efficacy of telaprevir for 12 weeks in combination with peginterferon and RBV for 24 weeks [13,14]. These studies demonstrated that the SVR rates of the telaprevir regimen were significantly higher compared with SOC (PROVE 1: 61% *vs* 41%, $P = 0.02$, PROVE 2: 69% *vs* 46%, $P = 0.004$). A subsequent phase II study (PROVE 3) for treatment-failure patients with genotype 1 gave SVR rates for nonresponders, relapsers and breakthroughs in the telaprevir regimen of 39%, 69% and 57%, respectively [9].

In Japan, a phase III study was conducted for the treatment of naïve patients with genotype 1 to compare the efficacy and safety between the telaprevir regimen and SOC. It has demonstrated that the SVR rate for the telaprevir regimen was significantly higher than that for SOC (73.0% *vs* 49.2%, $P = 0.0020$) [15]. We decided to conduct a phase III study to assess the efficacy and safety of telaprevir in combination with PEG-IFN and RBV in relapsers and nonresponders who had not achieved SVR to a previously administered IFN-based regimen in Japan.

PATIENTS AND METHODS

Study patients

Relapsers and nonresponders were enrolled in Study 1 (ClinicalTrials.gov Identifier: NCT00780910) and Study 2 (ClinicalTrials.gov Identifier: NCT00781274), respectively. Relapsers were defined as patients who had been previously treated for CHC and had undetectable HCV RNA during interferon or peginterferon therapy (including combination with RBV). Nonresponders were defined as patients who were previously treated for CHC and had never had undetectable HCV RNA for more than 24 weeks with interferon or peginterferon therapy (including combination with RBV).

The patients were enrolled from 17 sites in Japan. Patients considered eligible were of 20–65 years of age, had CHC because of HCV genotype 1 (defined by NS5B sequence) [16] and ≥5.0 $\log_{10}$ IU/mL HCV RNA level at the screening test, had been previously treated for CHC with interferon or peginterferon therapy (including combination with RBV), had a body weight of 40 kg or more and below 120 kg, could be hospitalized for at least 2 weeks after the first administration, were not pregnant and agreed to contraception from the screening period to 24 weeks after the last dosing of the study drug. The patients were excluded if they had a haemoglobin level of <12 g/dL, neutrophil count of <1500/mm^3, platelet count of <100 000/mm^3, were positive for HBs antigen and HIV antibodies at the screening test, had chronic renal failure or creatinine clearance of ≤50 mL/min, depression, schizophrenia or its history, history of suicide attempt, decompensated cirrhosis, previous or current HCC or other malignancies, autoimmune hepatitis, alcoholic liver disease or haemochromatosis.

All patients provided written informed consent before participating in the study. These studies were approved by each site's institutional review board and 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 subcutaneously, RBV (Rebetol®; MSD) at a dose of 600 mg per day (for body weight ≤60 kg), 800 mg per day (for body weight >60 to ≤80 kg) or 1000 mg per day (for body weight >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 period after the last dosing of study drugs to assess SVR.

Dose modification of study drugs

Specified dose modification of RBV that differed from the dose for SOC was introduced to alleviate anaemia. The initial dose of RBV was reduced by 200 mg per day in case of a haemoglobin level <13 g/dL at baseline. The RBV dose was reduced by 200 mg per day in patients receiving 600 or 800 mg per day (by 400 mg per day in those receiving 1000 mg) when the haemoglobin level was <12 g/dL and was reduced by an additional 200 mg per day when the haemoglobin level was <10 g/dL. The RBV dose was also reduced by 200 mg per day if the haemoglobin level dropped ≥1 g/dL within 1 week, and this level was <13 g/dL. Telaprevir was withdrawn when the haemoglobin level was <8.5 g/dL. PEG-IFN/RBV were withdrawn or interrupted when the haemoglobin level was <8.5 g/dL. The dose modifications of PEG-IFN were followed by SOC. Dose modification and interruption of telaprevir were not allowed. Telaprevir was withdrawn if serious adverse events appeared. The use of erythropoietin was not allowed for elevating the haemoglobin level.

Stopping rules

Patients could be discontinued from the study at any time if the investigator or sponsor determined that it was not in the interest of the patient to continue the study or the patient wished to withdraw from the study. The study drugs were discontinued if the patients had a haemoglobin level of <8.5 g/dL, white blood cell count of <1000/mm^3, neutrophil count of <500/mm^3 or platelet count of <50 000/mm^3.

In case of the following criteria for serum HCV RNA viral kinetics measured during the treatment period, discontinuation of the study drugs was decided at the investigator's discretion. (i) When the following criteria applied twice consecutively: (a) the amount of change from the lowest value for HCV RNA level exceeded 2.0 $\log_{10}$ IU/mL and (b)

HCV RNA level exceeded 2.0 $\log_{10}$ IU/mL after it had been confirmed to be <1.2 $\log_{10}$ IU/mL. (ii) When the serum HCV RNA level at 13 weeks after administration of study drugs did not decrease by >2.0 $\log_{10}$ IU/mL from the baseline level.

Efficacy assessments

Serum HCV RNA levels were measured using the COBAS TaqMan HCV test (Roche Diagnostics Co. Ltd., Tokyo, Japan). The linear dynamic range was 1.2–7.8 $\log_{10}$ IU/mL. Samples with undetectable HCV RNA were reported as '<1.2 $\log_{10}$ IU/mL (no detectable HCV RNA)'. Measurements were obtained at week 4 before day 1 of the screening period: at days 1 (predose), 2 and 3; weeks 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 of the treatment period; and weeks 2, 4, 8, 12, 16, 20 and 24 of the follow-up period.

The primary endpoint was a SVR defined as an undetectable HCV RNA level 24 weeks after the end of treatment. Relapse, breakthrough, and nonresponse were defined based on AASLD Guidelines as follows [4]: 'relapse' was a state of undetectable serum HCV RNA at the end of treatment and reappearance of serum HCV RNA during the follow-up period; 'breakthrough' was a state of undetectable serum HCV RNA and reappearance of serum HCV RNA during the treatment period; and 'nonresponse' was a state of continuously detectable serum HCV RNA during the treatment period.

Safety assessments

All adverse events were recorded up to the last visit and coded using MedDRA/J version 13.0. (MedDRA Japanese Maintenance Organization, Tokyo, Japan) Measurements for chemical laboratory data were obtained at week 4 before day 1 of the screening period: at day 1 (predose); weeks 1, 2, 4, 8, 10, 12, 14, 16, 18, 20 and 24 of the treatment period; and weeks 2, 4, 8, 12 and 24 of the follow-up period. Electrocardiogram (ECG) and fundus examinations were performed once during the screening period. Adverse events, haematological and chemical laboratory data, and vital signs were assessed and summarized. The severity of rash was categorized into three grades.

Statistical analysis

Sustained virological response rates were evaluated for the full analysis set. Categorical variables were compared by Fisher's exact test. Statistical analyses were performed using the statistical software SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA), and a P value < 0.05 was considered significant.

Table 1 Baseline characteristics of study patients

	Study 1 (relapsers) N = 109	Study 2 (nonresponders) N = 32
Gender – n (%)		
Men	66 (60.6)	17 (53.1)
Women	43 (39.4)	15 (46.9)
Age, years – median (range)	57.0 (20, 65)	57.5 (40, 65)
Weight, kg – median (range)	62.50 (41.0, 92.5)	61.30 (44.9, 92.5)
BMI, kg/m^2 – median (range)*	23.10 (18.0, 32.4)	22.60 (17.1, 31.2)
ALT (IU/L) – median (range)†	36.0 (16, 302)	48.0 (17, 190)
Haemoglobin (g/dL) – median (range)	14.70 (12.0, 17.8)	14.50 (12.3, 16.6)
White blood cell count (/mm^3)	4680.0 (2490, 15940)	4830.0 (3040, 8000)
Platelet count (×10^4/mm^3) – median (range)	17.80 (9.9, 33.8)	17.85 (9.1, 26.2)
HCV RNA ($\log_{10}$ IU/mL) – median (range)‡	6.75 (5.2, 7.6)	6.78 (6.0, 7.7)
HCV genotype 1 subtype – n (%)		
1a	0 (0.0)	1 (3.1)
1b	109 (100.0)	31 (96.9)
Prior therapy for chronic hepatitis C – n (%)		
Interferon	13 (11.9)	1 (3.1)
Interferon plus ribavirin	14 (12.8)	2 (6.3)
Peginterferon	3 (2.8)	0 (0.0)
Peginterferon plus ribavirin	79 (72.5)	29 (90.6)

HCV, hepatitis C virus.
*The body mass index (BMI) is the weight in kilograms divided by the square of the height in metres; †Alanine aminotransferase; ‡The HCV RNA level was measured using the COBAS TaqMan HCV test (Roche).

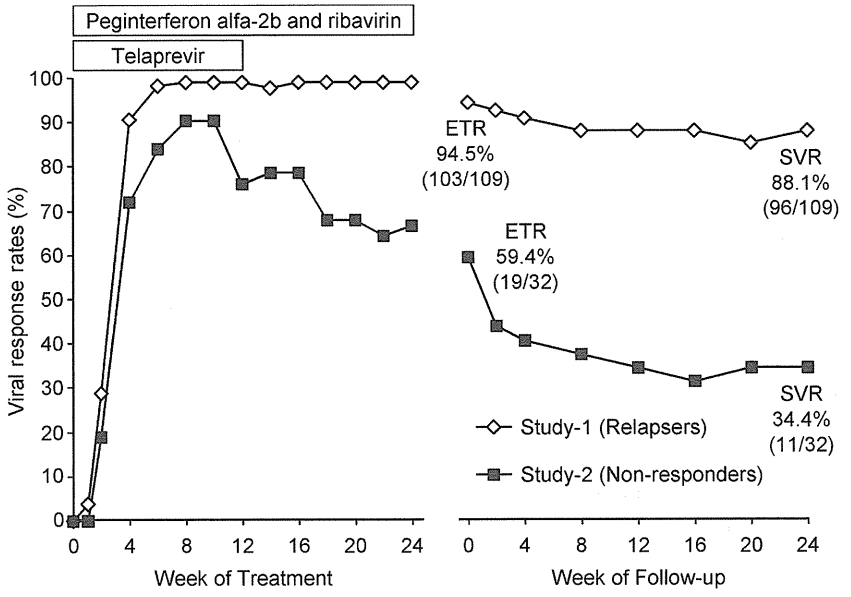

Fig. 1 Undetectable hepatitis C virus RNA rates at each measurement point. SVR, sustained virological response; ETR, end-of-treatment response.

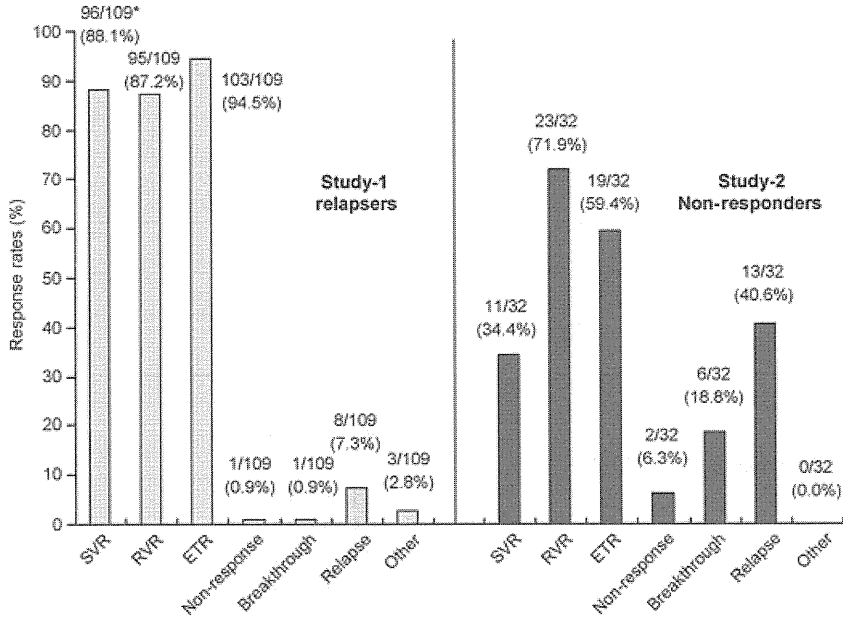

Fig. 2 Response rates of patients with virological response. *Number of patients who achieved SVR in each subgroup/*N* (%). SVR, sustained virological response; RVR, rapid viral response; ETR, end-of-treatment response.

RESULTS

Study patients

From November 2008 to August 2009, a total of 168 patients [Study 1 (*N* = 135) and Study 2 (*N* = 33)] were screened, and 141 patients [Study 1 (*N* = 109) and Study 2 (*N* = 32)] received at least one dose of a study drug. The baseline characteristics of the study patients are shown in Table 1. Patients previously treated with PEG-IFN (with or without RBV) and IFN (with or without RBV) in Study 1 and Study 2 accounted for 75.2% (82 of 109) and 24.7% (27 of 109) and 90.6% (29 of 32) and 9.4% (3 of 32), respectively. The median of age, weight, haemoglobin level, platelet count and HCV RNA level for Study 1 and Study 2 were 57.0 and 57.5 years, 62.5 and 61.3 kg, 14.7 and 14.5 g/dL, 17.8

and $17.85 \times 10^4/mm^3$, and 6.75 and 6.78 log_{10} IU/mL, respectively. Patients over 50 years of age accounted for 81.7% (89 of 109) and 81.3% (26 of 32), respectively.

Efficacy in study 1 (relapsers)

Figure 1 shows the change in the undetectable HCV RNA rates at each measurement point. The rapid viral response (RVR) rate and the end of treatment response (ETR) rate were 87.2% (95/109) and 94.5% (103/109), respectively. The SVR rate, nonresponse, breakthrough and relapse were 88.1% (96/109), 0.9% (1/109), 0.9% (1/109) and 7.3% (8/109), respectively (Fig. 2).

Factors influencing the SVR rate are compared in Table 2. The SVR rate in the patients who achieved undetectable HCV RNA at ≤week 4 was significantly higher than that in the patients who achieved undetectable HCV RNA at >week 4 (91.8% *vs* 66.7%, $P = 0.0487$). Also, the SVR rate for men was significantly higher than that for women (93.9% *vs* 79.1%, $P = 0.0316$). The SVR rate with discontinuation of all the study drugs was significantly lower than that with discontinuation of only telaprevir or no discontinuation of the study drugs (all the study drugs: 60.0%, only telaprevir: 95.0% and no discontinuation: 94.2%, $P = 0.0007$). In contrast, there was no difference in the SVR rate in relation to HCV RNA level and prior therapy for CHC. SVR rates by the ratio of the actual total RBV dose to the anticipated total RBV dose were evaluated (Fig. 3). The SVR rates did not depend on RBV dose reduction for 20–100% of the planned dose (87.5–100%, $P < 0.05$).

Efficacy in study 2 (nonresponders)

The RVR and ETR rates were 71.9% (23/32) and 59.4% (19/32), respectively (Fig. 1). The SVR rate, nonresponse, breakthrough and relapse were 34.4% (11/32), 6.3% (2/32), 18.8% (6/32) and 40.6% (13/32), respectively (Fig. 2). There was no difference in the SVR rate in relation to

Table 2 SVR rates stratified by demographic, undetectable HCV RNA and discontinuation of study drug treatment

	Study 1 (relapsers) *N* = 109	Study 2 (nonresponders) *N* = 32
Gender – *n/N* (%)		
Male	62/66 (93.9)	8/17 (47.1)
Female	34/43 (79.1)	3/15 (20.0)
P-value	0.0316	0.1475
Age – *n/N* (%)		
≤49	18/20 (90.0)	2/6 (33.3)
≥50	78/89 (87.6)	9/26 (34.6)
P-value	1.0000	1.0000
HCV RNA (log_{10} IU/mL) – *n/N* (%)		
≥7.0	26/30 (86.7)	5/10 (50.0)
<7.0	70/79 (88.6)	6/22 (27.3)
P-value	0.7498	0.2515
Prior therapy for chronic hepatitis C – *n/N* (%)		
Interferon	12/13 (92.3)	1/1 (100.0)
Interferon plus ribavirin	13/14 (92.9)	2/2 (100.0)
Peginterferon	3/3 (100.0)	– (–)
Peginterferon plus ribavirin	68/79 (86.1)	8/29 (27.6)
P-value	0.9271	0.0333
Undetectable – *n/N* (%)		
≤Week 4	90/98 (91.8)	9/23 (39.1)
>Week 4 ≤end of treatment	6/9 (66.7)	2/7 (28.6)
P-value	0.0487	1.0000
Discontinuation of study drug treatment – *n/N* (%)		
No discontinuation	65/69 (94.2)	9/20 (45.0)
Telaprevir only	19/20 (95.0)	2/7 (28.6)
All study drugs	12/20 (60.0)	0/5 (0.0)
P-value	0.0007	0.1711

SVR, sustained virological response; HCV, hepatitis C virus.
SVR was defined as an undetectable HCV RNA level 24 weeks after the end of treatment.

baseline characteristics, HCV RNA level and prior treatment for CHC. The SVR rates for the patients who received 40–80% RBV dose reduction were over 30% (Fig. 3).

Safety

Adverse events were observed in all the patients in Study 1 and Study 2. Adverse events observed in at least 15% of the patients in each clinical study are listed in Table 3. Adverse events were similar between Study 1 and Study 2. Most of the adverse events were mild and moderate. Serious adverse events in Study 1 and Study 2 were reported in 11.9% (13/109) and 9.4% (3/32) of the patients, respectively. The ratios of discontinuation of all the study drugs because of adverse events in Study 1 and Study 2 were 17.4% (19/109) and 12.5% (4/32), respectively. A frequent adverse event leading to discontinuation was anaemia. Discontinuation rates of all the study drugs because of anaemia in Study 1 and Study 2 were 10.1% (11/109) and 9.4% (3/32), respectively. One death was reported in Study 1. One patient in Study 1 died of pulmonary embolism. Causality of PEG-IFN and RBV was classified as 'probably related' and that of telaprevir was classified as 'possibly related'.

Adverse events related to skin disorders were observed in 82.3% (116/141) of the patients. Skin disorders reported in over 10% of the patients were rash in 39.0% (55/141), drug eruption in 24.1% (34/141), injection site reaction in 12.8% (18/141) and injection site erythema in 12.8% (18/141) of the patients. Most of the skin disorders were controllable by anti-histamine and/or steroid ointments. Grade 3 (severe) skin disorders in Study 1 and Study 2 were reported in 6.4% (7/109) and 6.3% (2/32) of the patients, respectively. Discontinuation of all the study drugs because of skin disorders in Study 1 amounted to 3.7% (4/109). No discontinuation because of skin disorders occurred in Study 2.

Figure 4 shows the changes in haemoglobin levels, platelet counts and neutrophil counts during the treatment and follow-up periods. Changes in the haematological parameters were similar between Study 1 and Study 2. The platelet count and neutrophil count decreased sharply within 4 weeks and then gradually decreased. Despite the modification of RBV, the median haemoglobin levels in Study 1 and Study 2 decreased to 10.6 and 10.4 g/dL at week 12, respectively. No patient discontinued all the study drugs because of neutrophil decrease. The haematological parameters recovered to the baseline level at the end of the follow-up period.

DISCUSSION

This phase III study was planned and conducted to assess the efficacy and safety of telaprevir in combination with PEG-IFN/RBV for relapsers and nonresponders. Most of the patients who participated in this study had received a prior PEG-IFN/RBV regimen. Despite a shorter treatment period, the SVR rates for relapsers and nonresponders were 88.1% and 34.4%, respectively. The result indicates that the HCV RNA response to previous treatment history should be one of the diagnostic factors for predicting SVR.

The SVR rate for men was significantly higher than that for women in the relapser group (93.9% *vs* 79.1%, $P = 0.0316$). There was no significant difference in other characteristics of the patients in that group. Once the relapsers had achieved undetectable HCV RNA, this condi-

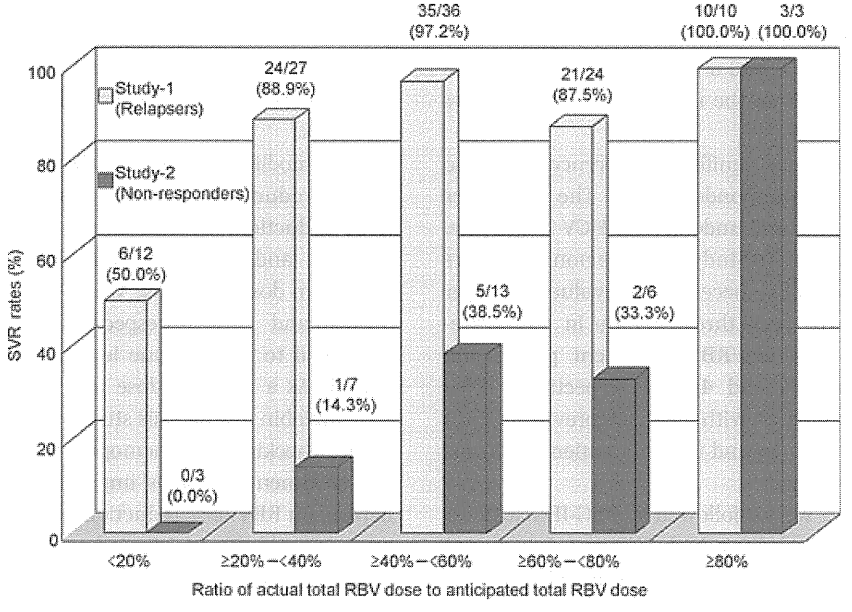

Fig. 3 Sustained virological response rates according to adherence to the ribavirin dose.

Table 3 Most common adverse events

MedDRA/J (Version.13.0) preferred term – *n* (%)	Study 1 (relapsers) *N* = 109	Study 2 (nonresponders) *N* = 32	Total *N* = 141
Anaemia	96 (88.1)	32 (100.0)	128 (90.8)
Pyrexia	90 (82.6)	30 (93.8)	120 (85.1)
White blood cell count decreased	83 (76.1)	22 (68.8)	105 (74.5)
Blood uric acid increased	72 (66.1)	25 (78.1)	97 (68.8)
Platelet count decreased	73 (67.0)	22 (68.8)	95 (67.4)
Malaise	60 (55.0)	23 (71.9)	83 (58.9)
Decreased appetite	56 (51.4)	15 (46.9)	71 (50.4)
Hyaluronic acid increased	56 (51.4)	15 (46.9)	71 (50.4)
Rash	39 (35.8)	16 (50.0)	55 (39.0)
Headache	42 (38.5)	10 (31.3)	52 (36.9)
Blood creatinine increased	36 (33.0)	12 (37.5)	48 (34.0)
Insomnia	34 (31.2)	11 (34.4)	45 (31.9)
Blood bilirubin increased	34 (31.2)	10 (31.3)	44 (31.2)
Alopecia	35 (32.1)	7 (21.9)	42 (29.8)
Diarrhoea	31 (28.4)	7 (21.9)	38 (27.0)
Dysgeusia	29 (26.6)	6 (18.8)	35 (24.8)
Vomiting	26 (23.9)	8 (25.0)	34 (24.1)
Drug eruption	24 (22.0)	10 (31.3)	34 (24.1)
Nausea	24 (22.0)	4 (12.5)	28 (19.9)
Abdominal discomfort	22 (20.2)	6 (18.8)	28 (19.9)
Blood triglycerides increased	19 (17.4)	8 (25.0)	27 (19.1)
Pruritus	20 (18.3)	2 (6.3)	22 (15.6)
Arthralgia	18 (16.5)	4 (12.5)	22 (15.6)
Nasopharyngitis	19 (17.4)	2 (6.3)	21 (14.9)
Stomatitis	13 (11.9)	6 (18.8)	19 (13.5)
Back pain	12 (11.0)	5 (15.6)	17 (12.1)
Blood phosphorus decreased	10 (9.2)	6 (18.8)	16 (11.3)

The adverse events listed are those that were reported in at least 15% of patients in each clinical study.

tion was sustained until the end of the treatment period. The patients who achieved RVR had a higher SVR rate than the patients who had no RVR in the relapser group (91.8% *vs* 66.7%, $P = 0.0487$).

In contrast, there was no significant difference related to characteristics in the nonresponder group. The SVR rates between men and women and undetectable HCV RNA were, however, slightly different. As Study 2 for the nonresponders was of a small scale, it will be necessary to evaluate a larger number of patients. The breakthrough ratio in the nonresponders during the PEG-IFN/RBV treatment period and relapse ratio were 18.8% and 40.6%, respectively. Two patients were nonresponders with high telaprevir-resistant variants; one was subtype 1a and the only patient with this characteristic in the study.

Triple therapy for 12 weeks, followed by PEG-IFN/RBV for 12 weeks for the relapsers led to a high SVR rate. In contrast to the relapsers, all breakthroughs were observed in 18.8% of nonresponder patients after the end of telaprevir treatment, and relapse were observed in 40.6% of nonresponder patients after the end of treatment period. Continuation of telaprevir over 12 weeks and PEG-IFN/RBV over 24 weeks might be needed to achieve a higher SVR rate for nonresponders.

Dose modification of RBV that differed from that for SOC was introduced to prevent anaemia in the patients [17]. Dose reductions of RBV were observed in 98.6% of the patients, and those who had 200 mg RBV per day as a minimum dose and those who discontinued it accounted for 41.8% and 29.8%, respectively. The haemoglobin level recovered to the baseline level at the end of the follow-up period. As a result of dose modification, the change in the haemoglobin level in this study was similar to that in PROVE 3 [9]. Checking the haemoglobin level once a week during the treatment period is important. The SVR rates did not depend on RBV dose reduction among the relapsers who had over 20% of the anticipated total RBV dose (87.5–100%). Thus, it is important to monitor haemoglobin levels and continue RBV dosing appropriately to achieve SVR, even with a low RBV dose.

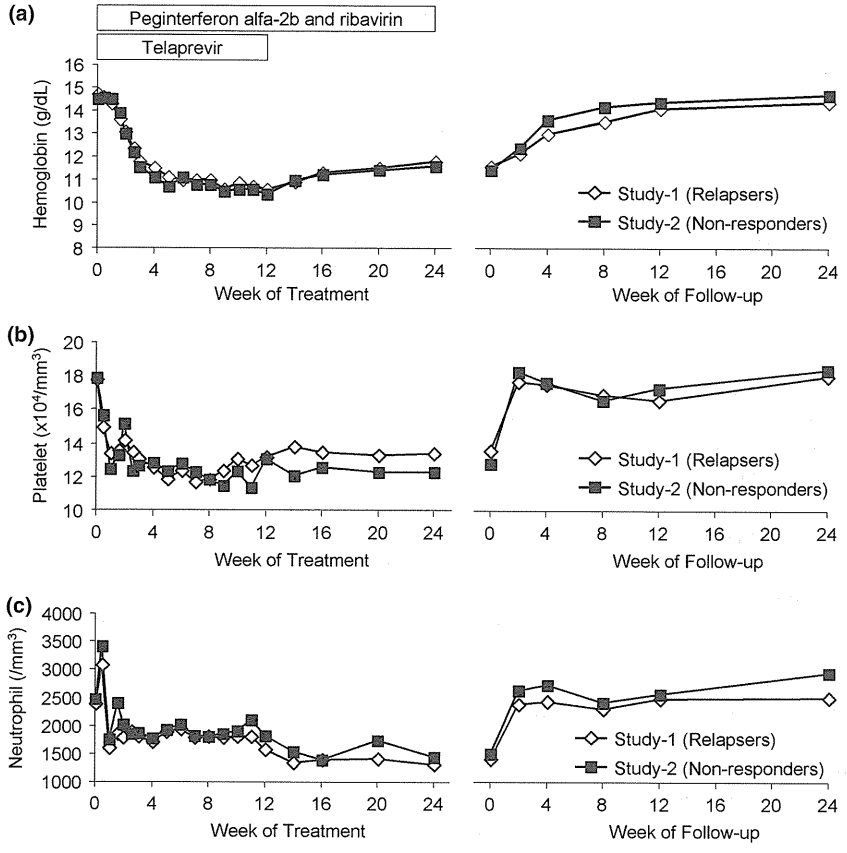

Fig. 4 Changes in hematology parameters. Median haemoglobin levels (a), median platelet counts (b) and median neutrophil counts (c) were plotted during treatment and follow-up periods.

Adverse events related to skin disorder were reported by 82.3% of the subjects. Of the nine cases of severe skin disorders, seven occurred within 8 weeks. Telaprevir was likely to be related to the occurrence of the severe skin disorders. The mechanism of skin disorders is unknown. All the patients who discontinued treatment received immediate care from dermatologists and recovered eventually. Skin disorders should be carefully monitored by physicians in collaboration with dermatologists.

The relationship between the SVR rates and the difference in SNPs in gene IL28B or near IL28B has become clear [18,19]. With genetic variation in rs8099917, SVR rates of 83.8% and 27.6% were achieved for patients with genotype TT and non-TT who were treated with telaprevir in combination with PEG-IFN/RBV, respectively [20]. Also, genetic variations in gene ITPA related to haemoglobin decrease and reduction of RBV has been discussed for patients treated with PEG-IFN/RBV [21,22]. We did not evaluate IL28B and ITPA in this study. As anaemia was the most frequent adverse event leading to the discontinuation of the study drugs in the present study, it should become a valuable pharmacogenetic diagnostic tool to optimize the triple therapy.

In conclusion, this phase III study conducted in Japan demonstrated that telaprevir in combination with PEG-IFN/RBV had a high SVR rate for relapsers and shows promise as a potential therapy for nonresponders even with a short treatment period. Prolongation of telaprevir and PEG-IFN/RBV treatment should be a better option for achieving high SVR for nonresponders. As the data demonstrated convincingly that the benefits greatly outweigh the risks, telaprevir-based regimen is at the lead for the next generation of HCV therapies.

DISCLOSURES

None to declare.

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APPENDIX

The members of the phase III study were as follows: Sapporo Kosei General Hospital, Toranomon Hospital, Juntendo University Hospital, Musashino Red Cross Hospital, Toranomon Branch Hospital, University of Yamanashi Hospital, Shinshu University Hospital, Gifu Municipal Hospital, Ogaki Municipal Hospital, Nagoya University Hospital, Osaka University Hospital, Ikeda Municipal Hospital, Saiseikai Suita Hospital, Hiroshima University Hospital, Shin-Kokura Hospital, Kurume University Hospital and Kagoshima University Medical and Dental Hospital.

Dual Therapy With the Nonstructural Protein 5A Inhibitor, Daclatasvir, and the Nonstructural Protein 3 Protease Inhibitor, Asunaprevir, in Hepatitis C Virus Genotype 1b–Infected Null Responders

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Patients with chronic hepatitis C virus (HCV) infection and previous null response to pegylated interferon (Peg-IFN) and ribavirin (RBV) have limited therapeutic options. HCV genotype 1 is the most common worldwide and the most difficult to treat; genotype 1b is the most common subtype of genotype 1 outside North America. The enhanced antiviral activity achieved by combining two direct-acting antiviral (DAA) agents may improve clinical outcomes. This open-label, phase IIa study included 10 patients with chronic HCV genotype 1b infection and previous null response (<2 $\log_{10}$ reduction in HCV RNA after 12 weeks) to Peg-IFN and RBV. Patients received dual DAA treatment for 24 weeks with the nonstructural protein 5A replication complex inhibitor, daclatasvir (60 mg once-daily), and the nonstructural protein 3 protease inhibitor, asunaprevir (initially 600 mg twice-daily, then subsequently reduced to 200 mg twice-daily). The primary efficacy endpoint was the proportion of patients with sustained virologic response (SVR) at 12 weeks post-treatment (SVR_{12}). Nine patients completed 24 weeks of treatment; 1 patient discontinued treatment after 2 weeks. In the 9 patients who completed the full course of treatment, HCV RNA was undetectable at week 8 and remained undetectable through the end of treatment; all 9 patients achieved SVR_{12} and SVR_{24}. HCV RNA also remained undetectable post-treatment in the patient who discontinued after 2 weeks. There was no viral breakthrough. Diarrhea and headache, generally mild, were the most common adverse events; transaminase elevations were reported in 3 patients, but did not result in discontinuation. *Conclusions*: Dual therapy with daclatasvir and asunaprevir, without Peg-IFN and RBV, can achieve high SVR rates in difficult-to-treat patients with HCV genotype 1b infection and previous null response to Peg-IFN and RBV. (HEPATOLOGY 2012;55:742-748)

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Chronic hepatitis C virus (HCV) infection affects approximately 180 million individuals worldwide and is a common cause of chronic liver disease and hepatocellular carcinoma (HCC) in Japan, the United States, and many European countries.[1,2] Among the six major HCV genotypes, genotype 1 is the most common and the most difficult to treat, and its two main subtypes may differentially influence therapeutic outcomes.[3,4] Genotype 1b is the most prevalent worldwide and predominates in Japan and China, whereas genotype 1a is most common in the United States; subtype prevalence in Europe is similar.[5-7]

Abbreviations: ALT, alanine aminotransferase; cEVR, complete early virology response: undetectable HCV RNA at week 12; DAA, direct-acting antiviral; EOTR, end-of-treatment response: undetectable HCV RNA at week 24; eRVR, extended rapid virologic response: undetectable HCV RNA at weeks 4 and 12; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IL28B, *interleukin-28B; INR, international normalized ratio; LLQ, lower limit of quantitation; NS3, nonstructural protein 3; NS5A, nonstructural protein 5A; Peg-IFN-α, pegylated interferon alpha; PCR, polymerase chain reaction; RBV, ribavirin; RVR, rapid virologic response: undetectable HCV RNA at week 4; SNP, single-nucleotide polymorphism; SVR, sustained virologic response: undetectable HCV RNA post-treatment; SVR_{12}, sustained virologic response 12 weeks post-treatment; SVR_{24}, sustained virologic response 24 weeks post-treatment; ULN, upper limit of normal.*

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Treatment of chronic HCV infection with pegylated interferon alpha (Peg-IFN-α) and ribavirin (RBV) elicits a sustained virologic response (SVR) in 40%-50% of treatment-naïve patients with genotype 1 infections; SVR rates in this population increase to 66% or 75% when boceprevir or telaprevir, respectively, is added to the regimen.[8-12] Response rates are influenced by viral load and genotype and by patient demographics, disease history, and genetics.[10] Peg-IFN/RBV retreatment of patients with previous nonresponse to Peg-IFN/RBV is frequently unsuccessful, with SVR rates of only 6%-9%.[13,14] Null responders are the subset of nonresponders who have responded most poorly to Peg-IFN/RBV, and their urgent need for more potent therapies has prompted the evaluation of regimens containing direct-acting antivirals (DAAs). SVR rates of 27% (genotype 1a) and 37% (genotype 1b) were achieved in null responders with a regimen combining telaprevir with Peg-IFN/RBV in a study of nonresponders.[15] These results suggest that DAA-containing regimens can benefit this population, but greater antiviral potency is needed to increase response rates further.

Combinations of two DAAs may overcome IFN nonresponsiveness in null responders by increasing antiviral activity and reducing the risk of developing resistance-associated variants.[16] In HCV-infected human hepatocyte chimeric mice, dual DAA treatment eradicated HCV without resistance, whereas resistance emerged rapidly with single DAA treatment.[17] In a clinical study that included null responders, marked antiviral effects were observed after 13 days of dual DAA treatment, supporting the evaluation of longer term dual DAA therapy reported in this study.[18] Daclatasvir (BMS-790052) is a first-in-class, highly selective nonstructural protein 5A (NS5A) replication complex inhibitor with picomolar potency and broad genotypic coverage; asunaprevir (BMS-650032) is a nonstructural protein 3 (NS3) protease inhibitor active against HCV genotypes 1a and 1b.[19,20] Daclatasvir and asunaprevir are associated with different resistance-associated variants, consistent with their different molecular targets, and showed no meaningful pharmacokinetic interactions in healthy volunteers.[20-22]

In a 24-week study of null responders in the United States, daclatasvir and asunaprevir demonstrated potent antiviral effects, both as a dual DAA regimen and in a quadruple regimen that included Peg-IFN/RBV.[23] Overall, 36% of dual-therapy recipients achieved SVR, including both of the 2 patients with genotype 1b infection. However, patients with genotype 1a experienced frequent viral breakthrough with the dual regimen and only 2 of 9 achieved SVR, suggesting subtype-associated differences in resistance barrier and response. We present the results of an open-label trial evaluating dual therapy with daclatasvir and asunaprevir in Japanese patients with chronic HCV genotype 1b infection and previous null response to Peg-IFN/RBV.

Patients and Methods

Study Design. This open-label, phase IIa study (clinicaltrials.gov identifier NCT01051414) evaluated the antiviral activity and safety of daclatasvir combined with asunaprevir in patients with HCV genotype 1 infection and previous null response to treatment with Peg-IFN/RBV, defined as <2 $\log_{10}$ reduction of HCV RNA after 12 weeks of therapy. This sentinel cohort provided safety data for review by an independent study safety committee before the enrollment of additional cohorts that will be described in a subsequent report. 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. Patients eligible for enrollment in the sentinel cohort included men and women 20-75 years in age (women of childbearing potential were required to use adequate contraception) with chronic HCV genotype 1 infection for at least 6 months (all enrolled patients were genotype 1b because of the high prevalence of this subtype in Japan) and HCV RNA $\geq 10^5$ IU/mL. Eligible patients met criteria defining null responders and had no evidence of cirrhosis documented by laparoscopy, imaging, or liver biopsy within 2 years.

Patients were excluded if they had a history of HCC, coinfection with hepatitis B virus or human immunodeficiency virus, other chronic liver disease, or

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evidence of hepatic decompensation. Patients were also excluded if they had other severe or unstable conditions or evidence of organ dysfunction in excess of that consistent with the age of the patient, were unable to tolerate oral medication or had conditions that could affect the absorption of study drug, or were exposed to any investigational drug within 4 weeks of study participation or had any previous exposure to inhibitors of NS5A or NS3 protease. Laboratory findings that excluded participation were the following: alanine aminotransferase (ALT) >5× the 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 <1,500/mm^3; absolute neutrophil count <750/mm^3; platelets <50,000/mm^3; or creatinine >1.8× ULN.

Prohibited concomitant medications included inducers or inhibitors of cytochrome P450/3A4, non-study medications with anti-HCV activity, any prescription medication or herbal product not prescribed for a specific condition, liver-protection drugs, proton pump inhibitors, and erythropoiesis-stimulating agents. H_2 receptor antagonists were permitted, but administered ≥10 hours before or ≥2 hours after daclatasvir; other acid-modifying agents had to be taken ≥2 hours before or after daclatasvir.

Study Drug Dosing. All patients received oral combination therapy with daclatasvir and asunaprevir from the beginning of the study. Daclatasvir was dosed as two 30-mg tablets once-daily. Asunaprevir was initially dosed as three 200-mg tablets twice-daily; subsequently, the dose of asunaprevir was reduced to 200 mg twice-daily after reports of hepatic enzyme elevations in a clinical study of asunaprevir and Peg-IFN/RBV.[24]

Treatment was continued to week 24 for patients with HCV RNA below the assay lower limit of quantitation (LLQ; 15 IU/mL) on or after week 2; treatment was discontinued for patients with <2 $\log_{10}$ IU/mL decrease of HCV RNA from baseline or on or after week 2. For patients with viral rebound on or after week 2, or HCV RNA above LLQ on or after week 4, treatment was discontinued or weight-based Peg-IFN-RBV therapy was added for up to 48 additional weeks at the investigator's discretion, based on expected tolerance of Peg-IFN-RBV. Viral rebound was defined as an increase ≥1 $\log_{10}$ IU/mL from nadir at more than one time point or HCV RNA ≥15 IU/mL after declining to below that level.

Safety and Efficacy Assessments. Assessments, including HCV RNA, physical examination, vital signs, adverse events, laboratory tests, and review of concomitant medications, were conducted at screening, on study days 1 (baseline) through 7 and days 9, 11, and 14, at weeks 3, 4, 6, 8, 10, 12, 16, 20, and 24, and at post-treatment weeks 4, 8, 12, and 24. Twelve-lead electrocardiograms were recorded at all visits, except those at weeks 3 and 6. Additional pretreatment assessments included HCV genotype and host interleukin-28B (*IL28B*) genotype.

Serum HCV RNA levels were determined at a central laboratory using the Roche COBAS TaqMan HCV Auto assay (LLQ = 15 IU/mL; Roche Diagnostics KK, Tokyo, Japan). HCV genotype and subtype were determined at the central laboratory by polymerase chain reaction (PCR) amplification and sequencing. *IL28B* genotype was determined by PCR amplification and sequencing of the rs12979860 single-nucleotide polymorphism (SNP).

Outcome Measures. The primary efficacy endpoint was the proportion of patients with undetectable HCV RNA at 12 weeks post-treatment (SVR_{12}). Secondary endpoints included the proportions of patients with rapid virologic response (RVR; defined as undetectable HCV RNA at week 4), extended RVR (eRVR; undetectable HCV RNA at weeks 4 and 12), complete early virologic response (cEVR; undetectable HCV RNA at week 12), end-of-treatment response (EOTR; undetectable HCV RNA at week 24), and SVR at 24 weeks post-treatment (SVR_{24}).

The possible presence of HCV-resistance polymorphisms was analyzed using stored specimens. Resistance testing was performed on all samples at baseline and on samples indicative of virologic failure, defined as either (1) <2 $\log_{10}$ HCV RNA decrease from baseline at week 2, (2) virologic rebound (HCV RNA detectable after previously undetectable or ≥1 $\log_{10}$ increase from nadir), or (3) detectable HCV RNA at weeks 4 or 12 or at the end of therapy. Resistance analysis methodology included isolation of HCV RNA, PCR amplification, and population sequencing of HCV NS3 protease and NS5A domains.

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

Results

Patient Characteristics and Disposition. Twelve patients were screened; 2 patients failed to meet entry criteria (for HCC and elevated direct bilirubin, respectively), and 10 were enrolled and treated. Enrolled patients were generally older (median, 62 years); 6

Table 1. Baseline Demographic and Disease Characteristics

Parameter	Value
N	10
Age, median years (range)	62 (52-70)
Male sex, n (%)	4 (40)
Japanese race, n (%)	10 (100)
Host *IL28B* genotype,* n (%)	
CC	2 (20)
CT	8 (80)
HCV genotype 1b, n (%)	10 (100)
HCV RNA, mean $\log_{10}$ IU/mL (SD)	6.8 (0.61)
ALT, mean U/L (SD)	60.6 (32.9)
Platelets $\times 10^9$ cells/mL, median (min, max)	150.5 (84.0, 166.0)
Total bilirubin, median mg/dL (min, max)	0.8 (0.6, 1.2)
Albumin, median g/dL (min, max)	3.9 (3.1, 4.2)
INR, median (min, max)	1.0 (1.0, 1.1)

*SNP rs12979860.

Abbreviation: *IL28B*, interleukin-28B; HCV, hepatitis C virus; SD, standard deviation; ALT, alanine aminotransferase; min, minimum; max, maximum; INR, international normalized ratio; SNP, single-nucleotide polymorphism.

were female and all were Japanese (Table 1). All enrolled patients were infected with genotype 1b, reflecting the predominance of this subtype in Japan, although the study protocol did not exclude patients with HCV genotype 1a.[6] Two patients were *IL28B* genotype CC (SNP rs12979860) and 8 were CT. Nine patients completed 24 weeks of therapy; 1 patient discontinued at week 2 because of a grade 4 total bilirubin elevation (see below). Among the 9 patients treated for 24 weeks, asunaprevir was dosed at 600 mg twice-daily for 12-21 weeks before the dose was reduced to 200 mg twice-daily (Fig. 1).

Virologic Response. Serum HCV RNA levels decreased rapidly in all patients (Fig. 2); mean reductions from baseline were 4.4 $\log_{10}$ IU/mL at week 1,

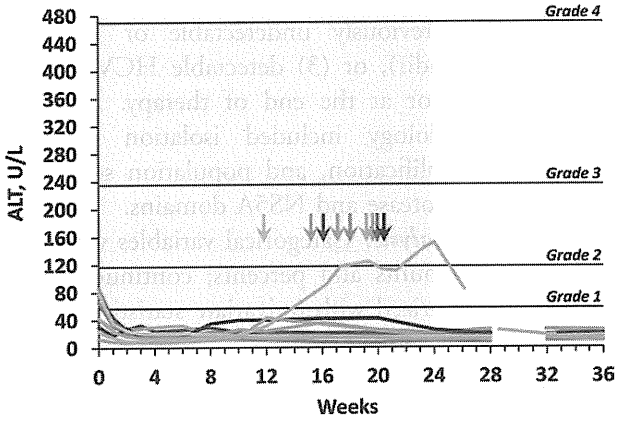

Fig. 1. ALT levels: individual patients. Serum ALT levels for the 9 patients who completed 24 weeks of treatment; the patient who discontinued at week 2 is not presented. Shaded area indicates the treatment period; arrows indicate the points at which the dose of asunaprevir was reduced from 600 to 200 mg twice-daily. Arrow and line colors are the same for each patient.

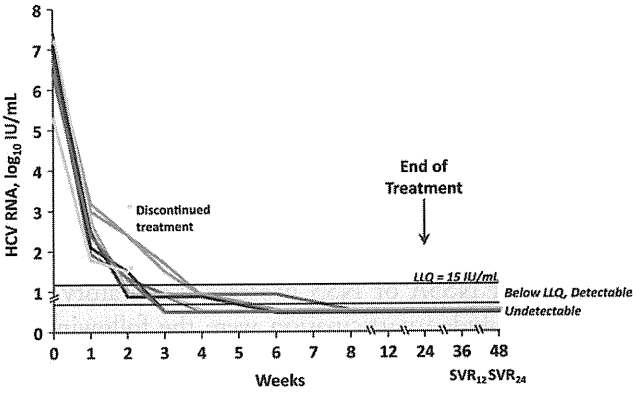

Fig. 2. HCV RNA levels: individual patients. Individual patient plasma HCV RNA levels during 24 weeks of treatment and through 24 weeks post-treatment (week 48) are shown. LLQ = 15 IU/mL.

5.3 $\log_{10}$ IU/mL at week 2, and 5.8 $\log_{10}$ IU/mL from week 4 through the end of treatment. At week 4, HCV RNA was undetectable (RVR) in 4 of 10 (40%) patients and below the assay LLQ in 9 of 10 (90%; Fig. 3). No patients qualified for discontinuation or addition of pegIFN/RBV. At week 8, HCV RNA was undetectable in 9 of 10 patients (all who remained on treatment) and remained undetectable through the end of treatment and follow-up. SVR_{12}, the primary endpoint, and SVR_{24} were achieved by 90% of patients, including all 9 who completed 24 weeks of therapy. The patient who discontinued treatment at week 2 had low-level HCV RNA at discontinuation (1.8 $\log_{10}$ IU/mL), but HCV RNA was undetectable at follow-up visits 2, 3, 4, 13, and 24 weeks after discontinuation.

Viral Breakthrough and Relapse. There was no viral breakthrough during treatment or relapse of HCV RNA post-treatment. Analysis of baseline samples revealed variants reported to confer minimal to low

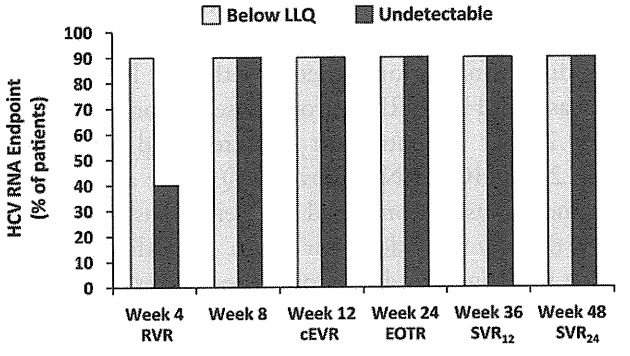

Fig. 3. HCV RNA endpoints. Categorical HCV RNA endpoints are indicated for the 10 study patients. One patient discontinued at week 2 and was counted as a treatment failure at the time points shown. However, HCV RNA was undetectable in this patient at 2, 3, 13, and 24 weeks post-treatment.

Table 2. On-Treatment Adverse Events Occurring in ≥2 Patients

Event	Patients, n (%)
Diarrhea	7 (70)
Headache	4 (40)
ALT increased	3 (30)
AST increased	3 (30)
Lymphopenia	2 (20)
Abdominal discomfort	2 (20)
Malaise	2 (20)
Pyrexia	2 (20)
Nasopharyngitis	2 (20)
Lipase increased	2 (20)
Back pain	2 (20)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

levels of resistance to daclatasvir.[22] NS5A substitutions L28M and L31M were detected in 1 patient each, and Y93H was detected in 2 other patients. NS3 protease substitutions reported to confer resistance to telaprevir, boceprevir, and TMC-435 were detected[25]; T54S was identified in 1 patient, and Q80L was identified in 3. In 1 patient, both NS3 protease substitutions (T54S and Q80L) and an NS5A substitution (Y93H) were detected. There was no consistent association between detection of these variants and virologic outcomes.

Safety. The most frequently reported adverse events were diarrhea and headache, all of which were mild (grade 1) (Table 2). The patient who discontinued (see below) experienced multiple grade 3 or 4 adverse events and laboratory abnormalities on treatment. In the other 9 patients, there were no grade 3 or 4 transaminase elevations or other grade 3 or 4 events, no clinically relevant changes in electrocardiogram parameters, and no lymphopenia of any severity. Two transient grade 1 ALT elevations were reported, and 1 grade 2 elevation that began at week 16 and persisted until the end of treatment, after which it normalized within 2 weeks (Fig. 1). There were no notable differences in ALT before and after asunaprevir dose reduction.

There were two serious adverse events. A 54-year-old male was hospitalized with grade 3 pyrexia and persistent diarrhea 11 days after initiating study treatment. Loxoprofen was initiated, and body temperature normalized and diarrhea improved after 4 days. The patient remained on study treatment. The second event concerned a 60-year-old woman with a history of ulcerative colitis who discontinued study treatment after 2 weeks because of a grade 4 bilirubin elevation with multiple complicating features. Five days before discontinuation, she presented with infectious gastroenteritis and was treated with cefotiam and was subsequently hospitalized with fever, vomiting, and diarrhea. Meropenem, human serum albumin, and furosemide were initiated. At discontinuation of study drugs, laboratory findings included total bilirubin of 7.7 mg/dL and grade 3 lymphopenia and serum phosphorus reduction; transaminases and alkaline phosphatase were within normal ranges. In the week after discontinuation, white cell and eosinophil counts became elevated; total bilirubin improved and transaminases remained normal. Two weeks after discontinuation, grade 4 ALT and aspartate-aminotransferase elevations and a grade 3 lipase elevation were reported. Six weeks after discontinuation, bilirubin and transaminase elevations were resolved and lipase improved to within 2× ULN.

Discussion

This study assessed combination oral DAA therapy in a difficult-to-treat population with multiple adverse prognostic features, including HCV genotype 1b infection, primarily *IL28B* CT genotype, generally older age, and null response to previous Peg-IFN/RBV therapy.[10,13,14] These patients represent a group with a significant need for new therapeutic options.

A DAA-only therapeutic strategy may be particularly appropriate for null responders, who have previously shown only marginal response to Peg-IFN/RBV.[13,14] The combination of two highly potent DAAs cleared detectable virus rapidly in this study; HCV RNA was undetectable by week 8 in all 9 patients treated for 24 weeks. This outcome compares favorably with those observed when null responders received a combination of Peg-IFN/RBV and a single NS3 protease inhibitor, telaprevir or TMC435.[15,26] In these studies, HCV RNA remained detectable in 36% to approximately 50% of patients after 12 weeks.

HCV RNA remained undetectable 12 (SVR_{12}) and 24 weeks (SVR_{24}) post-treatment in all patients who completed treatment. This contrasts with the poor results obtained with Peg-IFN/RBV retreatment and the reported 37% SVR rate of genotype 1b null responders who received Peg-IFN/RBV and telaprevir.[10,13-15] Additional follow-up of patients from this study will assess whether SVR_{24} is predictive of long-lasting viral clearance with this dual DAA therapy, as it is with Peg-IFN/RBV. It is interesting that HCV RNA was persistently undetectable post-treatment in the patient who discontinued after only 2 weeks of treatment. With early discontinuation data from only this single case, at present, the result must be considered an anomaly. The factors that contributed to viral

clearance are uncertain, although the patient's *IL28B* CC genotype suggests increased sensitivity to endogenous interferon[27]; the possible influence of concurrent acute gastroenteritis or other complicating factors is unknown. However, coupled with the attainment of SVR_{12} in all other patients, this outcome suggests that required duration of therapy, which is currently predicated on data from Peg-IFN-based regimens, may need reassessment for DAA-only regimens, and, possibly, that certain patient populations can be treated for very short durations.

The high SVR rate is consistent with limited data from a related U.S.-based study, in which 2 of 2 null responders with HCV genotype 1b and who were treated with daclatasvir and asunaprevir achieved SVR_{24}.[23] However, only 2 of 9 patients with genotype 1a achieved SVR_{24} with the dual DAA regimen, compared with 9 of 10 patients who received both DAAs and Peg-IFN/RBV. These differences suggest that viral genotype can influence responses to DAA regimens that do not include Peg-IFN/RBV, and outcomes can be optimized with individualized therapy that considers viral genotype, among other factors. Because of the high SVR rate, the potential influence of other baseline and on-treatment parameters could not be assessed, other than to observe that unfavorable predictors of Peg-IFN/RBV response, such as older age and *IL28B* CT genotype,[27,28] had no measureable impact on outcomes.

There was no viral breakthrough on treatment. In view of the rapid emergence of resistance in some studies of short-term DAA monotherapy,[29,30] these findings support the concept that dual DAA therapy reduces the risk of viral breakthrough, in addition to increasing antiviral activity. Resistance analyses revealed that before treatment, some patients carried NS5A and NS3 polymorphisms predicted to reduce sensitivity to daclatasvir and some HCV protease inhibitors, respectively.[22,25] There was no clear relationship between the presence of these polymorphisms and minor interpatient differences in the rate of early virologic response; however, further study in larger patient cohorts will help determine whether baseline polymorphisms can influence virologic response with this regimen.

The adverse event profile of the dual DAA regimen compares favorably with the more frequent and severe events reported with Peg-IFN/RBV, although patient numbers in this study were limited. The mild diarrhea experienced by several patients has been reported previously with asunaprevir and is common with other drugs of this class.[15,18,24] Though a role for daclatasvir and/or asunaprevir in the two serious adverse events could not be ruled out and the investigator considered these events drug related, multiple confounding factors existed. The case of pyrexia was consistent with a viral infection and resolved with treatment. In the case of hyperbilirubinemia that led to discontinuation, the time course of laboratory abnormalities and related events suggests a link to the use of cefotiam and meropenem for treatment of infectious gastroenteritis. Both of these agents have been associated with vomiting, diarrhea, and hyperbilirubinemia.[31,32]

The asunaprevir dose was reduced during treatment because of transaminase elevations observed with 600 mg twice-daily in a concurrent study.[24] In this sentinel cohort, viral suppression was maintained in all patients after dose reduction, and no grade 3 or 4 transaminase elevations occurred during treatment at either dose of asunaprevir. One patient experienced grade 2 transaminase elevations that began at week 16 and persisted during treatment, despite asunaprevir dose reduction at week 19. Although these elevations were not severe, their rapid normalization post-treatment suggests a possible relationship to study treatment. None of the 9 patients treated for 24 weeks experienced transaminase elevations post-treatment. Although grade 4 transaminase elevations occurred 2 weeks post-treatment in the patient who discontinued, the timing of these events and multiple other complications suggest that they were not related directly to study treatment.

In conclusion, the combination of daclatasvir and asunaprevir achieved a high rate of SVR_{24} in patients with HCV genotype 1b infections and previous null response to Peg-IFN/RBV. These results support the concept that HCV infection can be cured with two DAAs without Peg-IFN/RBV, even in difficult-to-treat populations that lack robust IFN responsiveness. Further research will assess the benefits of DAA combinations in larger, more diverse patient populations.

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REVIEW

Treatment of chronic hepatitis C virus infection in Japan: update on therapy and guidelines

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Abstract Hepatitis C virus (HCV) infection is a serious health problem leading to cirrhosis, liver failure and hepatocellular carcinoma. The recent introduction of telaprevir, which was approved in November 2011, in combination with peg-interferon and ribavirin is expected to markedly improve the eradication rate of the virus. However, side effects of triple therapy may be severe. In a phase three III clinical trial, 2250 mg of telaprevir, which is the same dosage used in clinical trials in Western countries, was given to Japanese patients. As this dosage is considered to be relatively high for Japanese patients, who typically have lower weight than patients in Western countries, reduction of telaprevir is recommended in the 2012 revision of the guidelines established by the Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis published by the Ministry of Health, Labour and Welfare of Japan. Other protease inhibitors with fewer side effects are now in clinical trials in Japan. Alternatively, treatment of patients with combination of direct acting antivirals without interferon has been reported. In this review we summarize current treatment options in Japan and discuss how we treat patients with chronic HCV infection.

Keywords Telaprevir · Triple therapy · Antiviral resistance · Anemia · Dose reduction

Abbreviations

HCV	Hepatitis C virus
DAAs	Direct acting anti-virals
SVR	Sustained virological response
RVR	Rapid virological response

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Introduction

At least 1.5 million people in Japan and more than 200 million people worldwide are chronically infected with the hepatitis C virus [1, 2]. Due to an aging patient population, the health burden of chronic HCV infection in Japan is expected to increase over the next several decades [3]. Chronic infection develops in 60–80 % of symptomatic patients, leading to higher risk of cirrhosis, hepatocellular carcinoma, and end-stage liver disease. Chronic HCV infection is also one of the primary indications for liver transplantation [3], and ultimately 5–7 % of patients die from complications related to HCV infection [4–7].

The goal of HCV therapy is successful eradication of the virus and resolution of liver disease. Success is defined as

the absence of detectable virus 24 weeks following the end of treatment. In some patients, the virus becomes undetectable by the end of treatment (end of treatment response) but then rebounds in the absence of therapy (relapse or transient response). Viral breakthrough occurs when the virus rebounds during the course of therapy. In non-responders, the virus remains detectable throughout therapy.

Therapy for chronic HCV infection

Hepatitis C virus genotypes vary by region and susceptibility to interferon treatment [8]. Genotype 1 is the most common genotype worldwide and in Japan [8]. Weekly injections of pegylated interferon (peg-interferon) and daily oral administration of ribavirin constitute the standard therapy for genotype 1 chronic HCV [9]. However, combination therapy is costly and poorly tolerated, requires long-term treatment (48 weeks), and is successful in only 42–52 % of patients [10–12].

The success rate of HCV therapy in Japan is expected to improve greatly following the November 2011 approval of telaprevir (VX-950/MP-424; Incivek; Vertex Pharmaceuticals, Inc., Cambridge, MA, USA), the first in a class of new direct acting antiviral (DAA) drugs. Teleprevir and a related drug, boceprevir (Victrelis), were also recently approved for treatment of genotype 1 in the US, Canada, and the European Union. While boceprevir is not approved for use in Japan, a meta-analysis found no difference in outcomes between the two drugs, except for slightly higher efficacy among prior relapsers using telaprevir [13].

Telaprevir and direct acting antiviral drugs

DAAs act by specifically inhibiting essential viral targets. Telaprevir is an NS3/4 serine protease inhibitor that mimics the carboxy-terminal region of the NS3 protease and binds slowly and tightly to the protease [14]. The NS3-4A protein is also an attractive target due to its additional role in degrading immune signaling molecules [15]. Consequently, targeting NS3-4A may not only disrupt viral replication but may also help to restore innate antiviral responses [16, 17]. However, treatment with telaprevir alone often results in a rapid decline in viral load followed by viral breakthrough due to rapid selection for resistance mutations [18, 19]. Triple therapy with peg-interferon, ribavirin, and telaprevir appears to be required to suppress viral breakthrough and achieve SVR [20].

Telaprevir clinical trials outside of Japan

Phase II studies

Several phase II and III clinical trials have established the safety and efficacy of telaprevir in the treatment of HCV genotype 1 (Table 1). The PROVE I [20] and PROVE II [21] phase II studies showed SVR rates significantly higher for triple therapy compared to the standard of care (61 vs. 41 %, 69 vs. 46 %, respectively) after 12 weeks of triple therapy followed by another 12 weeks of peg-interferon plus ribavirin combination therapy. Both studies found that reducing the length of peg-interferon and ribavirin to 12 weeks erased the advantage of triple therapy over standard therapy, and PROVE II revealed that ribavirin is required to suppress viral breakthrough [20, 21]. PROVE III examined the efficacy of triple therapy in patients who failed to achieve SVR during prior interferon therapy and reported improved SVR rates among patients with prior nonresponse (39 %), relapse (69 %), or viral breakthrough (57 %) [22].

Phase III studies

The phase III ADVANCE study compared duration of telaprevir therapy in treatment-naive patients using three treatment arms, a control peg-interferon plus ribavirin group and 8 and 12 week telaprevir triple therapy groups followed by response-guided peg-interferon plus ribavirin combination therapy [23] (Table 1). SVR rates were 69 % for the 8 week telaprevir treatment and 75 % for the 12 week telaprevir treatment, compared to 44 % for standard peg-interferon plus ribavirin combination therapy. The phase III REALISE study assessed response to triple therapy in patients with prior treatment failure [24]. Prior relapsers, partial responders, and null responders were randomized to a 48 week peg-interferon plus ribavirin control group or to 48 week triple therapy groups with 12 weeks of telaprevir with or without a 4 week peg-interferon plus ribavirin lead-in phase. SVR rates in the triple therapy group were 66 % with the lead-in phase and 64 % without it, compared to only 17 % in the control group. When analyzed by response to prior treatment, prior relapsers showed the strongest improvement in SVR rates, but triple therapy also appears to benefit prior null and partial responders as well [24–26]. Based on these studies, the U.S. Food and Drug Administration (FDA) approved response-guided therapy (RGT) for prior relapsers who achieved extended rapid virological response (eRVR) [27]. This allows prior relapsers to discontinue all treatment after 24 weeks if HCV RNA is undetectable at weeks 4 and 12. In Japan, duration of triple therapy is 24 weeks without regard for response to prior treatment.

Table 1 Summary of telaprevir clinical trials

Study	Design	Results
PROVE I	Phase II; $N = 233$	SVR
McHutchison et al. [20]	T12PR24: 12 week TVR + 24 week PR	PR: 41 %
	T12P48: 12 week TVR + 24 week PR	T12PR24: 61 %
	PR48: 12 week placebo + 48 week PR	T12P48: 67 %
PROVE II	Phase II; $N = 334$	SVR
Hezode et al. [21]	T12PR24: 12 week TVR + 24 week PR	PR48: 46 %
	PR48: 12 week placebo + 48 week PR	T12PR24: 69 %
PROVE III	Phase II; $N = 465$	SVR
McHutchison et al. [22]	Patients with prior PR treatment failure	T12PR24: 51 %
	T12PR24: 12 week TVR + 24 week PR	T24PR48: 53 %
	T24PR48: 12 week TVR + 24 week PR	T24P24: 24 %
	T24P24: 12 week TVR + 24 week PR	PR48: 14 %
	PR48: 12 week placebo + 48 week PR	
ADVANCE	Phase III double-blind; $N = 1088$	SVR
Jacobson et al. [23]	Treatment-naïve patients	T8PR: 69 %
	T8PR: 8 week TVR + 24 or 48 week PR RGT	T12PR: 75 %
	T12PR: 12 week TVR + 24 or 48 week PR RGT	PR: 44 %
	PR: 12 week placebo + 48 week PR	
ILLUMINATE	Phase III open-label; $N = 540$	SVR
Sherman et al. [55]	Treatment-naïve patients	T12PR24: 92 %
	T12PR24: 12 week TVR + 24 or 48 week PR RTG	T12PR48: 88 %
	T12PR48: 12 week TVR + 48 week PR	
REALIZE	Phase III; $N = 662$	SVR by treatment
Zeuzem et al. [24]	Patients with prior PR treatment failure	T12P48: 64 %
	T12PR48: 8 week TVR + 48 week PR	Lead-in T12P48: 66 %
	Lead-in T12PR48: 4 week PR + 8 week TVR + 48 week PR	PR48: 17 %
	PR48: 12 week placebo + 48 week PR	SVR by prior history
		Relapsers: 83–88 %
		Partial responders: 54–58 %
		Non-responders: 29–33 %
Yamada et al. [32]	Phase Ib; $N = 10$	ETR: 10 %
	Treatment-naive Japanese patients	
	TVR monotherapy: 12 week	
Ozeki et al. [19]	Phase IIa; $N = 4$; single-arm, open label	SVR (off-study): 100 %
	Older female Japanese patients with prior PR treatment failure	
	TVR monotherapy: 24 week + off-study PR	
Toyota et al. [33]	Phase II; $N = 15$; single-arm, open-label	SVR: 7 %
	Treatment-naive Japanese patients	
	TVR monotherapy: 24 week	
Kumada et al. [28]	Phase III; $N = 189$	SVR
	Treatment-naïve Japanese patients	TR12P24: 73 %
	TR12P24: 12 week TVR + 12 week PR	P48: 49 %
	P48: 48 week PR	

Table 1 continued

Study	Design	Results
Hayashi et al. [31]	Phase III; $N = 141$ Patients with prior PR treatment failure TR12P24: 12 week TVR + 12 week PR P48: 48 week PR	SVR Relapsers: 88 % PR48: 34 %

TVR telaprevir, *PR* peg-interferon plus ribavirin combination therapy, *RGT* response-guided therapy—24 week PR if undetectable HCV RNA at weeks 4 and 12 (eRVR); otherwise 48 week PR, *ETR* end-of-treatment response

Clinical trials of telaprevir in Japan

Triple therapy in treatment-naive patients

Although Asians are under-represented in the above studies (1–2 %), several phase II and III clinical trials have also been performed in Japan (Table 1). In Kumada et al. [28], 126 patients were randomly assigned to 12 weeks of telaprevir triple therapy followed by 12 weeks of combination therapy, and 63 patients were assigned to 48 weeks of combination therapy. Early viral dynamics varied greatly between the two groups, with more rapid and extensive loss of HCV RNA and a significantly higher rate of SVR in the triple therapy group (73.0 vs. 49.2 %). Rates of viral breakthrough and relapse did not differ between the treatment groups. However, patients who underwent triple therapy experienced a significantly higher incidence of side effects during the telaprevir phase of the treatment. Because HCV patients in Japan tend to be more than 10 years older than patients in Western countries and include a higher proportion of women, ribavirin-induced anemia is of particular concern [29]. Moderate or severe anemia developed in 38.1 % of patients in the triple therapy group compared to 17.5 % in the combination therapy group [30]. The ribavirin dose was adjusted accordingly, resulting in a lower total ribavirin dose in the triple therapy group. However, ribavirin dose reduction did not significantly impact treatment efficacy. Skin disorders were about twice as common in triple therapy patients (46.8 vs. 23.8 %), and severe skin lesions were only observed in this group. Due to the higher SVR rate and shorter duration of triple therapy, the study authors recommend triple therapy over combination therapy for treatment of HCV genotype 1 in Japan but stress the need for careful monitoring of hemoglobin levels and close coordination with a dermatologist.

Triple therapy in patients with prior treatment failure

In a second phase III clinical trial in Japan, Hayashi et al. [31] examined the safety and efficacy of triple therapy for difficult-to-treat patients who either relapsed (109) or failed to respond to prior interferon therapy (32). As in the previous studies, patients were treated to 12 weeks of triple therapy followed by 12 weeks of combination therapy. SVR rates were 88.1 % for prior relapsers and 34.4 % for prior non-responders. Adverse events were common but moderate. 82 % of patients experienced rash or other skin disorders, mainly during the telaprevir phase, and nearly all (98.6 %) patients required ribavirin dose reduction for anemia, although ribavirin dose reduction had no effect on SVR rate down to about 20 % of the planned dose. Telaprevir was discontinued in 21.3 % of patients, and all drugs were discontinued in 16.3 % of patients. SVR rates in prior relapsers were significantly higher among men than women (93.9 vs. 79.1 %), but there was no difference among prior non-responders. Rates of viral breakthrough (18.8 %) and relapse (40.6 %) were significantly higher among prior non-responders and were more common after completion of the telaprevir phase, suggesting that extension of telaprevir therapy past 12 weeks or continuation of combination therapy past 24 weeks may improve response for prior non-responders. The study authors recommend weekly hemoglobin monitoring and note that even sharp reductions in ribavirin dose my allow therapy to continue without adversely affecting outcome.

Side effects of telaprevir in clinical trials in Japan

An early phase Ib study was conducted in Japan to examine the safety, tolerability, and antiviral profile of telaprevir monotherapy over 12 weeks in 10 treatment-naive patients with high viral loads of genotype 1b [32]. Telaprevir was well tolerated and no serious adverse events occurred, but 80 % of patients developed a rash and 70 % experienced anemia. Telaprevir monotherapy demonstrated potent antiviral activity, with HCV RNA levels decreasing by 2.3 $\log_{10}$ by 16 h and by 5.2 $\log_{10}$ after 2 weeks. HCV RNA dropped to the limit of detection or became undetectable in all patients during the course of therapy, but only one patient achieved an end-of-treatment response. Viral breakthrough occurred in 8 patients, mainly due to Ala156 mutation. However, resistance mutants reverted to wild type during the 24 week follow-up period.

Another study examined safety and efficacy of telaprevir monotherapy over a longer duration of 24 weeks with a larger number of patients and a greater range of viral loads [33]. The only patient who achieved SVR also had the lowest baseline viral load (3.55 $\log_{10}$ IU/ml), but three other patients were able to achieve an end-of-treatment response. HCV RNA levels decreased rapidly (average −5 $\log_{10}$ IU/ml), and HCV RNA became undetectable in 5 patients within 8 weeks. 10 out of 15 patients (66 %) discontinued the drug due to viral breakthrough, adverse events, or other causes. Incidence of adverse events was high (14/15 patients) and 7 out of 15 patients (47 %) developed anemia, but most incidences were mild to moderate, and anemia did not lead to discontinuation of therapy. T54A and A156V variants were the most common and were not detectable at earlier time points. Secondary substitutions at V158I and I132L were also observed.

SVR rates tend to be lower among women than men over 50 in Japan (53 vs. 22 %), and dose reductions and discontinuation of treatment in standard therapy are high in this group [34]. Ozeki et al. [19] examined 24 weeks of telaprevir monotherapy in a group of four older female patients predicted to be difficult to treat due to age, sex, and Core70 and ISDR substitutions. All patients required telaprevir dose reduction due to anemia but did not require discontinuation. Resistance variants were detected in three patients, and two patients experienced viral breakthrough. Additional substitutions and variants emerged as therapy progressed. However, at the end of the telaprevir administration, all four patients were given at least 48 weeks of standard therapy, and all patients were able to achieve SVR. Although this approach results in longer duration of therapy, it avoids the need for simultaneous administration of the three drugs and takes advantage of the fact that resistance mutants selected during telaprevir therapy often have reduced fitness compared to the wild type and are more susceptible to standard therapy.

Telaprevir antiviral resistance

Pre-existence of resistance mutations and selection for resistance may be an inevitable consequence of DAA therapy [35]. The high replication rate of HCV high (10^{12} viruses per day) coupled with the low fidelity of HCV polymerase results in a high mutation rate (10^{-3}–10^{-5} per day) and the presence of viral quasispecies. Single and double substitutions from the consensus sequence are expected to exist at low frequency prior to therapy. The relative proportion of these variants increases rapidly in the viral population as the wild-type virus is eradicated. De novo mutations appear to play only a minor role in the emergence of resistance mutations, suggesting that a genetic barrier of three to four mutations might be sufficient to reduce selection based on pre-existing mutants. At the same time, mutations conferring resistance often have reduced fitness and may require compensatory mutations in order to compete with wild-type viruses. Nonetheless, HCV sub-genotypes vary substantially in sequence, and some are likely to have a reduced genetic barrier against certain DAAs. For example, viral genotypes 1a and 1b already have different genetic barriers to telaprevir resistance; amino acid substitution of amino acid 155 requires only one nucleotide change in genotype 1a, whereas genotype 1b requires two nucleotide substitutions [36, 37]. Resistance substitutions at six major sites within the NS3 HCV protease have been reported, including at amino acids 36, 54, 155, 156, 168, and 170, and some substitutions are known to act synergistically [35]. At least 50 direct-acting antiviral drugs are at some stage of development, but these belong to a small number of distinct drug classes, increasing the risk of cross-resistance. Although wild-type strains are typically restored following removal of the drug due to viral breakthrough, prior treatment experience with DAAs, especially in high-risk subpopulations such as injection drug users, may increase the risk of transferring partially resistant strains during new infections.

Patient selection and predictive factors for triple therapy

Telaprevir triple therapy is an extension of peg-interferon plus ribavirin combination therapy. Therefore, factors that predict the outcome of combination therapy might also help to predict outcome of triple therapy. Age, fibrosis, obesity, hepatic steatosis [38], LDL cholesterol, gamma-GTP [39], insulin resistance [40], baseline viral titer [38, 41], and IL28B SNP genotype [42–44] are known to affect response to combination therapy. HCV genotype [41] and genetic variants within the viral genome, including amino acid substitutions at positions 70 (Core70) and 91 (Core91) of the HCV core protein and substitutions within the NS5A interferon sensitivity determining region (ISDR) [45, 46], are also thought to influence response to combination therapy. Akuta et al. [47] reported that Core70 substitution and partial response to prior therapy were significant predictors of SVR for triple therapy, and partial response and alpha-fetoprotein levels were significant predictors of end-of-treatment response. Chayama et al. [26] reported that IL28B SNP genotype, rapid virological response (RVR), and response to prior therapy were predictive of outcome of triple therapy. Prior relapsers achieved high levels of SVR (93 %), whereas patients who failed to respond to combination therapy were also less likely to respond to triple therapy. ITPA SNP genotype did