than 80 %) to detect a clinically meaningful effect size (odds ratio ≥ 2.4).

The *PNPLA3* rs738409 variant (I148 M) has now been associated with the progression of chronic liver diseases

with different etiologies, including NAFLD, alcoholic liver disease, and CHC. Moreover, the association appears to be common in different populations. Elucidation of the physiological functions of *PNPLA3* and the pathological effects of the I148 M variant will reveal the common underlying mechanisms involved in chronic liver diseases and may hopefully lead to identification of therapeutic targets for these diseases.

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Conflict of interest The authors declare that they have no conflict of interest.

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Original Article

Efficacy of telaprevir-based therapy for difficult-to-treat patients with genotype 2 chronic hepatitis C in Japan

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Aim: This study assessed the efficacy and safety of telaprevir in combination with peginterferon-α-2b (PEG IFN) and ribavirin (RBV), for Japanese difficult-to-treat patients with hepatitis C virus (HCV) genotype 2 who had not achieved sustained virological response (SVR) during prior treatment.

Methods: In total, 108 relapsed (median age, 59.0 years) and 10 non-responding (median age, 59.0 years) patients with genotype 2 HCV participated. Patients received telaprevir (750 mg, every 8 h) for 12 weeks and PEG IFN/RBV for 24 weeks.

Results: The SVR rates for relapsers and non-responders were 88.0% (95/108) and 50.0% (5/10), respectively. The SVR rates did not differ significantly between patients with rs8099917 TT and non-TT. The SVR rates for relapsers and non-responders with extended rapid viral response (eRVR) were 97.6% (82/84) and 100% (5/5), respectively. On the other hand, the SVR rates for relapsers and non-responders completing the treatment protocol were 98.4% (61/62) and 100% (5/5), respectively. The overall safety profiles of telaprevir-based regimens were similar for Japanese patients with genotype 1 and 2 HCV infection who experienced treatment failure.

Conclusion: Telaprevir, in combination with PEG IFN/RBV, provided a high SVR rate for genotype 2 HCV, difficult-to-treat patients who had not achieved SVR during prior IFN-based treatment. The eRVR had a strong influence on the cure rate of telaprevir-based therapy. In addition, the continuation of telaprevir-based treatment for up to 24 weeks was a significant predictor of SVR.

Key words: genotype 2, peginterferon, ribavirin, sustained virological response, telaprevir, treatment failure

INTRODUCTION

HEPATITIS C VIRUS (HCV) affects approximately 185 million people worldwide;[1] patients with chronic hepatitis C (CHC) eventually develop cirrhosis and hepatocellular carcinoma (HCC).[2,3] Viral clearance is associated with improvements in histological outcomes, morbidity and mortality. Six HCV genotypes and over 100 subtypes have been documented,[4] each with a distinct geographical distribution. Among the HCV genotypes, genotype 1 is the most prevalent and difficult to cure. Several protease inhibitors, including telaprevir,[5–7] boceprevir[8] and simeprevir,[9,10] are approved for the treatment of CHC. These direct-acting antivirals (DAA) combined with peginterferon (PEG IFN) and ribavirin (RBV) improve the rates of sustained virological response (SVR) in genotype 1-infected,

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treatment-naïve patients and in those previously treated with IFN-based therapy.

However, clinical data are not available regarding the efficacy of the HCV NS3/4A protease inhibitors in patients with HCV genotype 2, which predominates in the Mediterranean and Asia. In treatment-naïve patients with HCV genotype 2, a combination of PEG IFN and RBV results in SVR rates of 70–90%, although some patients respond less well.[11–14] HCV patients who fail to achieve SVR with the combination therapy remain at high risk for decompensated cirrhosis, HCC and liver-related mortality. Therefore, establishing a new regimen to increase SVR rates and shorten the treatment period for these patients is important.

Recently, sofosbuvir was approved for use with RBV for patients with HCV genotype 2 in the USA and Europe.[15,16] Sofosbuvir is a new generation DAA that can shorten treatment duration, improve tolerability and raise SVR rates, even in difficult-to-treat patients. However, sofosbuvir has not been approved in Asia, including Japan. Nonetheless, telaprevir, alone and in combination with PEG IFN and RBV, results in a rapid decrease in genotype 2 HCV RNA, with a median decline of -3.66 $\log_{10}$ IU/mL and -5.51 $\log_{10}$ IU/mL at day 15, respectively.[17] The results indicate that telaprevir-based triple therapy also may be considered as an option for difficult-to-treat patients with genotype 2 HCV.

We conducted two phase 3 studies, in Japan, to assess the efficacy and safety of telaprevir, in combination with PEG IFN-α-2b and RBV, in patients with HCV genotype 2 who experienced relapses or were non-responders to a prior IFN-based regimen.

METHODS

Study patients

BETWEEN NOVEMBER 2011 and September 2013, a total of 132 (Study-1, $n = 120$; Study-2, $n = 12$) patients were screened, and 118 (Study-1, $n = 108$; Study-2, $n = 10$) patients received at least one dose of study drug. Those HCV genotype 2 patients who relapsed or did not respond to previous IFN-based therapy were enrolled in Study-1 (Clinical Trials.gov Identifier, NCT01466192) and Study-2 (ClinicalTrials.gov Identifier, NCT01468584), respectively. Relapsers were defined as patients who had been previously treated for CHC and achieved undetectable levels of HCV RNA during IFN or PEG IFN therapy (including in combination with RBV). Non-responders were defined as patients who were similarly treated for CHC, but never achieved undetectable levels of HCV RNA during treatment. The patients were recruited at 34 sites across Japan. Eligible patients were 20–65 years of age, had CHC due to HCV genotype 2 (defined by NS5B sequence),[18] had been previously treated for CHC with IFN or PEG IFN therapy (including in combination with RBV), had a bodyweight of 40–120 kg, were hospitalized for at least 2 weeks prior to the first study drug administration, and were not pregnant and agreed to use contraception from the screening period through to 24 weeks after the last dose of study drug. Patients were excluded if they had low hemoglobin (Hb) levels (<12 g/dL), neutrophil counts (<1500/mm^3) or platelet counts (<100 000/mm^3); were positive for hepatitis B surface antigen or HIV antibodies at the screening test; had chronic renal failure or creatinine clearance of 50 mL/min or less; had a history of depression, schizophrenia or attempted suicide; or had decompensated cirrhosis, previous or current HCC or other malignancies, autoimmune hepatitis, alcoholic liver disease or hemochromatosis.

All patients provided written informed consent before participating in the study. These studies were approved by each site's institutional review board and were conducted in accordance with Good Clinical Practice and the Declaration of Helsinki.

Study design

All patients received PEG IFN (PegIntron; MSD, Tokyo, Japan) at a dose of 1.5 μg/kg per week, s.c.; RBV (Rebetol; MSD) at a dose of 600 mg/day (bodyweight, ≤60 kg), 800 mg/day (bodyweight, >60 kg to ≤80 kg) or 1000 mg/day (bodyweight, >80 kg); and telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) at a dose of 750 mg every 8 h, after food. The patients were treated with telaprevir, PEG IFN and RBV for 12 weeks, followed by PEG IFN and RBV (PEG IFN/RBV) for 12 weeks. All patients had a 24-week follow up after the last dose of study drugs to assess SVR.

Dose modification of study drugs

Ribavirin dose modification, which differed from the dosing for PEG IFN/RBV therapy, was introduced. The initial dose of RBV was reduced by 200 mg/day in patients with baseline Hb levels of less than 13 g/dL. It was also reduced by 200 mg/day in patients receiving 600 or 800 mg/day (or by 400 mg/day in those receiving 1000 mg/day) when Hb levels were less than 12 g/dL, and reduced by an additional 200 mg/day when Hb levels were less than 10 g/dL. Additionally, the dose of RBV was also reduced by 200 mg/day when Hb levels dropped by 1 g/dL or more within 1 week and the level

was less than 13 g/dL. Telaprevir was withdrawn and PEG IFN/RBV was withdrawn or interrupted if Hb levels fell to less than 8.5 g/dL. Dose modification and interruption of telaprevir was not allowed; it was withdrawn if serious adverse events (AE) appeared.

Stopping rules

Patients were discontinued from the study at any time if the investigator determined that it was not in the patient's interest to continue, or if a patient withdrew from the study. The study drugs were discontinued if a patient's Hb level (<8.5 g/dL), white blood cell count (<1000/mm^3), neutrophil count (<500/mm^3) or platelet count (<50 000/mm^3) fell to below the indicated values.

Efficacy assessments

Serum HCV RNA levels were measured using the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan), with a linear dynamic range of 1.2–7.8 $\log_{10}$ IU/mL; an RNA level of less than 1.2 $\log_{10}$ IU/mL was considered undetectable. Measurements were obtained 4 weeks before dosing (screening period); on days 1 (pre-dose), 2 and 3, and at weeks 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 (treatment period); and at weeks 2, 4, 8, 12, 16, 20 and 24 in the follow-up period. The primary end-point was an SVR, defined as an undetectable HCV RNA level 24 weeks after treatment completion. In cases of study drug discontinuation, the day of discontinuation was regarded as the day of completion of treatment. The definitions of relapse, breakthrough and non-response were based on the American Association for the Study of Liver Diseases Guidelines: relapse, undetectable serum HCV RNA levels at the end of treatment, followed by reappearance of serum HCV RNA during the follow-up period; breakthrough, undetectable serum HCV RNA during treatment, followed by the reappearance of serum HCV RNA during the treatment period; and non-response, detectable serum HCV RNA levels were observed continuously during the treatment period.[19]

Sequence analysis at HCV NS3 protease domain

Hepatitis C virus RNA was isolated from serum samples collected for the measurement of HCV RNA levels. The DNA fragment containing the 543-base (181 amino acids) NS3 protease domain was amplified using nested reverse transcription polymerase chain reaction and cloned. At least 39 clones per specimen were bidirectionally sequenced. The limit of detection of the HCV RNA for the sequencing analysis was 3.0 $\log_{10}$ IU/mL.

Safety assessments

All AE were recorded at each visit and coded using MedDRA/J version 16.0 (International Conference on Harmonization, Geneva, Switzerland). Measurements for hematological and chemical laboratory data were obtained at approximately the same times as defined for efficacy assessments (above). AE, hematological and chemical laboratory data, and vital signs were assessed and summarized. Rash severity was categorized into four grades.

Determination of interleukin (IL) 28B and inosine triphosphate pyrophosphatase (ITPA) genotype

Patient IL 28B (rs8099917 and rs12979860) and ITPA (rs1127354) were genotyped by the invader assay, as described elsewhere.[20–22]

Statistical analysis

The SVR rates and other virological response rates were evaluated in the full analysis set. Categorical variables for relapsers were compared using Fisher's exact test. Statistical analyses were performed using the statistical software SAS version 9.2 (SAS Institute, Cary, NC, USA), and $P < 0.05$ was considered statistically significant.

RESULTS

Study patients

THE BASELINE CHARACTERISTICS of the study patients are shown in Table 1. Patients over 60 years of age comprised 48.1% (52 of 108) and 40.0% (4 of 10) of the Study-1 (relapsers) and Study-2 (non-responders) patients, respectively; 91 of 108 patients in the relapser group and nine of 10 patients in the non-responder group were previously treated with PEG IFN/RBV.

Efficacy

Figure 1 compares the time courses of the undetectable HCV RNA rates between relapsed and non-response patients. The SVR rates for relapsed and non-response patients were 88.0% and 50.0%, respectively (Fig. 2). The rapid viral response (RVR) rates and the end-of-treatment response (ETR) rates were 87.0% and 94.4% for the relapsers, and 70.0% and 60.0% for the non-responders, respectively. The SVR rates in patients

Table 1 Baseline characteristics of patients

	Study-1 (relapsers) $n = 108$	Study-2 (non-responders) $n = 10$
Male, *n* (%)	53 (49.1)	8 (80.0)
Age (years), median (range)	59.0 (29–65)	59.0 (46–65)
Weight (kg), median (range)	60.45 (40.8–111.1)	67.15 (53.3–103.2)
BMI† (kg/m^2), median (range)	23.47 (17.6–33.6)	24.12 (19.9–33.7)
Hemoglobin (g/dL), median (range)	14.20 (12.0–18.0)	14.95 (13.6–15.9)
White blood cells (/mm^3), median (range)	5100.0 (3100–11 000)	5355.0 (3200–7300)
Platelets (×10^4/mm^3), median (range)	18.05 (9.4–30.1)	14.50 (11.8–22.0)
ALT‡ (U/L), median (range)	26.0 (10–355)	34.0 (15–141)
HCV RNA ($\log_{10}$ IU/mL), median (range)	6.48 (3.9–7.5)	6.28 (3.6–6.7)
HCV genotypes, *n* (%)		
2a	63 (58.3)	8 (80.0)
2b	45 (41.7)	2 (20.0)
rs8099917 (TT/TG/GG), *n*‡	74/29/3	8/2/0
rs12979860 (CC/CT/TT), *n*‡	73/30/3	8/2/0
rs1127354 (CC/CA/AA), *n*‡	87/18/1	9/1/0
History of IFN-based therapy, *n* (%)		
IFN monotherapy	7 (6.5)	1 (10.0)
IFN/RBV	4 (3.7)	0 (0.0)
PEG IFN monotherapy	6 (5.6)	0 (0.0)
PEG IFN/RBV	91 (84.3)	9 (90.0)

†Body mass index (BMI) is the weight in kilograms divided by the square of the height in meters.
‡$n = 106$.
ALT, alanine aminotransferase; HCV, hepatitis C virus; IFN, interferon; PEG IFN, peginterferon; RBV, ribavirin.

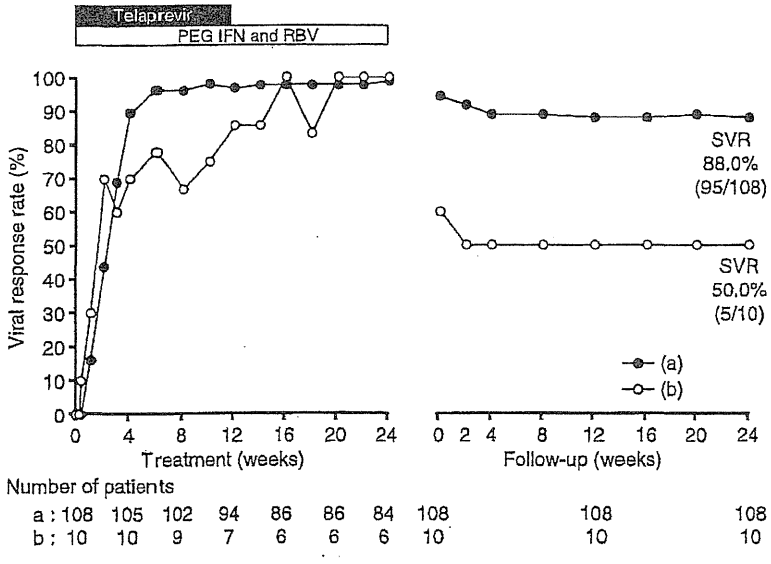

Figure 1 Time courses of undetectable HCV RNA rates. (a) Study-1 (relapsers). (b) Study-2 (non-responders). HCV, hepatitis C virus; PEG IFN, peginterferon-α-2b; RBV, ribavirin; SVR, sustained virological response.

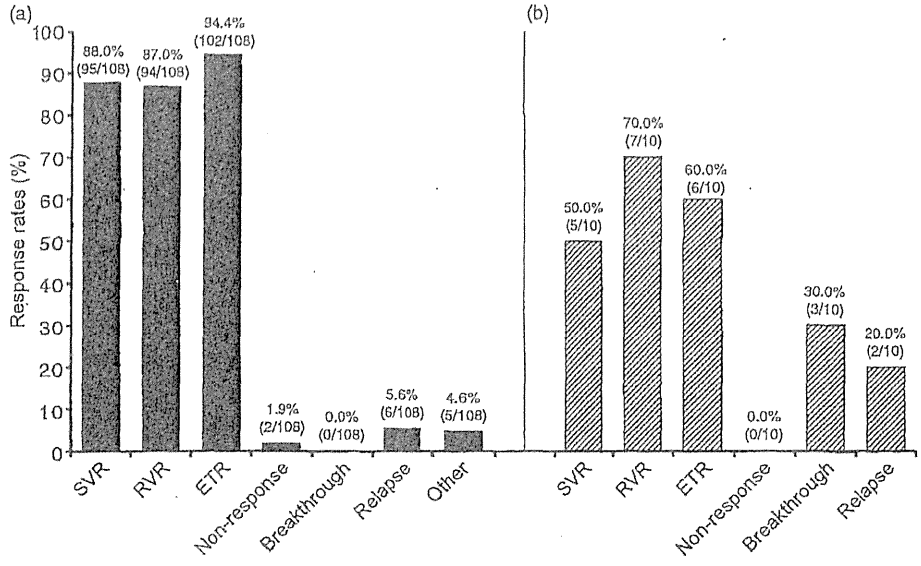

Figure 2 Response rates of patients with virological response. (a) Study-1 (relapsers). (b) Study-2 (non-responders). Number of patients achieving SVR in each subgroup/*n* (%). †Two patients had stopped all study drugs within a week. Two patients dropped out. One patient did not attend the last visit. ETR, end-of-treatment response; RVR, rapid viral response; SVR, sustained virological response.

previously treated with PEG IFN/RBV were 86.8% (79/91) and 55.6% (5/9), respectively. Table 2 shows that the SVR rate in older relapsers tended to be lower than in younger patients, but the difference in the SVR rates (≤59 vs ≥60 years, $P = 0.3805$) were not significant. Similarly, there were no differences in the SVR rates according to sex, baseline HCV RNA level (<7.0 vs ≥7.0 $\log_{10}$ IU/mL), HCV subtype (2a vs 2b) or IL 28B single-nucleotide polymorphism (rs8099917; TT vs non-TT). The SVR rates in patients achieving RVR and extended RVR (eRVR) were 93.6% and 97.6% for relapsers, and 71.4% and 100.0% for non-responders, respectively. The SVR rate in patients achieving RVR and/or eRVR was significantly higher than in patients not achieving those virological responses ($P < 0.001$). On the other hand, the SVR rates among those discontinuing the study drugs early were significantly lower than in those completing the treatment protocol ($P < 0.001$). Among the non-responders, the SVR rate for those discontinuing any study drug was 0.0%. One of the 13 non-SVR patients who relapsed during a prior IFN-based regimen had R155K and A156S as the predominant HCV clones during the telaprevir-based triple treatment. On the other hand, three of the five non-SVR non-responders had T54A and A156S/T/V as the predominant clones at the time of viral breakthrough. Figure 3 shows the SVR rates according to adherence to the study drug doses for relapsers and non-responders. Relapsers who received 60% or more of the prescribed doses of telaprevir (96.5% vs 54.5%, $P < 0.001$) and PEG IFN (96.5% vs 56.5%, $P < 0.001$), and 20% or more of the prescribed dose of RBV (94.7% vs 38.5%, $P < 0.001$) had higher SVR rates than did the less adherent patients, respectively. On the other hand, non-responders who received 80% or more of the prescribed doses of telaprevir (71.4% vs 0.0%) and PEG IFN (80.0% vs 20.0%), and 60% or more of the prescribed dose of RBV (100% vs 28.6%) had higher SVR rates than did the less adherent patients, respectively. This study demonstrated that differences in adherence–response relationships were observed in genotype 2 patients among relapsers and non-responders.

Safety

The AE experienced by at least 15% of the patients in each clinical trial are listed in Table 3. Because the AE profiles were similar between Study-1 and Study-2, only the pooled analysis of both studies is reported. Most of the AE were mild or moderate; serious AE were reported

Table 2 Sustained virological response rates, stratified by demographics, undetectable hepatitis C virus RNA and study drug discontinuation

	Study-1 (relapsers) $n = 108$	Study-2 (non-responders) $n = 10$
Sex, *n/n* (%)		
Male	46/53 (86.8)	4/8 (50.0)
Female	49/55 (89.1)	1/2 (50.0)
Age, *n/n* (%)		
≤59 years	51/56 (91.1)	3/6 (50.0)
≥60 years	44/52 (84.6)	2/4 (50.0)
HCV RNA (log_{10} IU/mL), *n/n* (%)		
≥7.0	15/19 (78.9)	–
<7.0	80/89 (89.9)	5/10 (50.0)
HCV genotypes, *n/n* (%)		
2a	57/63 (90.5)	4/8 (50.0)
2b	38/45 (84.4)	1/2 (50.0)
IL 28B (rs8099917), *n/n* (%)		
TT	66/74 (89.2)	4/8 (50.0)
Non-TT	28/32 (87.5)	1/2 (50.0)
Undetectable, *n/n* (%)		
RVR	88/94 (93.6)	5/7 (71.4)
Non-RVR	7/14 (50.0)	0/3 (0.0)
eRVR	82/84 (97.6)	5/5 (100.0)
Non-eRVR	13/24 (54.2)	0/5 (0.0)
Discontinuation of study drug, *n/n* (%)		
No discontinuation	61/62 (98.4)	5/5 (100)
Telaprevir only	21/23 (91.3)	0/1 (0.0)
All study drugs	13/23 (56.5)	0/4 (0.0)

Undetectable was defined as <1.2 log_{10} IU/mL. Sustained virological response was defined as undetectable hepatitis C virus RNA, 24 weeks after treatment completion.
eRVR, extended rapid viral response; HCV, hepatitis C virus, IL, interleukin; RVR, rapid viral response.

in 10.2% (12/118) of patients. The proportion of patients discontinuing all study drugs due to AE was 17.8% (21/118). The most frequent AE leading to discontinuation was anemia. Discontinuations of all study drugs due to anemia, malaise and renal failure were 11.9% (14/118), 2.5% (3/118) and 1.7% (2/118), respectively. AE related to skin disorders were observed in 89.0% (105/118) of the patients. The skin AE reported by more than 10% of the patients were rash (44/118, 37.3%), injection site erythema (33/118, 28.0%), injection site reactions (30/118, 25.4%) and drug eruptions (27/118, 22.9%). Most of the skin disorders were controlled with antihistamines and/or steroid ointments. Grade 3 (severe) skin disorders were reported in seven (5.9%) patients. Discontinuation of all study drugs due to skin disorders occurred in one patient (0.8%); Grade 4 skin disorders were not observed.

DISCUSSION

GENOTYPE 2 ACCOUNTS for approximately 30% of chronic HCV infections in Japan,[23] and the SVR rates among treatment-naïve patients are quite high.[11–14] Thus, the number of difficult-to-treat genotype 2 patients is small, as reflected by the limited number of patients (108 relapsers and 10 non-responders) participating in this prospective, multicenter, confirmative study. Because retreatment with PEG IFN/RBV for patients not responding to initial PEG IFN/RBV therapy is not recommended,[24] a control group was not included in the present clinical trials due to ethical concerns.

The SVR rate in this study was 88.0% among patients who had previously experienced a relapse and 50.0% among patients previously non-responsive to treatment. The overall SVR rates were similar to those for patients who had previously received PEG IFN/RBV (86.8% for

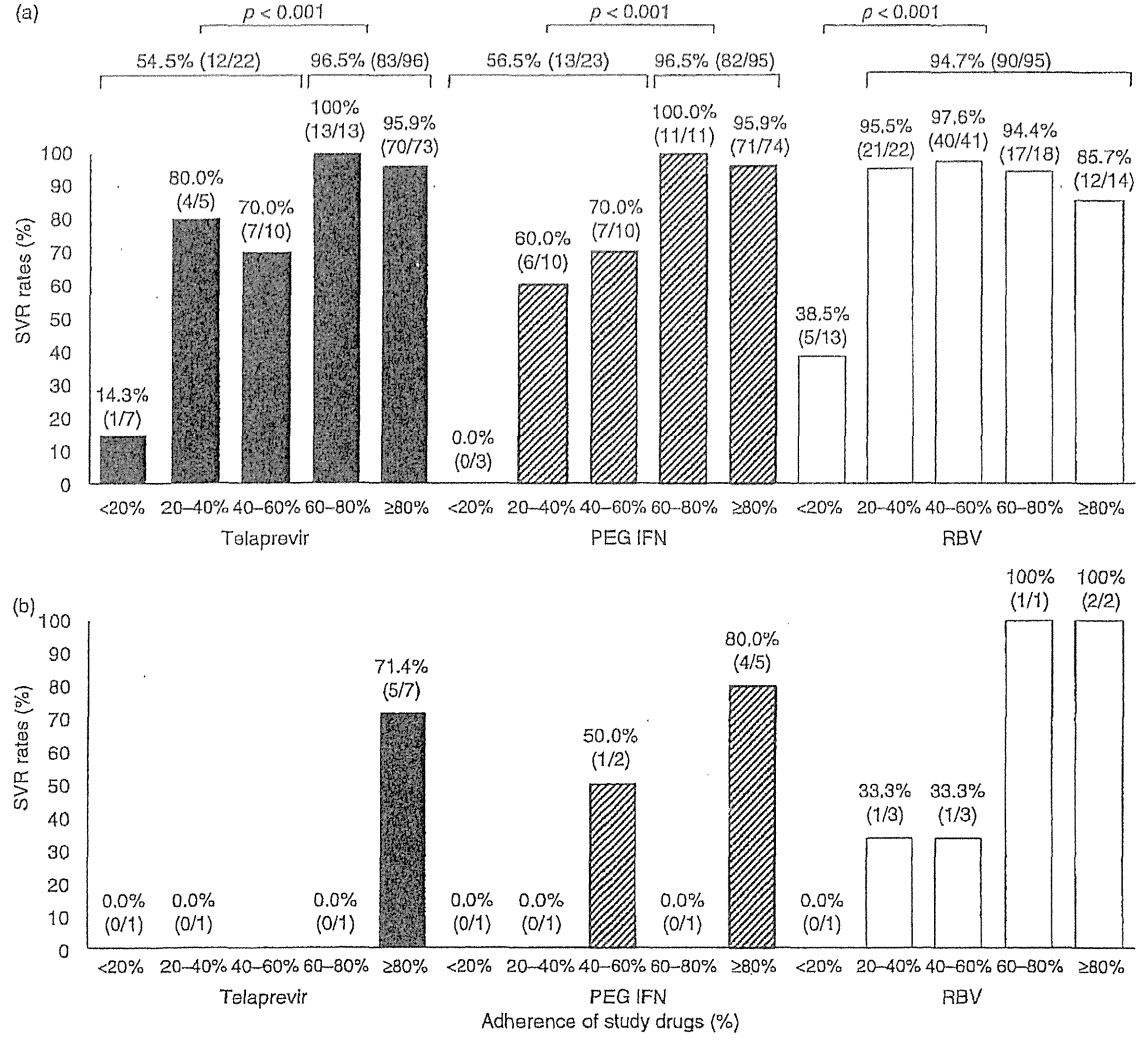

Figure 3 Sustained virological response rates according to adherence to study drug dosages. (a) Study-1 (relapsers). (b) Study-2 (non-responders). SVR, sustained virological response.

prior relapse and 55.6% for prior non-response). Subgroup analyses did not reveal significant differences in SVR rates based on sex (male or female), age (≤59 or ≥60 years), baseline HCV RNA levels (≥7.0 or <7.0 $\log_{10}$ IU/mL), subtype (2a or 2b), or IL 28B genotype (rs8099917, TT or non-TT). Previous studies also showed that the rs8099917 genotype did not influence SVR rates among Asian patients with genotype 2 HCV treated with PEG IFN/RBV.[25] On the other hand, Kawaoka *et al.* reported that the rs8099917 genotype is a significant predictor of SVR in Japanese genotype 2 patients.[26] Thus, the IL 28B genotype may have limited utility in guiding the use of telaprevir-based regimens for difficult-to-treat patients with genotype 2.

The SVR rates in patients achieving RVR and eRVR were 93.6% and 97.6% for relapsers, and 71.4% and 100.0% for non-responders, respectively. Because of the emergence of telaprevir-resistant variants, the SVR rate among patients achieving eRVR was higher than in patients achieving RVR. An eRVR was associated with an SVR rate of more than 97% for difficult-to-treat Japanese patients with genotype 2 HCV, suggesting that eRVR was

Table 3 Most common adverse events

MedDRA/J (ver. 16.0) Preferred term	Study-1 (relapsers), $n = 108$, n (%)		Study-2 (non-responders), $n = 10$, n (%)		Total, $n = 118$, n (%)	
Anemia	86	(79.6)	7	(70.0)	93	(78.8)
Pyrexia	86	(79.6)	4	(40.0)	90	(76.3)
Malaise	72	(66.7)	4	(40.0)	76	(64.4)
White blood cell count decreased	68	(63.0)	3	(30.0)	71	(60.2)
Platelet count decreased	61	(56.5)	4	(40.0)	65	(55.1)
Decreased appetite	56	(51.9)	4	(40.0)	60	(50.8)
Headache	48	(44.4)	3	(30.0)	51	(43.2)
Nausea	43	(39.8)	3	(30.0)	46	(39.0)
Nasopharyngitis	42	(38.9)	3	(30.0)	45	(38.1)
Rash	42	(38.9)	2	(20.0)	44	(37.3)
Increased blood uric acid	39	(36.1)	4	(40.0)	43	(36.4)
Increased blood creatinine	40	(37.0)	0	(0.0)	40	(33.9)
Dysgeusia	36	(33.3)	1	(10.0)	37	(31.4)
Abdominal discomfort	35	(32.4)	0	(0.0)	35	(29.7)
Alopecia	33	(30.6)	2	(20.0)	35	(29.7)
Injection site erythema	30	(27.8)	3	(30.0)	33	(28.0)
Hyperuricemia	28	(25.9)	2	(20.0)	30	(25.4)
Injection site reaction	24	(22.2)	6	(60.0)	30	(25.4)
Vomiting	28	(25.9)	1	(10.0)	29	(24.6)
Drug eruption	25	(23.1)	2	(20.0)	27	(22.9)
Arthralgia	26	(24.1)	1	(10.0)	27	(22.9)
Insomnia	25	(23.1)	1	(10.0)	26	(22.0)
Increased hyaluronic acid	23	(21.3)	3	(30.0)	26	(22.0)
Increased blood bilirubin	17	(15.7)	5	(50.0)	22	(18.6)
Diarrhea	21	(19.4)	0	(0.0)	21	(17.8)
Stomatitis	18	(16.7)	1	(10.0)	19	(16.1)
Pruritus	16	(14.8)	2	(20.0)	18	(15.3)
Increased blood triglycerides	18	(16.7)	0	(0.0)	18	(15.3)

The adverse events listed are those reported by ≥15% of patients in each study.

a strong indicator of cure rate during telaprevir-based triple therapy. Among relapsers, the SVR rate in patients discontinuing all study drugs was significantly lower than among those not discontinuing the study drugs ($P < 0.001$) or only discontinuing telaprevir ($P = 0.0165$). Similarly, the SVR rate among non-responders discontinuing any study drugs was 0.0%. Continuation of telaprevir-based regimens has been previously associated with SVR rates of more than 98%. We evaluated the impact of adherence to each study drug on SVR among relapsers and non-responders. The probability of SVR depended on proper adherence to the telaprevir-based regimen. However, the SVR rates did not depend on achieving 60% or more of telaprevir and PEG IFN doses and 20% or more of RBV dose for relapsers. The patients with genotype 1 HCV receiving telaprevir (1500 mg/day) had a lower discontinuation rate and higher 24-week adherence to RBV and PEG IFN.[27,28] However, both groups had similar SVR rates. Therefore, a lower dose (1500 mg/day) regimen is an option for relapsers with genotype 2 HCV. On the other hand, in non-responders, administration of 80% or more of telaprevir and PEG IFN doses was critical to improving the likelihood of achieving an SVR. These results suggest that adherence is an important consideration for achieving SVR among non-responders.

Amino acid substitutions in the HCV NS3 protease domain were monitored in patients with HCV RNA levels of more than 3.0 $\log_{10}$ IU/mL. None of the patients had detectable telaprevir-resistant variants at baseline. Fifteen of the 18 non-SVR patients had available NS3 sequence data; the HCV NS3 domain sequences were not analyzed in three patients because two dropped out and the final data were not obtained for one patient. Ten of the 15 patients had wild-type domains detected at every point; four patients had

R155K, T54A and A156S/T/V mutations as the predominant clones at the time of recurrent elevation of HCV RNA levels. One other patient had one R155K clone (1.9%) at week 4 of the follow-up period. Novel telaprevir-resistant mutations were not observed in this study. Similar to those of Meyer *et al.*,[29] our findings indicated that the telaprevir resistance profile of genotype 2 HCV appears to involve similar amino acid substitutions, as previously observed for genotype 1.

Adverse events in telaprevir-based triple regimens are similar between genotype 1[6] and genotype 2 (this study) patients. Serious AE rates among genotype 1 and 2 patients experiencing treatment failures were 11.3% (16/141)[6] and 10.2% (12/118), respectively. A frequent AE leading to discontinuation, for both patient populations, was anemia; discontinuation rates for all study drugs, due to anemia, in genotype 1 and 2 patients were 9.9% (14/141) and 11.9% (14/118), respectively. Grade 3 (severe) skin disorders in genotype 1 and 2 patients were reported as 6.4% (9/141) and 5.9% (7/118), respectively. Thus, the overall safety profiles of telaprevir-based regimens are similar among Japanese patients with genotype 1 and 2 HCV infections who experienced treatment failure.

In Japan, effective treatment is not available for genotype 2 patients who do not achieve SVR following PEG IFN/RBV treatment. This study demonstrated that telaprevir, in combination with PEG IFN/RBV, has a high SVR rate for genotype 2, difficult-to-treat patients not achieving SVR following a prior IFN-based regimen. The SVR rates did not differ significantly between patients with different IL 28B single nucleotide polymorphisms, but the eRVR had a strong influence on the cure rate for telaprevir-based therapy. Additionally, continuation of telaprevir-based treatment for up to 24 weeks is a significant predictor of SVR. However, the small numbers of non-responder patients in this study limits the conclusions that can be drawn; an additional larger study is essential.

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Hepatology Research

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Original Article

Simeprevir (TMC435) once daily with peginterferon-α-2b and ribavirin in patients with genotype 1 hepatitis C virus infection: The CONCERTO-4 study

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Aim: The efficacy and safety of simeprevir in combination with peginterferon-α-2b and ribavirin (PEG IFN-α-2b/RBV) were investigated in patients infected with hepatitis C virus (HCV) genotype 1 who were treatment-naïve or had previously received interferon (IFN)-based therapy.

Methods: CONCERTO-4 (NCT01366638) was an open-label, non-comparative, multicenter study of once-daily simeprevir (TMC435) 100 mg in combination with PEG IFN-α-2b/RBV in treatment-naïve and -experienced patients (prior relapsers or non-responders to IFN-based therapy) with chronic HCV genotype 1 infection. Twelve-week combination treatment was followed by 24/48-week response-guided PEG IFN-α-2b/RBV therapy for treatment-naïve patients and prior relapsers, and 48-week PEG IFN-α-2b/RBV therapy for prior non-responders. Patients were followed for 72 weeks after treatment initiation. The proportions of patients with sustained viral response (SVR; undetectable HCV RNA) at treatment end and 12 weeks after the last treatment (SVR12) were among the major efficacy end-points. Safety, including adverse events (AE), was monitored.

Results: Of the 79 patients treated, the proportion achieving SVR12 was highest among treatment-naïve patients (91.7%) and prior relapsers (100%) versus 38.5% of prior non-responders. All treatment-naïve patients and prior non-responders who achieved SVR12 also achieved SVR at treatment end and 24 weeks after last dose; 96.6% of prior relapsers achieved both end-points. Most AE were of grade 1 or 2 severity. Grade 3 AE occurred in 17 patients, most frequently neutropenia (6.3%).

Conclusion: Simeprevir combined with PEG IFN-α-2b/RBV was effective in patients infected with HCV genotype 1, both for initial treatment of naïve patients and for retreatment of patients in whom previous IFN-based therapy had failed.

Key words: chronic hepatitis C, direct-acting antiviral, protease inhibitor, simeprevir (TMC435), sustained virologic response

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INTRODUCTION

APPROXIMATELY 1.5–2 MILLION Japanese people are infected with hepatitis C virus (HCV), with the majority being infected with HCV genotype 1b.[1] Chronic infection with HCV is a major cause of liver disease,[2] and is estimated to account for more than 70% of hepatocellular carcinoma cases, one of the most common causes of cancer death in Japan.[3]

Combination therapy with weekly peginterferon-α (PEG IFN-α) injections and twice-daily oral ribavirin (RBV) for 48–72 weeks has been standard care for HCV genotype 1 infection for many years.[4–6] However, treatment discontinuations and dose reductions are common owing to the wide range of adverse events (AE) associated with PEG IFN-α/RBV therapy, including influenza-like symptoms, anemia and depression.[7–9]

Novel direct-acting antiviral agents, including protease inhibitors (PI) that target the HCV NS3/4A serine protease, have recently become available and are recommended for use in combination with PEG IFN-α/RBV.[6] The addition of PI to PEG IFN-α/RBV has improved treatment outcomes substantially in both treatment-naïve and treatment-experienced patients.[6,10–16] Sustained virologic response (SVR) rates of 60–88% have been reported for the first-generation PI in combination with PEG IFN-α/RBV in untreated and previously treated relapsed HCV infection,[10–16] compared with rates of 40–50% with PEG IFN-α/RBV alone.[7–9,17,18] This has enabled the use of shorter courses of PEG IFN-α/RBV than the standard 48 weeks.[13,19] However, currently available PI in combination with PEG IFN-α/RBV are associated with higher incidences of anemia, dysgeusia, rash and nausea than PEG IFN-α/RBV alone,[10–13,15,16] and high rates of patient discontinuation.[11,14] In addition, currently available PI require multiple daily dosing. Patients infected with HCV would benefit from novel agents with improved tolerability and more convenient dosing schedules.

Simeprevir (TMC435) is a once-daily, oral HCV NS3/4A PI, with potent antiviral activity against HCV genotype 1,[20] as well as against isolates of genotypes 2 and 4–6.[21] Simeprevir combined with PEG IFN-α-2a/RBV has demonstrated good tolerability and high SVR rates in both treatment-naïve and treatment-experienced patients infected with HCV genotype 1 in international studies[22–24] and in phase III studies in Japan (CONCERTO-1,-2 and -3).[25,26]

We report the results of a phase III, open-label, non-comparative study (CONCERTO-4) conducted in Japan to investigate the efficacy and safety of simeprevir in combination with PEG IFN-α-2b/RBV in patients infected with HCV genotype 1 who were treatment-naïve or had previously received interferon (IFN)-based therapy.

METHODS

Patients

ELIGIBLE PATIENTS WERE aged 20–70 years with chronic HCV genotype 1 infection and plasma HCV RNA of 5.0 $\log_{10}$ IU/mL or more at screening. Treatment-naïve patients must not have received prior treatment with any approved or investigational HCV drug (including IFN). Patients who had previously received IFN-based therapy for 24 weeks or more were eligible provided their last treatment was administered 60 days or more before the study start. Treatment-experienced patients were classified as prior relapsers (i.e. patients who had undetectable levels of HCV RNA at the last assessment while on IFN-based therapy and subsequent detectable levels of HCV RNA within 12 months from their last treatment), or prior non-responders (i.e. patients who did not achieve undetectable HCV RNA on prior IFN-based therapy or who had discontinued IFN-based therapy within 24 weeks of treatment initiation due to <2 $\log_{10}$ IU/mL reduction from baseline in HCV RNA at week 12 of treatment). All patients provided written informed consent.

Exclusion criteria included liver cirrhosis or hepatic failure, liver disease of non-HCV etiology, infection/co-infection with non-genotype 1 HCV, hepatitis B virus, or HIV-1 or HIV-2, any condition that required caution with PEG IFN-α-2b or RBV therapy, and any other clinically significant disease, organ transplant or defined laboratory abnormalities at screening. In addition, treatment-experienced patients were not eligible if they had received treatment with any HCV therapy other than IFN, PEG IFN or RBV, or if they had discontinued previous therapy due to an AE considered likely to be treatment-limiting during PEG IFN-α-2b/RBV therapy.

Study design

This was an open-label, non-comparative, multicenter study to assess the efficacy and safety of simeprevir (TMC435) combined with PEG IFN-α-2b/RBV in treatment-naïve and treatment-experienced (prior relapsers or non-responders to IFN-based therapy) patients with chronic HCV genotype 1 (NCT01366638). The study was conducted at 14 sites in Japan from 1 April 2011 to 20 November 2012. The study was

approved by the relevant institutional review boards and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines.

Eligible patients received oral simeprevir 100 mg once daily (Q.D.) plus PEG IFN-α-2b/RBV for 12 weeks. In treatment-naïve patients and prior relapsers, this was followed by response-guided therapy (RGT) with PEG IFN-α-2b/RBV until week 24 or 48. Treatment-naïve patients and prior relapsers who achieved HCV RNA of less than 1.2 $\log_{10}$ IU/mL detectable or undetectable levels at week 4, with undetectable levels at week 12, stopped PEG IFN-α-2b/RBV therapy at week 24, while all others continued to week 48. All prior non-responders received PEG IFN-α-2b/RBV until week 48. All patients were followed for 72 weeks after treatment initiation.

Patients had to discontinue simeprevir but could continue with PEG IFN-α-2b/RBV if they experienced grade 4 elevations of aspartate aminotransferase (AST) or alanine aminotransferase (ALT) and the value was more than 2 times baseline, or if they experienced grade 4 blood bilirubin elevations and bilirubin values were the same or higher at retesting. All study medications were stopped if patients experienced grade 4 AE or laboratory abnormalities that were not considered to be related to simeprevir specifically or were not expected toxicities of PEG IFN-α-2b/RBV or HCV infection, or if patients experienced grade 3/4 skin events/allergic reactions, or worsening of hepatic disease. Additionally, all study medications were stopped if the following predefined virologic stopping criteria were met: less than 2 $\log_{10}$ IU/mL reduction in HCV RNA at week 12 relative to baseline (treatment-naïve patients and prior relapsers); HCV RNA levels of more than 2 $\log_{10}$ IU/mL at week 12 (prior non-responders); and confirmed detectable HCV RNA of 1.2 $\log_{10}$ IU/mL or more at weeks 24 or 36 (all patients). Patients who discontinued therapy proceeded immediately into follow-up.

The major efficacy end-point was the proportion of patients with undetectable HCV RNA at the end of treatment and 12 weeks after the last treatment (SVR12). Other efficacy end-points included the proportion of patients with: undetectable HCV RNA at end of treatment and 24 weeks after the last treatment (SVR24); undetectable HCV RNA at end of treatment and 4 weeks after the last treatment (SVR4); rapid virologic response (RVR; undetectable HCV RNA at week 4); complete early virologic response (cEVR; undetectable HCV RNA at week 12); undetectable HCV RNA at the end of treatment; viral breakthrough (increase of >1 $\log_{10}$ IU/mL in plasma HCV RNA level from the lowest level reached or plasma HCV RNA level >2.0 $\log_{10}$ IU/mL in patients whose plasma HCV RNA level had previously been <1.2 $\log_{10}$ IU/mL detectable or undetectable); viral relapse (detectable or quantifiable plasma HCV RNA during the post-treatment follow-up period in patients who had undetectable plasma HCV RNA at the end of treatment); and normalization of ALT. Tolerability and safety (AE, clinical laboratory parameters and vital signs) were secondary end-points.

Treatment administration

Simeprevir 100 mg was administered orally Q.D. as a single capsule. No simeprevir dose adjustments were permitted but, at the investigator's discretion, dosing could be interrupted for 4 days or less due to AE. PEG IFN-α-2b (PegIntron®; Merck Sharp & Dohme, Whitehouse Station, NJ, USA) was administered weekly as an s.c. injection (1.5 μg/kg body weight), and RBV (Rebetol®, Merck Sharp & Dohme) was administered as oral capsules (600–1000 mg total daily dose, according to body weight). Dose change, temporary interruption or discontinuation of PEG IFN-α-2b and RBV had to be conducted in accordance with the manufacturer's prescribing information. Patients were hospitalized for at least 1 week, starting on the first day of treatment. Use of erythropoiesis-stimulating agents and medications acting on the immune system was not permitted during treatment.

Study assessments

Plasma HCV RNA was quantified at screening, at baseline, on day 3, and at weeks 1, 2, 3, 4, 8, 12, 16, 20 and 24 (all patients), and weeks 28, 36, 48, 60 and 72 (patients receiving PEG IFN-α-2b/RBV until week 24), or weeks 28, 36, 42, 48, 52, 60 and 72 (patients receiving PEG IFN-α-2b/RBV until week 48). Levels were determined at a central laboratory using Roche COBAS® TaqMan® HCV Auto (Roche Molecular Diagnostics, Pleasanton, CA, USA) with a lower limit of quantification of 1.2 $\log_{10}$ IU/mL.

Sequence analysis of the HCV NS3 protease domain was performed at baseline and in patients with simeprevir treatment failure (viral breakthrough, meeting virologic stopping rule, detectable HCV RNA at end of treatment or viral relapse). The analysis of baseline polymorphisms focused on detecting previously characterized HCV genotype 1 amino acid substitutions in the NS3 region at positions 36, 43, 54, 80, 122, 138, 155, 156, 168 and 170 that have been associated with reduced susceptibility to simeprevir and other HCV NS3 PI *in vitro*.[27,28]

Safety and tolerability were evaluated throughout the entire treatment period, from first study medication intake until 28 days after the last dose. Severity of AE was graded by investigators according to the World Health Organization (WHO) grading scale. Vital sign monitoring, electrocardiograms, physical examinations and clinical laboratory tests were performed at regular intervals during the study period. Severity of laboratory abnormalities was classified according to the WHO grading scale.

Statistical analysis

A sample size of 70 patients was deemed sufficient to give a 97% probability of detecting an AE of special interest with 5% or more incidence.

Efficacy analyses were performed on the full analysis set (i.e. all patients who received the study medication and had post-baseline efficacy assessment data). The safety population included all patients who received at least one dose of simeprevir.

Ninety-five percent confidence intervals (CI) around the SVR12, SVR24 and SVR4 rates were calculated for each group. Descriptive statistics and tabulation were used to summarize baseline characteristics. All statistical analyses were performed using SAS® version 9.2 (SAS Institute, Cary, NC, USA).

RESULTS

Patients

IN TOTAL, 97 patients were screened and 79 received treatment (24 treatment-naïve patients, 29 prior relapsers and 26 prior non-responders) (Fig. 1). All 79 patients who received treatment were included in the full analysis set and safety populations. All study medications were completed by 65 patients (82.3%). The rate of treatment completion was lowest among prior non-responders (57.7% vs 91.7% for treatment-naïve patients and 96.6% for prior relapsers). Of the 14 patients who discontinued medications, one patient discontinued simeprevir and subsequently discontinued PEG IFN-α-2b/RBV, nine patients discontinued PEG IFN-α-2b/RBV after completing simeprevir treatment and four patients discontinued all study medications at the same time. The main reason for treatment discon-

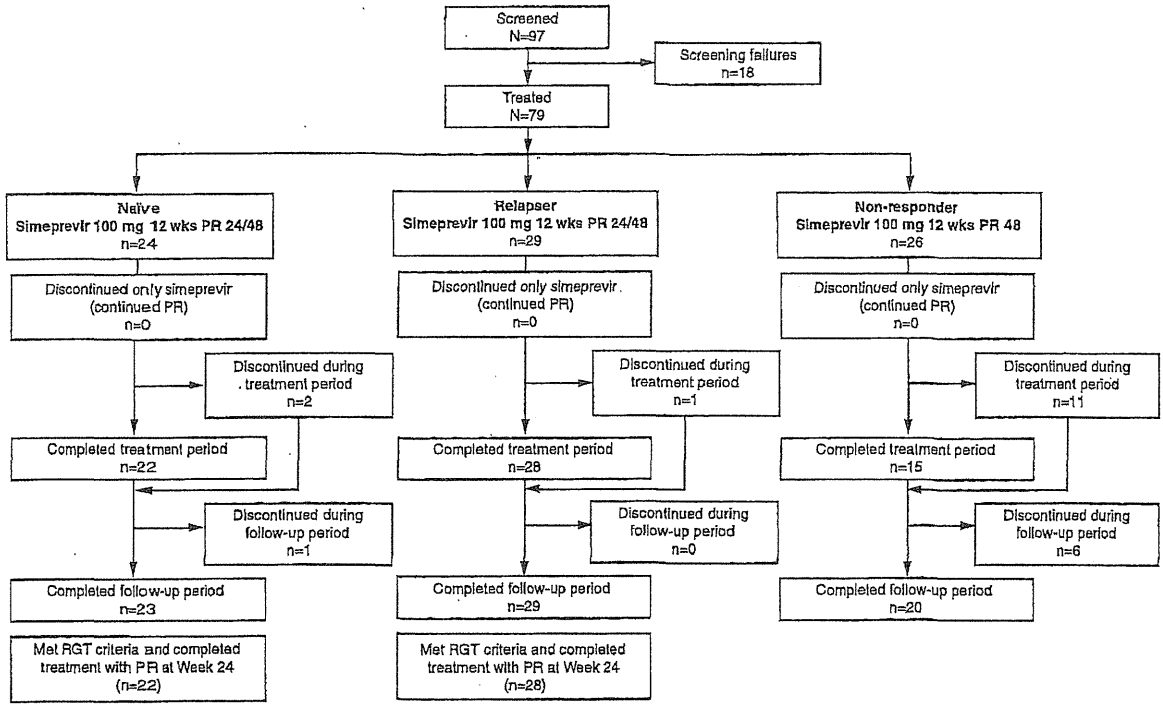

Figure 1 Patient disposition. PR, peginterferon-α-2b and ribavirin; RGT, response-guided treatment; wks, weeks.

Table 1 Patient demographics and baseline characteristics (full analysis set)

Characteristic	Treatment-naïve ($n=24$)	Prior relapsers ($n=29$)	Prior non-responders ($n=26$)
Male, *n* (%)	8 (33.3)	16 (55.2)	13 (50.0)
Age, years, median (range)	60 (37–68)	60 (38–70)	53 (45–69)
Age <65 years, *n* (%)	19 (79.2)	20 (69.0)	22 (84.6)
BMI, kg/m², median (range)	22.95 (18.1–30.2)	22.5 (18.1–31.9)	22.4 (16.9–34.3)
IL28B genotype (SNP rs8099917)			
TT, n (%)	16 (66.7)	26 (89.7)	2 (7.7)
TG/GG, *n* (%)	8 (33.3)	3 (10.3)	24 (92.3)
IL28B genotype (SNP rs12979860)			
CC, *n* (%)	16 (66.7)	26 (89.7)	2 (7.7)
CT/TT, *n* (%)	8 (33.3)	3 (10.3)	24 (92.3)
Genotype 1b, *n* (%)	24 (100.0)	29 (100.0)	25 (96.2)
Baseline HCV RNA, $\log_{10}$ IU/mL, median (range)	6.6 (5.4–7.0)	6.6 (4.9–7.4)	6.5 (5.1–7.4)
METAVIR score, category, *n* (%)†	$n=6$	$n=6$	$n=7$
F0	0	0	0
F1	5 (83.3)	4 (66.7)	5 (71.4)
F2	1 (16.7)	1 (16.7)	2 (28.6)
F3	0	1 (16.7)	0
F4	0	0	0
Platelets ($\times10^9$/L), *n* (%)			
<150	5 (20.8)	9 (31.0)	11 (42.3)
≥150	19 (79.2)	20 (69.0)	15 (57.7)
Prior therapy, *n* (%)			
IFN only	N/A	1 (3.4)	0
IFN/RBV	N/A	0	3 (11.5)
PEG IFN only	N/A	0	0
PEG IFN/RBV	N/A	28 (96.6)	23 (88.5)
ALT			
<50 IU/mL	16 (66.7)	20 (69.0)	13 (50.0)
≥50 IU/mL	8 (33.3)	9 (31.0)	13 (50.0)
Total bilirubin (mg/dL), median (range)	0.7 (0.3–1.8)	0.8 (0.4–2.2)	0.8 (0.3–1.1)
Hemoglobin (g/dL), median (range)	14.2 (12.4–16.3)	14.4 (11.5–17.0)	13.9 (12.2–16.6)
Neutrophils ($\times10^2$/μL), median (range)	25.4 (12.1–51.2)	25.4 (10.1–48.1)	22.2 (9.6–35.8)
Platelets ($\times10^4$/μL), median (range)	17.1 (12.2–27.5)	16.3 (9.6–33.3)	15.4 (11.0–20.5)

†Available for patients who had a liver biopsy within two years prior to informed consent or during the screening period.
ALT, alanine aminotransferase; BMI, body mass index; HCV, hepatitis C virus; IFN, interferon; N/A, not applicable; PEG IFN, peginterferon; RBV, ribavirin; SNP, single nucleotide polymorphism.

tinuation was meeting the virologic stopping criteria (eight patients, all prior non-responders).

Demographic and disease characteristics at baseline were generally comparable across the three patient groups (Table 1), with a few notable exceptions for sex, *IL28B* genotype and baseline platelet counts. Median age was 60 years (range, 37–70), with 22.8% of patients aged 65 years or more. Most treatment-naïve patients and prior relapsers had major allele TT and CC genotypes for *IL28B* rs9088817 and rs12979860 polymorphisms, respectively. By contrast, most prior non-responders had minor alleles TG/GG and CT/TT at these loci. All but one patient (prior non-responder) had HCV genotype 1b; median HCV RNA at baseline was 6.5 $\log_{10}$ IU/mL. Most prior relapsers and prior non-responders had previously been treated with PEG IFN plus RBV. Platelet counts at baseline were slightly lower in prior non-responders, with 42.3% having counts of less than 150×10^9/L versus 31.0% or less of patients in the other groups.

Efficacy

SVR

The proportion of patients achieving SVR4, SVR12 (major efficacy end-point), and SVR24 is shown in Fig. 2. The proportion of patients achieving SVR12 was

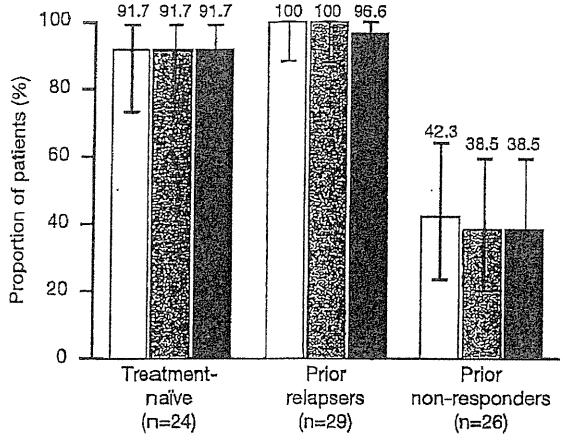

Figure 2 Sustained virologic response at 4, 12 (major end-point) and 24 weeks after the end of treatment. Bars indicate 95% confidence intervals. †$n = 12$. SVR, sustained virologic response (undetectable hepatitis C virus RNA); SVR4, SVR at end of actual treatment and at 4 weeks after the last treatment; SVR12, SVR at end of actual treatment and at 12 weeks after the last treatment (major efficacy end-point); SVR24, SVR at end of actual treatment and at 24 weeks after the last treatment. □, SVR4; ▨, SVR12; ■, SVR24.

highest among treatment-naïve patients (91.7%; 95% CI, 73.0–99.0%) and prior relapsers (100%; 95% CI, 88.1–100%). Only two patients in the treatment-naïve group did not achieve SVR12; both had undetectable HCV RNA at end of treatment, but had viral relapse at the SVR4 time point. Among prior non-responders, 10 patients (38.5%; 95% CI, 20.2–59.4%) achieved SVR12, 11 patients had detectable HCV RNA at end of treatment, four patients had viral relapse at the SVR4 time point, and one patient had discontinued follow-up before the SVR12 time point.

The SVR24 rate was 91.7% (95% CI, 73.0–99.0%) among treatment-naïve patients and 96.6% (95% CI, 82.2–99.9%) for prior relapsers (Fig. 2). All treatment-naïve patients and prior non-responders who achieved SVR12 also achieved SVR24, while 28 of 29 prior relapsers achieved both end-points (one patient experienced viral relapse at week 24 of follow-up).

Twenty-two (91.7%) treatment-naïve patients and 28 (96.6%) prior relapsers met RGT criteria and completed PEG IFN-α-2b/RBV treatment at week 24. The remaining three patients had discontinued treatment before the week 24 assessment. Rates of SVR12 and SVR24 for patients stopping treatment at week 24 were 90.9% (20/22) for treatment-naïve patients and 100% (28/28) for prior relapsers.

Virologic response

A rapid decline in mean plasma HCV RNA levels was evident in all patient groups up to week 2 (Fig. 3), by which time most patients had achieved levels below the lower limit of quantification.

Most patients in all three groups achieved RVR (60.0–86.2%; Table 2) and cEVR (79.2–100%; Table 2). All treatment-naïve patients and prior relapsers had undetectable levels of HCV RNA at the end of treatment (Table 2). In prior non-responders, 57.7% of patients had undetectable HCV RNA at end of treatment; all patients in this group had a reduction in HCV RNA from baseline of 1 $\log_{10}$ IU/mL or more at week 4.

Viral breakthrough and viral relapse

No viral breakthrough was observed in treatment-naïve patients or prior relapsers. Six prior non-responders (23.1%) had viral breakthrough (Table 2). Two of these six patients experienced breakthrough at week 8 during the simeprevir treatment period. One patient had viral breakthrough at week 8 after discontinuing simeprevir at week 5 upon meeting virologic stopping criteria. The remaining three patients experienced viral breakthrough during PEG IFN-α-2b/RBV-only treatment (weeks 12–24).

Two treatment-naïve patients experienced viral relapse at week 4 of follow-up. One prior relapser experienced viral relapse at week 24 of follow-up. Four of 15 prior non-responders with undetectable HCV RNA at

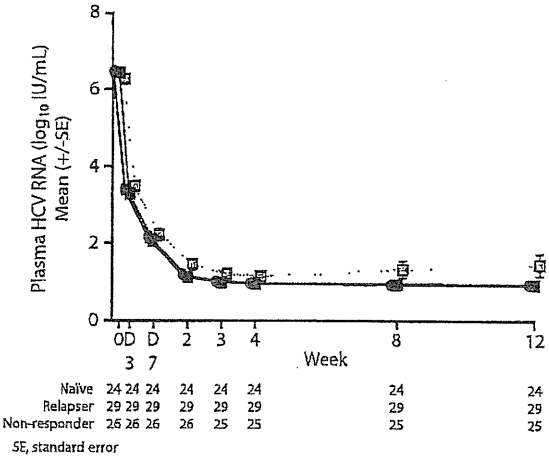

Figure 3 Mean (±SE) change in hepatitis C virus (HCV) RNA levels from baseline to week 12. D, day. —●—, naïve; —■—, relapser; □, non-responder.

Table 2 Virologic response rates

Response, *n*/N (%)	Treatment-naïve ($n = 24$)	Prior relapsers ($n = 29$)	Prior non-responders ($n = 26$)
RVR,[†] *n* (%)	19/24 (79.2)	25/29 (86.2)	15/25 (60.0)
cEVR,[‡] *n* (%)	23/23 (100.0)	28/28 (100.0)	19/24 (79.2)
End of treatment response,[§] *n* (%)	24/24 (100.0)	29/29 (100.0)	15/26 (57.7)
Viral breakthrough,[¶] *n* (%)	0/24 (0)	0/29 (0)	6/26 (23.1)
Viral relapse,[††] *n* (%)	2/24 (8.3)	1/29 (3.4)	4/15 (26.7)

Note: RVR and cEVR are assessed while on treatment. If a subject discontinues all study medications prior to the time point of the parameter of interest, then the subject is not included in the denominator.
[†]Undetectable HCV RNA at week 4 (i.e. while on treatment). [‡]Undetectable HCV RNA at week 12. [§]Undetectable HCV RNA at end of treatment. [¶]An increase of >1.0 $\log_{10}$ IU/mL in HCV RNA level from the lowest level reached, or HCV RNA level of >2.0 $\log_{10}$ IU/mL in patients whose HCV RNA had previously been <1.2 $\log_{10}$ IU/mL detectable or undetectable. [††]Detectable HCV RNA during the post-treatment follow-up period of sustained viral response assessment in patients who had undetectable plasma HCV RNA at end of treatment. The incidence of viral relapse was calculated only for patients with undetectable HCV RNA levels at end of treatment and with ≥1 follow-up HCV RNA measurement. The denominator for prior non-responders was $n = 15$.
cEVR, complete early virologic response; HCV, hepatitis C virus; N, number of patients with data at specific time point assessed; *n*, number of patients with observation; RVR, rapid virologic response.

end of treatment had documented viral relapse at the week 4 follow-up visit.

Emerging mutations in treatment failures

Sequencing analysis of the NS3 protease domain of HCV was available for 17 (two treatment-naïve patients, one prior relapser and 14 non-responders) of the 18 simeprevir-treated patients classified as treatment failures (i.e. met predefined virologic stopping criteria, detectable HCV RNA at end of treatment, viral breakthrough or viral relapse). Emerging mutations were identified for 16 patients at the time of failure, including six patients with viral breakthrough. D168V was the most frequent emerging mutation, accounting for eight single mutations, followed by Q80R+D168E (three patients), D168E (two patients), and R155K, D168T and Q80K+D168E (one patient each).

SVR according to selected demographic and baseline disease characteristics

A summary of SVR12 rate by selected demographic and baseline disease characteristics is presented in Table 3. Most treatment-naïve patients and all prior relapsers achieved SVR12; therefore, no apparent trend was noted

Table 3 SVR12 rates by selected demographic and baseline disease characteristics

Characteristic	SVR12 rate, *n*/N (%)		
	Treatment-naïve ($n = 24$)	Prior relapsers ($n = 29$)	Prior non-responders ($n = 26$)
Sex			
Male	7/8 (87.5)	16/16 (100.0)	5/13 (38.5)
Female	15/16 (93.8)	13/13 (100.0)	5/13 (38.5)
Age			
<65 years	19/19 (100.0)	20/20 (100.0)	7/22 (31.8)
≥65 years	3/5 (60.0)	9/9 (100.0)	3/4 (75.0)
IL28B genotype (rs8099917)			
TT	16/16 (100.0)	26/26 (100.0)	0/2 (0.0)
TG/GG	6/8 (75.0)	3/3 (100.0)	10/24 (41.7)
IL28B genotype (rs12979860)			
CC	16/16 (100.0)	26/26 (100.0)	0/2 (0.0)
CT/TT	6/8 (75.0)	3/3 (100.0)	10/24 (41.7)

SVR12, undetectable hepatitis C virus RNA 12 weeks after the last treatment.