Ⅳ．研究成果の刊行物・別刷

Research Article

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Telaprevir with peginterferon and ribavirin for treatment-naive patients chronically infected with HCV of genotype 1 in Japan

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Background & Aims: To evaluate the efficacy and safety of telaprevir in combination with peginterferon-α2b (PEG-IFN) and ribavirin (RBV) in patients with chronic hepatitis C.

Methods: In a multi-center randomized clinical trial in Japan, on patients infected with HCV of genotype 1, 126 patients were assigned to telaprevir for 12 weeks along with PEG-IFN and RBV for 24 weeks (Group A), while 63 to PEG-IFN and RBV for 48 weeks (Group B).

Results: HCV RNA disappeared more swiftly in patients in Group A than B, and the frequency of patients without detectable HCV RNA at week 4 (rapid virological response (RVR)) was higher in Group A than B (84.0% *vs.* 4.8%, p <0.0001). Grade 3 and 4 skin disorders, including Stevens–Johnson syndrome and drug rashes with eosinophilia and systemic symptoms, as well as Grade 3 anemia (<8.0 g/dl), occurred more frequently in Group A than B (skin disorders, 11.9% *vs.* 4.8%; anemia, 11.1% *vs.* 0.0%). The total RBV dose was smaller in Group A than B (47.0% *vs.* 77.7% of the target, p <0.0001). Despite these drawbacks, sustained virological response (SVR) was achieved more frequently in Group A than B (73.0% *vs.* 49.2%, p = 0.0020).

Conclusions: Although the triple therapy with telaprevir-based regimen for 24 weeks resulted in more adverse events and less total RBV dose than PEG-IFN and RBV for 48 weeks, it was able to achieve higher SVR within shorter duration by carefully monitoring adverse events and modifying the RBV dose as required.

Keywords: Telaprevir; Chronic hepatitis C; Peginterferon; Ribavirin; Sustained virological response; Genotypes.

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Abbreviations: PEG-IFN, peginterferon; RBV, ribavirin; SVR, sustained virological response; SOC, standard of care; DAA, direct acting antiviral.

Introduction

Over the world, an estimated 170 million people are persistently infected with hepatitis C virus (HCV) [1]. Most individuals with persistent HCV infection can fulfill the life expectancy, while about 30% of them develop life-threatening liver disease such as decompensated cirrhosis and hepatocellular carcinoma [2,3].

Currently, interferon (IFN) is the only antiviral drug capable of terminating HCV infection. The present standard-of-care (SOC) therapy for patients infected with HCV of genotype 1, the most prevalent genotype over the world, is peginterferon (PEG-IFN) combined with ribavirin (RBV) for 48 weeks. However, sustained virological response (SVR), judged by the loss of detectable HCV RNA from serum 24 weeks after the completion of therapy, can be achieved in only 42–52% of the patients [4–6]. To cope with this grim situation, a number of direct acting antivirals (DAAs) have been designed and developed, represented by NS3/4A protease inhibitors and NS5B polymerase or NS5A inhibitors [7]. Among them, telaprevir has shown promising results, when combined with PEG-IFN and RBV, in the phase 2 [8,9] and 3 clinical trials [10,11], by improving SVR to ~70% in patients infected with HCV-1.

Previous trials with the triple therapy were conducted in Europe and the United States, respectively. Hence, Asians were under-represented, accounting only for 1.6–2.1% of studied patients, and distributions of genotypes 1a (44–67%) and 1b (27–55%) varied widely [8–10]. In view of ethnic differences in response to IFN-based treatments [12,13], as well as profiles of resistance to telaprevir difference between genotypes 1a and 1b [14], a multi-center, randomized, and treatment-controlled clinical trial was conducted for comparison of therapeutic efficacy between the triple therapy and SOC in patients infected with HCV-1b in Japan.

Patients and methods

Patients

From November 2008 through August 2010, 220 patients, who were infected with HCV-1 and had not received antiviral treatments before, were recruited at 41 institutions in Japan. They joined the study for finding differences in the

Table 1. Baseline characteristics of patients.

Features[a]	Group A: T12PR24 (n = 126)	Group B: PR48 (n = 63)
Men (%)	66 (52.4%)	33 (52.4%)
Age (years)	53.0 (20-65)	55.0 (20-65)
Weight (kg)	60.2 (40.7-87.5)	64.1 (42.1-84.9)
BMI (kg/m^2)	22.6 (16.2-31.1)	23.3 (17.9-30.8)
Hemoglobin (g/dl)	14.3 (12.1-17.1)	14.5 (12.3-17.5)
White blood cells (/mm^3)	5300 (2900-10,670)	5130 (2950-11,050)
Platelets (x10^4/mm^3)	19.2 (9.0-36.2)	20.2 (8.7-37.0)
ALT (IU/L)	36.5 (12-252)	45.0 (18-259)
AST (IU/L)	34.0 (18-170)	38.0 (17-142)
Total bilirubin (mg/dl)	0.70 (0.3-1.9)	0.80 (0.4-1.8)
Total cholesterol (mg/dl)	182 (111-299)	180 (116-263)
HCV RNA ($\log_{10}$ IU/ml)	6.7 (5.1-7.5)	6.9 (5.1-7.4)
HCV genotypes		
1a	2 (1.6%)	0 (0.0%)
1b	124 (98.4%)	63 (100.0%)

[a]Values are the median with the range in parentheses, or number with the percentage in parentheses.

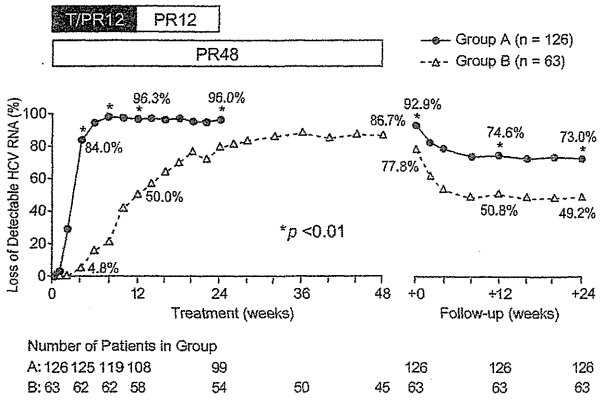

Fig. 1. Loss of detectable HCV RNA in patients in Groups A and B. Statistical tests were performed at weeks 4, 8, 12, and 24 in the treatment period, end of treatment, and weeks 12 and 24 in the follow-up period. An asterisk (*) indicates p <0.01 differences. The number of patients at each time point is indicated below the graph.

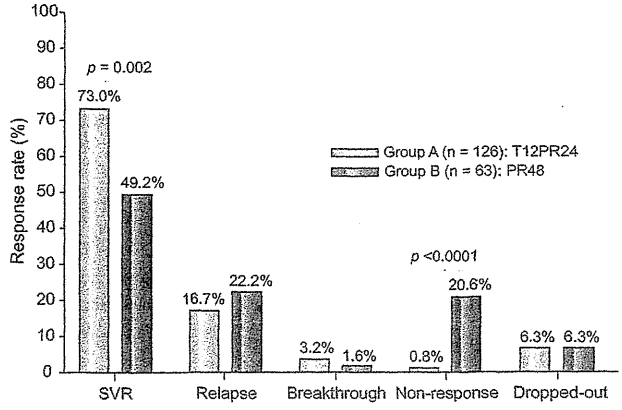

Fig. 2. Comparison of treatment responses between patients in Groups A and B. SVR, sustained virological response (HCV RNA negative 24 weeks after the completion of treatment); relapse, reappearance of HCV RNA in serum during follow-up period; breakthrough, reappearance of HCV RNA in serum during treatment period; non-response, HCV RNA continuously detectable in serum during treatment period.

treatment response and adverse events between the triple therapy involving telaprevir, PEG-IFN and RBV, and SOC with PEG-IFN and RBV. The study protocol complied with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the review board of each institution. Each patient gave a written informed consent before participating in this study.

Study design

This prospective, multi-center, and randomized study was planned on Japanese patients with chronic hepatitis C who met inclusion and did not meet exclusion criteria. Main inclusion criteria were: (a) diagnosed with chronic hepatitis C, and had not received antiviral treatments before; (b) infected with HCV-1 confirmed by the sequence analysis in the NS5B region; (c) had HCV RNA levels $\geqslant$5.0 $\log_{10}$ IU/ml determined by the COBAS TaqMan HCV test (Roche Diagnostics K.K. Tokyo, Japan); (d) Japanese aged from 20 to 65 years at the entry; (e) had the body weight between >40 and $\leqslant$120 kg; (f) were not pregnant and capable of contraception till 24 weeks after the treatment; and (g) agreed on the admission for 15 days since the treatment start. Main exclusion criteria were: (a) decompensated liver cirrhosis; (b) hepatitis B surface antigen; (c) hepatocellular carcinoma or other malignancy, or its history; (d) autoimmune hepatitis, alcoholic liver disease, hemochromatosis or chronic liver disease other than chronic hepatitis C; (e) depression or schizophrenia, or its history, or history of suicide attempts; (f) chronic renal disease or creatinine clearance $\leqslant$50 ml/min at the baseline; (g) hemoglobin <12 g/dl, neutrophil counts <1500/mm^3 or platelet counts <100,000/mm^3 at the baseline; and (h) pregnancy in progress or planned during the study period of either partner.

Patients were randomly assigned to either of the following two treatment groups in a 2:1 ratio, with stratification to balance sex and age: (1) the triple therapy with telaprevir, PEG-IFN, and RBV for 12 weeks, followed by PEG-IFN and RBV for an additional 12 weeks (Group A: T12PR24); and (2) SOC with PEG-IFN and RBV for 48 weeks (Group B: PR48). After the treatment was completed or discontinued, they were followed for $\geqslant$24 weeks for SVR evaluation. Patients were followed regularly for subjective symptoms and objective signs, as well as blood

Table 2. Comparison of SVR stratified by demographic and virological factors as well as discontinuation of study drugs between two groups with different therapeutic regimens.

	A: T12PR24 n = 126	B: PR48 n = 63	Differences *p* value
Gender			
Men	50/66 (75.8%)	18/33 (54.5%)	0.0400
Women	42/60 (70.0%)	13/30 (43.3%)	0.0214
Age (years)			
≤49	35/41 (85.4%)	13/21 (61.9%)	0.0543
≥50	57/85 (67.1%)	18/42 (42.9%)	0.0125
HCV RNA (log_{10} IU/ml)			
≥7	18/26 (69.2%)	5/18 (27.8%)	0.0132
<7	74/100 (74.0%)	26/45 (57.8%)	0.0556
Discontinuation of study drugs			
Not discontinued	66/79 (83.5%)	27/46 (58.7%)	0.0030
All drugs discontinued	14/27 (51.9%)	4/17 (23.5%)	0.1143

counts and chemistry. HCV RNA levels were monitored at day −28, days 1 (pre-dose), 2, and 3, weeks 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 (both groups), as well as weeks 26, 28, 32, 36, 40, and 48 (Group B), during the treatment period; they were monitored at weeks 2, 4, 8, 12, 16, 20, and 24 in the follow-up period (both groups).

HCV RNA and genotypes

HCV RNA was quantified using the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). The linear dynamic range of this assay was 1.2–7.8 log_{10} IU/ml, and samples with no HCV RNA detected were reported as: <1.2 log_{10} IU/ml (no HCV RNA detectable). Genotypes of HCV were determined by direct sequencing followed by phylogenetic analysis of the NS5B region [15].

Antiviral treatments

Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) 750 mg was administered three times a day at an 8-h interval (q8h) after each meal. Peginterferon-α2b (PegIntron®, MSD, Tokyo, Japan) was injected subcutaneously at a median dose of 1.5 μg/kg (range: 1.250–1.739 μg/kg) once a week. Ribavirin (Rebetol®, MSD, Tokyo, Japan) 200–600 mg was administered after breakfast and dinner. The daily dose of RBV was adjusted to the body weight: 600 mg for ≤60 kg; 800 mg for >60 kg ~≤80 kg; and 1000 mg for >80 kg.

RBV dose was diminished by 200 mg in patients receiving 600 or 800 mg (by 400 mg in those receiving 1000 mg) when hemoglobin decreased <12 g/dl, and by extra 200 mg when it lowered <10 g/dl. In addition, RBV was reduced by 200 mg in patients with hemoglobin <13 g/dl at baseline or in those in whom it decreased by 1 g/dl within a week and below 13 g/dl. Dose modification of RBV in Group B was conducted in accordance with SOC. PEG-IFN dose was reduced to one half, when leukocyte counts decreased <1500/mm^3, neutrophil counts <750/mm^3 or platelet counts <8 × 10^4/mm^3; PEG-IFN was discontinued when they decreased <1000/mm^3, 500/mm^3 or 5 × 10^4/mm^3, respectively. The triple therapy was discontinued or interrupted when hemoglobin decreased <8.5 g/dl. In patients whose hemoglobin increased ≥8.5 g/dl within 2 weeks after the interruption, treatment was resumed with PEG-IFN and RBV 200 mg. The reduction of telaprevir dose was not permitted.

Statistical analysis

SVR was evaluated in the full analysis set. The difference in SVR between Groups A and B with the 2-sided 95% confidence interval (CI) was calculated with the adjustment for sex and age, and *p* value was evaluated by the Wald-test. Continuous variables between groups were compared by the Mann–Whitney test (*U*-test), and categorical variables by the Fisher's exact test. Statistical analyses were performed using the statistical software SAS Version 9.1 (SAS Institute Inc., Cary, NC), and a *p* value <0.05 was considered significant.

Results

Patient cohorts

Of the 220 Japanese patients from whom an informed consent was obtained, 31 (14.1%) were found not eligible for the study entry. The remaining 189 patients were randomly assigned to T12PR24 (Group A [n = 126]) or PR48 (Group B [n = 63]). Overall, 114 out of the 126 (90.0%) patients in Group A and 54 out of the 63 (85.7%) in Group B completed the full study period. Table 1 compares baseline characteristics of studied patients in Groups A and B. There were no differences in demographic characters, hematology, biochemistry, or virology between the two groups of patients.

Loss of HCV RNA during treatment

Dynamics of HCV RNA during treatment was much different between Groups A and B. HCV RNA disappeared more frequently (98.4% *vs.* 79.4%, *p* <0.001) and swiftly (within 8 *vs.* 38 weeks) in patients in Group A than B. Time courses of the loss of HCV RNA are compared in Fig. 1. The loss of HCV RNA increased constantly, sharply, and swiftly in Group A. By contrast, in Group B, it gradually increased during the first 24 weeks of treatment. Rapid virological response at 4 weeks (RVR) occurred more frequently in Group A than B (84.0% *vs.* 4.8%, *p* <0.0001). HCV RNA was undetectable in >90% of patients in Group A, while it stayed undetectable in <80% of patients in Group B at the start of follow-up. After treatment completion, HCV RNA re-appeared in patients in both Groups A and B (16.7% *vs.* 22.2%, *p* = 0.4272).

Responses to treatments

Fig. 2 compares treatment responses between Groups A and B. SVR was achieved more frequently in Group A than B (73.0% *vs.* 49.2%, *p* = 0.0020). By contrast, non-response was less frequent in Group A than B (0.8% *vs.* 20.6%, *p* <0.0001). The difference in SVR between Groups A and B, adjusted for sex and age, was 23.8% (95% CI: 9.4–38.2%, *p* = 0.0012, Wald-test).

Table 3. Adverse events developing in more than 15% of patients in either Groups A or B.

	A: T12PR24 (n = 126)		B: PR48 (n = 63)	
Anemia	115	(91.3%)	46	(73.0%)
Pyrexia	98	(77.8%)	46	(73.0%)
Leukocytopenia	86	(68.3%)	46	(73.0%)
Thrombocytopenia	81	(64.3%)	23	(36.5%)
Malaise	73	(57.9%)	30	(47.6%)
Serum uric acid increased	65	(51.6%)	5	(7.9%)
Serum hyaluronic acid increased	64	(50.8%)	25	(39.7%)
Alopecia	51	(40.5%)	29	(46.0%)
Headache	48	(38.1%)	32	(50.8%)
Skin rashes	48	(38.1%)	18	(28.6%)
Anorexia	42	(33.3%)	17	(27.0%)
Insomnia	40	(31.7%)	17	(27.0%)
Vomiting	37	(29.4%)	9	(14.3%)
Drug eruption	37	(29.4%)	2	(3.2%)
Arthralgia	36	(28.6%)	15	(23.8%)
Serum triglycerides increased	36	(28.6%)	11	(17.5%)
Dysgeusia	34	(27.0%)	10	(15.9%)
Diarrhoea	34	(27.0%)	19	(30.2%)
Nausea	32	(25.4%)	7	(11.1%)
Serum creatinine increased	32	(25.4%)	0	
Erythema at the injection site	33	(26.2%)	21	(33.3%)
Reactions at the injection site	29	(23.0%)	16	(25.4%)
Stomatitis	24	(19.0%)	12	(19.0%)
Abdominal discomfort	23	(18.3%)	12	(19.0%)
Pruritus	23	(18.3%)	13	(20.6%)
Nasopharyngitis	23	(18.3%)	18	(28.6%)
Influenza-like symptoms	22	(17.5%)	16	(25.4%)
Serum bilirubin increased	22	(17.5%)	13	(20.6%)
Back pain	21	(16.7%)	12	(19.0%)
Hyperuricemia	20	(15.9%)	2	(3.2%)
Serum phosphorus decreased	16	(12.7%)	13	(20.6%)
Constipation	14	(11.1%)	13	(20.6%)
Erythema	9	(7.1%)	13	(20.6%)

Factors influencing the treatment response are compared in Table 2. SVR was higher in Group A than B, irrespective of different genders, age ranges, or HCV RNA loads. Of note, SVR in women in Group A was higher than that in Group B (70.0% *vs.* 43.3%, $p = 0.0214$). Likewise, SVR in patients ⩾50 years was higher in Group A than B (67.1% *vs.* 42.9%, $p = 0.0125$), and that in patients with high HCV RNA loads (⩾7 $\log_{10}$ IU/ml) at the baseline was higher in Group A than B (69.2% *vs.* 27.8%, $p = 0.0132$).

Adverse events

Adverse events occurred in all patients in both Groups A and B. Adverse events with a frequency >15% in either group are listed in Table 3. Of them, frequencies of anemia, thrombocytopenia, malaise, and elevated serum levels of uric acid as well as hyaluronic acid were >10% higher in Group A than B. Most of them were mild, and severe and serious adverse events occurred in small proportions of patients (9.5% and 11.9% in Group A, respectively, and 9.5% and 9.5% in Group B). All drugs were discontinued due to adverse events comparatively frequently in Groups A and B (16.7% and 22.2%, respectively), and telaprevir alone in 19.0% of patients in Group A. The total dose of RBV was less in Group A than B (47.0% *vs.* 77.7% of the target, $p < 0.0001$). Doses of antiviral treatments were reduced or discontinued in some patients with moderate to severe adverse events, patients were taken care of by specialists, and received specific therapies when necessary. Eventually, all patients recovered from adverse events.

Hematological disorders

Anemia occurred in 91.3% and 73.0% of patients in Groups A and B, respectively. Table 4 compares the severity of anemia between Groups A and B. Combined, Grade 1 and 2 anemia developed more frequently in Group A than B (38.1% *vs.* 17.5%, $p = 0.0045$). Grade 3 anemia occurred in 11.1% in Group A only. During the follow-up, hemoglobin increased both in Groups A and B, and returned to pretreatment levels 12 weeks after the completion of therapy and thereafter (Fig. 3A). Platelet counts decreased more extensively in Group A than B (Fig. 3B). They rebounded after the completion of therapy, and then returned to pretreatment values. Decreases in neutrophil counts were milder in Group A than B (Fig. 3C). Both in Groups A and B, neutrophils started to increase immediately after the treatment completion, and returned to pretreatment levels within 12 weeks.

Skin disorders

Skin disorders were monitored at every hospital visit for severity and extent, and they were categorized into four Grades (Table 4). When skin disorders of Grades 2–4 occurred, the attendant physician was instructed to consult with a dermatologist in each institution for the diagnosis and specific cares, and telaprevir was discontinued, while PEG-IFN and RBV were reduced or discontinued, as required. Skin disorders were mainly rash, drug eruptions, and erythema. They occurred comparably frequently in Groups A and B (89.7% and 84.1%, respectively). Most skin disorders were mild and categorized into Grade 1 in 75.4% and 76.2% of patients in Groups A and B, respectively. Combined, skin disorders of Grades 2–4 occurred more frequently in Group A than B (46.8% *vs.* 23.8%, $p = 0.0026$). Due to skin disorders, at least one drug was discontinued in merely 9.5% and 3.2% of patients in Groups A and B, respectively, and most skin disorders were controllable by anti-histamine and/or steroid ointments.

Serious skin disorders developed in three patients in Group A, but none in Group B. Stevens–Johnson syndrome occurred in one patient 35 days after the treatment start, and led to the discontinuation of treatment. Erythema spread widely in the trunk (Fig. 4A), as well as limbs and the face. Erosion of oral mucosae, epidermal detachment, conjunctival redness, high fever to reach 39.3 °C, and lymphadenopathy were also noted. Histopathology showed the epidermal necrosis, satellite-cell necrosis, and perivascular dermatitis with infiltration of lymphocytes, neutrophils, and eosinophils in the superficial dermis (Fig. 4B). The patient was admitted and received steroids intravenously, and recovered completely within 9 weeks. Drug rash with eosinophilia and

Table 4. Decreases in hemoglobin levels and skin disorders according to the grade.

Grade	A: T12PR24 n = 126	B: PR48 n = 63	Differences p value
A Hemoglobin levels			
Grade 1 (9.5- <11.0 g/dl)	50 (39.7%)	32 (50.8%)	0.1631
Grade 2 (8.0- <9.5 g/dl)	34 (27.0%)	11 (17.5%)	0.2043
Grade 3 (<8.0 g/dl)	14 (11.1%)	0	0.0055
Total	98 (77.8%)	43 (68.3%)	0.1613
B Skin disorders			
Grade 1[a]	95 (75.4%)	48 (76.2%)	1.0000
Grade 2[b]	44 (34.9%)	12 (19.0%)	0.0282
Grade 3[c]	13 (10.3%)	3 (4.8%)	0.2709
Grade 4[d]	2 (1.6%)	0 (0.0%)	0.5532
Any grade	113 (89.7%)	53 (84.1%)	0.3451

[a]Localized skin lesions.
[b]Diffuse or multiple skin lesions.
[c]Skin lesions covering >50% of the body surface or rashes with some characteristics such as bullae, ulceration of mucous membrane, epidermal detachment, target lesion or significant systemic signs.
[d]Stevens–Johnson syndrome and drug rashes with eosinophilia and systemic symptoms (DRESS) were categorized in Grade 4.

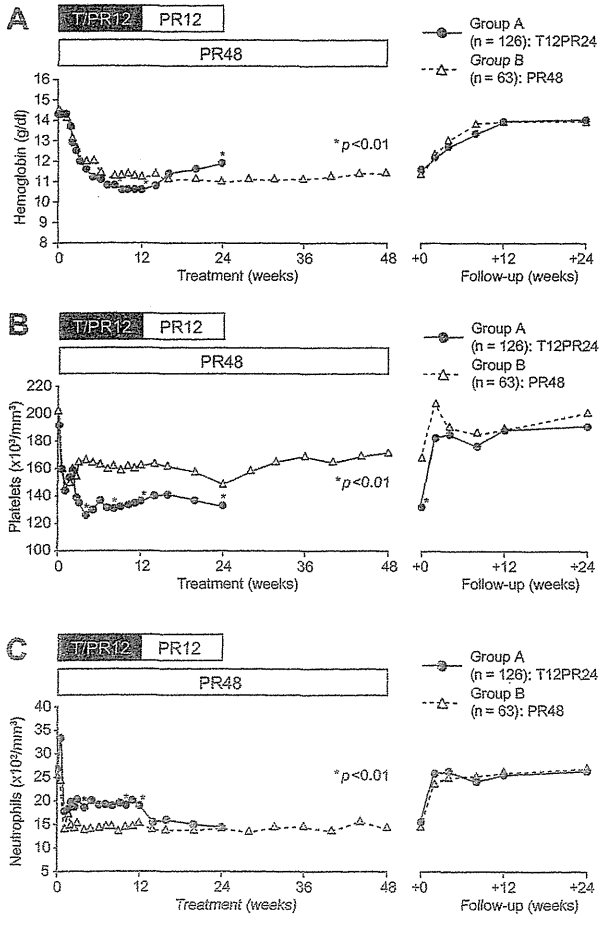

Fig. 3. Comparison of hematopoietic disorders between patients in Groups A and B. (A) Median hemoglobin levels, (B) platelet counts, and (C) neutrophil counts are plotted during treatment and follow-up. Ranges from 25% to 75% are omitted for visual clarity. Statistical tests were performed at weeks 4, 8, 12, and 24 in the treatment period, end of treatment, and at weeks 12 and 24 in the follow-up period. An asterisk (*) indicates p <0.01 difference.

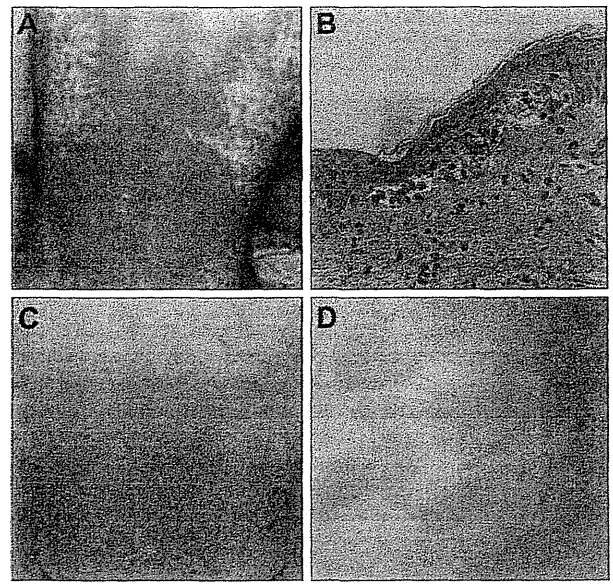

Fig. 4. Grade 4 skin regions in patients who received the triple therapy. (A) Erythema and (B) histopathology of the skin in the patient with Stevens–Johnson syndrome, as well as (C and D) generalized erythema in the patient developing drug rashes with eosinophilia and systemic symptoms (DRESS), are shown.

systemic symptoms (DRESS or drug-induced hypersensitivity syndrome) occurred in another patient. Fresh red erythema appeared on the whole body, and fresh red-colored target lesions (up to 3–4 cm in diameter) were also observed (Fig. 4C and D). Edema in the face, lymphadenopathy, fever up to 39.7 °C, and erosion of oral mucosae were noted, also. Maximum levels of white blood cells, eosinophils, and atypical lymphocytes were 46,300/mm^3, 45.7%, and 23.3%, respectively. Titers of IgG antibody to human herpes virus 6 were ×160 (29 days after the onset) and ×2560 (57 days). The remaining patient developed erythema multiforme. These two patients received steroids orally and recovered completely within 14 weeks.

Discussion

A prospective, randomized, and treatment-controlled clinical trial was planned and conducted in Japan to compare the therapeutic efficacy and safety profiles between the triple therapy with T12PR24 and the SOC treatment with PR48. In this trial, 126 patients were assigned to receive T12PR24 (Group A) and the 63 to receive PR48 (Group B). They all were treatment-naive, and infected with HCV-1 in high viral loads ($\geqslant 5$ $\log_{10}$ IU/ml) and of genotype 1b in the great majority (98.9%). Randomization was not adopted due to ethnical concerns against giving intravenous placebo weekly for 24 weeks to patients in Group A.

Dynamics of circulating HCV RNA during treatment was quite different between Groups A and B. HCV RNA disappeared more frequently (98.4% *vs.* 79.4%, $p<0.001$) and swiftly (within 8 *vs.* 38 weeks) in patients in Group A than B. Accordingly, SVR was achieved more frequently in patients with T12PR24 than PR48 (73.0% *vs.* 49.2%, $p = 0.0020$), while rates of relapse (16.7% *vs.* 22.2%) and breakthrough (3.2% and 1.6%) were not different between them. Due to the higher therapeutic efficacy and shorter treatment duration, T12PR24 would be more suitable for treatment of HCV-1 patients than the standard PR48, and lessen the total economic burden of patients and the nation.

Previous clinical trials with telaprevir were conducted in Europe or the United States and combined with PEG-IFN-α2a [8–11]. In the present study, Japanese patients have responded to a triple therapy with PEG-IFN-α2b, with an efficacy of 73% in comparison with 72–75% in phase 3 clinical trials [10,11]. In a recent report, PEG-IFN-α2a and -α2b were equally effective in triple therapies in combination with telaprevir and RBV [16]. Frequency of side effects demanding the discontinuation of all drugs is comparable between patients receiving the triple therapy with PEG-IFN-α2a in phase 3 trials [10,11] and -α2b in the present study (7–17% and 17%, respectively).

In our previous report [17], the IFN-responsive C/C genotype of *IL28B* at rs12979860 was detected in 42 out of the 72 (55%) patients infected with HCV-1 in Japan; the prevalence was not much different from that in 336 out of the 769 (44%) European-Americans [18]. The susceptibility to telaprevir depends on HCV genotypes, and is higher for genotypes 1 and 2 than genotypes 4 and 5 in *in vitro* experiments [19]. Further, it may differ between 1a and 1b, due to dissimilar evolution patterns of drug-resistant mutations [14]. Nevertheless, present patients infected with HCV-1b in the great majority (98.4%) were equally responsive to the triple therapy with telaprevir as those infected with HCV-1a [8,9,11].

High efficacy of T12PR24 was accompanied by increased adverse events, of which anemia and skin lesions were worrisome. Moderate and severe anemia (<9.5 g/dl) developed more frequently in Group A than B (38.1% *vs.* 17.5%, $p = 0.0045$). Since Japanese patients with chronic hepatitis C are older by >10 years than those in Western countries, with a higher proportion of women, they are prone to develop anemia during treatment with telaprevir. Stringent precaution had to be taken, therefore, by deducting the RBV dose in patients in whom hemoglobin levels decrease <12 g/dl, higher than the conventional threshold of <10 g/dl. The total RBV dose was lower in Group A than B (47.0% *vs.* 77.7% of the target, $p<0.0001$). However, decreased doses of RBV or PEG-IFN did not influence substantially the therapeutic efficacy of T12PR24.

Skin disorders of Grades 2–4 occurred more frequently in Group A than B (46.8% *vs.* 23.8%, $p = 0.0026$). It has to be noted that Grade 4 skin lesions, such as Stevens–Johnson syndrome and drug rashes with eosinophilia and systemic symptoms (DRESS), developed exclusively in patients in Group A. Since studied patients were monitored carefully and received immediate care by dermatologists, if and when skin lesions of Grades 2–4 developed, all patients eventually recovered. In the area of DAAs, potentially accompanying severe skin disorders, physicians would need close cooperation with dermatologists for the care of patients with hepatitis C.

In conclusion, this multicenter, randomized, and treatment-controlled study of T12PR24 in Japanese patients infected with HCV-1b has proven the efficacy and safety comparable to those in previous phase 3 studies [10,11]. Due to the excellence of T12PR24 over the standard PR48, we hope it will be used widely in patients with chronic hepatitis C over the world, who are expected to increase rapidly in the foreseeable future [20].

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Amino Acid Substitution in HCV Core Region and Genetic Variation near the *IL28B* Gene Affect Viral Dynamics during Telaprevir, Peginterferon and Ribavirin Treatment

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Key Words
Hepatitis C virus · Core region · *IL28B* · Telaprevir · Peginterferon · Ribavirin · Viral dynamics

Abstract
Objectives: Genetic variation near the *IL28B* gene and substitution of aa 70 and 91 in the core region of HCV-1b are useful as predictors of treatment efficacy to telaprevir/pegylated interferon (PEG-IFN)/ribavirin, but its impact on viral dynamics is not clear. ***Methods:*** This study investigated predictive factors of viral dynamics during 12- or 24-week regimen of triple therapy in 80 Japanese adults infected with HCV-1b. ***Results:*** After 24 h of commencement of treatment, the proportion of patients with Arg70 and Leu91 substitutions in the core region who showed ≥3.0 log drop in HCV RNA level was significantly higher than that of patients with Gln70 (His70) and/or Met91. At 8 and 12 weeks, HCV RNA loss rate of patients with rs8099917 genotype TT near *IL28B* gene was significantly higher than that of patients with non-TT. Multivariate analysis identified substitution of aa 70 and 91 as a predictor of ≥3.0 log fall in HCV RNA level at 24 h (Arg70 and Leu91) and SVR (Arg70), and rs8099917 (TT) as a predictor of HCV RNA loss at 12 weeks and SVR. ***Conclusions:*** This study identified genetic variation near *IL28B* gene and aa substitution of the core region as predictors of viral dynamics during triple therapy.

Introduction

Hepatitis C virus (HCV) usually causes chronic infection that can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) [1, 2]. At present, treatments based on interferon (IFN), in combination with ribavirin, are mainstay for combating HCV infection. In Japan, HCV genotype 1b (HCV-1b) in high viral loads (>100 kIU/ml) accounts for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis

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C [3]. Such a background calls for efficient treatments of Japanese patients with chronic HCV infection.

Even with pegylated IFN (PEG-IFN) combined with ribavirin, a sustained virological response lasting over 24 weeks after the withdrawal of treatment is achieved in at most 50% of the patients infected with HCV-1b and high viral loads [4, 5]. Recently, a new strategy was introduced in the treatment of chronic HCV infection by means of inhibiting protease in the NS3/NS4 of the HCV polyprotein. Of these, telaprevir (VX-950) was selected as a candidate agent for treatment of chronic HCV infection [6]. Later, it was found that telaprevir, when combined with PEG-IFN and ribavirin, gains a robust antiviral activity [7, 8]. Two previous studies (PROVE1 and PROVE2) showed that the 12- and 24-week regimen of telaprevir/PEG-IFN/ribavirin could achieve sustained virological response rates of 35–60 and 61–69% in patients infected with HCV-1, respectively [9, 10]. Furthermore, a recent study (PROVE3) also showed that the 24- and 48-week regimen of triple therapy could achieve sustained virological response rates of 51 and 53% in HCV-1 infected patients in whom initial PEG-IFN/ribavirin treatment failed, respectively [11].

Amino acid (aa) substitutions at positions 70 and/or 91 in the HCV core region of patients infected with HCV-1b and high viral loads are pretreatment predictors of poor virological response to PEG-IFN plus ribavirin combination therapy [12–14], and also affect clinical outcome, including hepatocarcinogenesis [15, 16]. Furthermore, genetic variations near the *IL28B* gene (rs8099917, rs12979860) on chromosome 19 as host-related factor, which encodes IFN-λ-3, are pretreatment predictors of virological response to 48-week PEG-IFN plus ribavirin combination therapy in individuals infected with HCV-1 [17–20], and also affect clinical outcome, including spontaneous clearance of HCV [21]. A recent report identified genetic variation near *IL28B* gene and aa substitution of the core region as predictors of sustained virological response to triple therapy of telaprevir/PEG-IFN/ribavirin in Japanese patients infected with HCV-1b [22]. However, it is not clear at this stage whether genetic variation near the *IL28B* gene and aa substitution of the core region can be used before therapy to predict viral dynamics during triple therapy.

The present study included 80 patients with HCV-1b and high viral loads, who received the triple therapy of telaprevir with PEG-IFN plus ribavirin. The aims of the study were to identify the pretreatment factors that could predict viral dynamics during treatment, including viral- (aa substitutions in the HCV core and NS5A regions) and host-related factors (genetic variation near *IL28B* gene).

Patients and Methods

Study Population

Between May 2008 and September 2009, 81 patients infected with HCV were recruited to this study at the Department of Hepatology in Toranomon Hospital in metropolitan Tokyo. The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the institutional review board. Each patient gave an informed consent before participating in this trial. Patients were divided into two groups: 20 (25%) patients were allocated to a 12-week regimen of triple therapy [telaprevir (MP-424), PEG-IFN and ribavirin] (the T12PR12 group), and 61 patients (75%) were assigned to a 24-week regimen of the same triple therapy for 12 weeks followed by dual therapy of PEG-IFN and ribavirin for 12 weeks (the T12PR24 group).

Eighty of the 81 patients met the following inclusion and exclusion criteria: (1) Diagnosis of chronic hepatitis C. (2) HCV-1b confirmed by sequence analysis. (3) HCV RNA levels of ≥5.0 log IU/ml determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). (4) Japanese (Mongoloid) ethnicity. (5) Age at study entry of 20–65 years. (6) Body weight ≥35 kg and ≤120 kg at the time of registration. (7) Lack of decompensated liver cirrhosis. (8) Negativity for hepatitis B surface antigen (HBsAg) in serum. (9) Negative history of HCC. (10) No previous treatment for malignancy. (11) Negative history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, and chronic liver disease other than chronic hepatitis C. (12) Negative history of depression, schizophrenia or suicide attempts, hemoglobinopathies, angina pectoris, cardiac insufficiency, myocardial infarction or severe arrhythmia, uncontrollable hypertension, chronic renal dysfunction or creatinine clearance of ≤50 ml/min at baseline, diabetes requiring treatment or fasting glucose level of ≥110 mg/dl, autoimmune disease, cerebrovascular disorders, thyroidal dysfunction uncontrollable by medical treatment, chronic pulmonary disease, allergy to medication or anaphylaxis at baseline. (13) Hemoglobin level of ≥12 g/dl, neutrophil count ≥1,500/mm^3, and platelet count of ≥100,000/mm^3 at baseline. Pregnant or breast-feeding women or those willing to become pregnant during the study and men with a pregnant partner were excluded from the study. In this study, all of the 80 patients were evaluated for the pretreatment predictors for viral dynamics during triple therapy, and 77 of the 80 patients were followed up for at least 24 weeks after the completion of treatment. The treatment efficacy was evaluated by 24 weeks after the completion of therapy (sustained virological response), based on the COBAS TaqMan HCV test (Roche Diagnostics).

Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) was administered at 750 or 500 mg three times a day at an 8-hour (q8) interval after the meal. PEG-IFNα-2b (PEG-Intron; Schering Plough, Kenklworth, N.J., USA) was injected subcutaneously at a median dose of 1.5 μg/kg (range 1.3–2.0 μg/kg) once a week. Ribavirin (Rebetol; Schering Plough) was administered at 200–600 mg twice a day after breakfast and dinner (daily dose 600–1,000 mg).

PEG-IFN and ribavirin were discontinued or their doses reduced, as required, upon reduction of hemoglobin level, leukocyte count, neutrophil count or platelet count, or the development of adverse events. Thus, the dose of PEG-IFN was reduced by 50% when the leukocyte count decreased below 1,500/mm^3, neutro-

Table 1. Profile and laboratory data at commencement of telaprevir, peginterferon and ribavirin triple therapy in Japanese patients infected with HCV-1b

Demographic data	
Number of patients	80
Sex, M/F	43/37
Age, years*	55 (23–65)
History of blood transfusion	24 (20.0%)
Family history of liver disease	13 (16.3%)
Body mass index*	22.5 (13.2–32.4)
*Laboratory data**	
Level of viremia, log IU/ml	6.8 (5.1–7.6)
Serum aspartate aminotransferase, IU/l	34 (15–118)
Serum alanine aminotransferase, IU/l	42 (12–175)
Serum albumin, g/dl	3.9 (3.3–4.6)
Gamma-glutamyl transpeptidase, IU/l	36 (9–229)
Leukocyte count, per mm^3	4,800 (2,800–8,100)
Hemoglobin, g/dl	14.3 (11.7–16.8)
Platelet count, $\times 10^4/mm^3$	17.3 (9.5–33.8)
α-Fetoprotein, μg/l	4 (2–39)
Total cholesterol, mg/dl	180 (112–276)
Fasting plasma glucose, mg/dl	92 (64–125)
Treatment	
PEG-IFNα-2b dose, μg/kg*	1.5 (1.3–2.0)
Ribavirin dose, mg/kg*	11.5 (7.2–18.4)
Telaprevir dose, 1,500/2,250 mg/day	10/70
Treatment regimen (T12PR12 group/T12PR24 group)	20/60
Amino acid substitutions in the HCV-1b	
Core aa 70, arginine/glutamine (histidine)	47/33
Core aa 91, leucine/methionine	43/37
ISDR of NS5A, wild-type/non-wild-type	76/4
Genetic variation near IL28B gene	
rs8099917 genotype, TT/TG/GG/ND	46/30/2/2
rs12979860 genotype, CC/CT/TT/ND	43/31/2/4
Past history of IFN therapy	
Treatment naive	27
Relapsers to previous treatment	33
Nonresponders to previous treatment	20

Data are numbers and percentages of patients, except those denoted by *, which represent the median (range) values.
ND = Not determined.

phil count below 750/mm^3 or platelet count below 80,000/mm^3; PEG-IFN was discontinued when these counts decreased below 1,000/mm^3, 500/mm^3 or 50,000/mm^3, respectively. When hemoglobin decreased to <10 g/dl, the daily dose of ribavirin was reduced from 600 to 400, 800 to 600 and 1,000 to 600 mg, depending on the initial dose. Ribavirin was withdrawn when hemoglobin decreased to <8.5 g/dl. However, the dose of telaprevir (MP-424) remained the same, and its administration was stopped when the discontinuation was appropriate for the development of adverse events. In those patients who discontinued telaprevir, treatment with PEG-IFNα-2b and ribavirin was also terminated.

Table 1 summarizes the profiles and laboratory data of the 80 patients at the commencement of treatment. They included 43 males and 37 females, aged 23–65 years (median 55 years).

Measurement of HCV RNA

The antiviral effects of the triple therapy on HCV were assessed by measuring plasma HCV RNA levels. In this study, HCV RNA levels during treatment were evaluated at least once every month before, during, and after therapy. Furthermore, to investigate the pretreatment predictors for viral dynamics, HCV RNA levels during treatment were evaluated at 7 time points; 24 h, 1, 2, 4, 6, 8 and 12 weeks after the commencement of treatment. HCV RNA levels during treatment were evaluated in 80 (100%), 80 (100%), 80 (100%), 79 (98.8%), 75 (93.8%), 74 (92.5%), and 69 (86.3%) of the 80 patients, at the above time intervals, respectively. HCV RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2–7.8 log IU/ml, and the undetectable samples were defined as loss of HCV RNA. Especially, falls in HCV RNA levels at 24 h relative to baseline were investigated as very early dynamics.

Detection of Amino Acid Substitutions in Core and NS5A Regions of HCV-1b

With the use of HCV-J (accession No. D90208) as a reference [23], the sequence of 1–191 aa in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed on 80 clinical samples to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [12]. The sequence of 2209–2248 aa in the NS5A of HCV-1b (IFN sensitivity-determining region; ISDR) reported by Enomoto et al. [24] was determined, and the numbers of aa substitutions in ISDR were defined as wild-type (0, 1) or non-wild-type (≥2). In the present study, aa substitutions of the core region and NS5A-ISDR of HCV-1b were analyzed by direct sequencing [22].

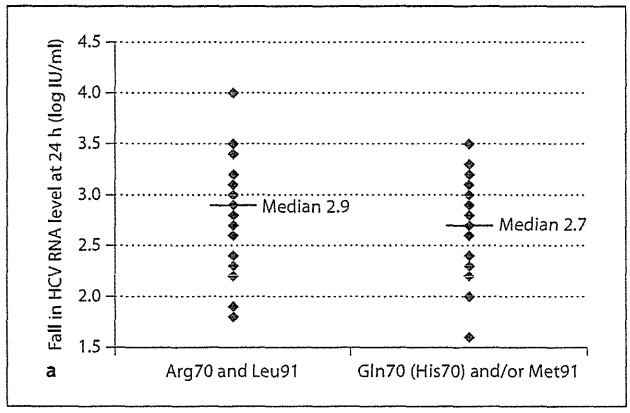
Genetic Variation near IL28B Gene

Samples for genomewide association survey were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip. Genotyping data were subjected to quality control before the data analysis. Genotyping for replication and fine mapping was performed by use of the Invader assay, TaqMan assay, or direct sequencing as described previously [25, 26].

In this study, genetic variations near *IL28B* gene (rs8099917, rs12979860), reported as the pretreatment predictors of treatment efficacy and clinical outcome [17–22], were investigated.

Statistical Analysis

Nonparametric tests (χ^2 test and Fisher's exact probability test) were used to compare the characteristics of the groups. Univariate and multivariate logistic regression analyses were used to determine those factors that significantly contributed to viral dynamics and sustained virological response. The ORs and 95%CI were also calculated. All p values less than 0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance ($p < 0.05$) on univariate analysis were entered into



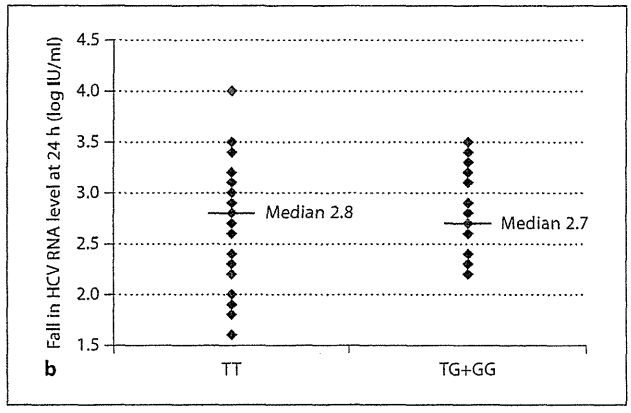


Fig. 1. a Very early dynamics according to amino acid substitutions in core region. After 24 h of commencement of the triple therapy, patients with Arg70 and Leu91 (median 2.9 log IU/ml; range 1.8–4.0 log IU/ml) significantly showed the steeper decline of HCV RNA level than those with Gln70 (His70) and/or Met91 (median 2.7 log IU/ml; range 1.6–3.5 log IU/ml). **b** Very early dynamics according to genetic variation near the *IL28B* gene. After 24 h of commencement of the triple therapy, the decline of HCV RNA level of patients with rs8099917 genotype TT (median 2.8 log IU/ml; range 1.6–4.0 log IU/ml) was not significantly different from that of patients with genotype TG and GG (median 2.7 log IU/ml; range 2.2–3.5 log IU/ml).

multiple logistic regression analysis to identify significant independent predictive factors. Each variable was transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. The potential pretreatment factors associated with treatment efficacy included the following variables: sex, age, history of blood transfusion, familial history of liver disease, body mass index, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, gamma-glutamyl transpeptidase (γGTP), leukocyte count, hemoglobin, platelet count, HCV RNA level, α-fetoprotein, total cholesterol, fasting blood sugar, PEG-IFN dose/body weight, ribavirin dose/body weight, telaprevir dose/day, treatment regimen of triple therapy, past history of IFN therapy, genetic variation near the *IL28B* gene, and amino acid substitution in the core region, and NS5A-ISDR. Statistical analyses were performed using the SPSS software (SPSS Inc., Chicago, Ill., USA).

Results

Virological Response to Therapy and Loss of HCV RNA during Treatment

Sustained virological response was achieved by 63.6% (49 of 77 patients). The disappearance rate of HCV RNA during treatment was 0% (0 of 80), 1.3% (1 of 80), 33.8% (27 of 80), 81.0% (64 of 79), 90.7% (68 of 75), 94.6% (70 of 74), and 89.9% (62 of 69) at 24 hours, 1, 2, 4, 6, 8, and 12 weeks, respectively.

Very Early Dynamics according to Amino Acid Substitutions in Core Region and Genetic Variation near the IL28B Gene

After 24 h of commencement of the triple therapy, the proportion of patients with Arg70 and Leu91 substitutions who showed ≥3.0 log drop in HCV RNA level (45.2%; 14 of 31 patients) was significantly higher than that of patients with Gln70 (His70) and/or Met91 (14.3%; 7 of 49) ($p = 0.004$). Thus, patients with Arg70 and Leu91 (median 2.9 log IU/ml; range 1.8–4.0 log IU/ml) significantly showed the steeper decline of HCV RNA level than those with Gln70 (His70) and/or Met91 (median 2.7 log IU/ml; range 1.6–3.5 log IU/ml) (fig. 1a).

After 24 h of commencement of treatment, the proportion of patients with rs8099917 genotype TT who showed ≥3.0 log drop in HCV RNA level (30.4%; 14 of 46 patients) was not significantly different from that of patients with genotype TG and GG (21.9%; 7 of 32). Thus, the decline of HCV RNA level of patients with genotype TT (median 2.8 log IU/ml; range 1.6–4.0 log IU/ml) was not significantly different from that of patients with genotype TG and GG (median 2.7 log IU/ml; range 2.2–3.5 log IU/ml) (fig. 1b).

Hence, the fall in HCV RNA level at 24 h was influenced by aa substitution patterns in the core region, but was independent of genetic variation near *IL28B* gene.

Rates of Loss of HCV RNA according to Amino Acid Substitutions in Core Region and Genetic Variation near the IL28B Gene

According to the substitution of core aa 70 and 91, the rate of HCV RNA loss of patients with Arg70 and Leu91 was not significantly different from that of patients with

Gln70 (His70) and/or Met91 at each time point (1, 2, 4, 6, 8 and 12 weeks).

According to genetic variation near the *IL28B* gene, the rate of HCV RNA loss at 1, 2, 4 and 6 weeks was not significantly different between rs8099917 genotype TT and non-TT (TG and GG). However, at 8 and 12 weeks, the rate of HCV RNA loss of patients with genotype TT was significantly higher than that of patients with genotype non-TT (fig. 2).

Predictive Factors Associated with ≥3.0 log Fall in HCV RNA Level at 24 Hours

Univariate analysis identified two parameters that correlated with ≥3.0 log fall in HCV RNA level at 24 h significantly: substitution of aa 70 and 91 (Arg70 and Leu91; OR 4.94, p = 0.003) and body mass index (≥25.0; OR 3.92, p = 0.022). Two factors were identified by multivariate analysis as independent parameters that either significantly ($p < 0.05$) or marginally ($p < 0.10$) influenced ≥3.0 log fall in HCV RNA level at 24 h [Arg70 and Leu91 (OR 3.99, p = 0.015) and body mass index ≥25.0 (OR 3.24, p = 0.061)] (table 2).

Predictive Factors Associated with Loss of HCV RNA at 2, 4 and 12 Weeks

Univariate analysis identified two parameters that correlated with loss of HCV RNA at 2 weeks significantly: platelet count (≥15.0 × 10^4/mm^3; OR 6.99, p = 0.014) and level of viremia (<7.0 log IU/ml; OR 3.13, p = 0.045). One factor was identified by multivariate analysis as independent parameter that either significantly or marginally influenced loss of HCV RNA at 2 weeks (platelet count ≥15.0 × 10^4/mm^3; OR 6.99, p = 0.014) (table 2).

Univariate analysis identified two parameters that correlated with loss of HCV RNA at 4 weeks significantly: history of blood transfusion (absence; OR 5.71, p = 0.006) and body mass index (≥20.0; OR 4.29, p = 0.019). Two factors were identified by multivariate analysis as independent parameters that either significantly or marginally influenced loss of HCV RNA at 4 weeks (history of blood transfusion: absence; OR 4.29, p = 0.026, and body mass index ≥20.0; OR 3.47, p = 0.069) (table 2).

Univariate analysis identified two parameters that correlated with loss of HCV RNA at 12 weeks significantly: sex (male; OR 9.52, p = 0.043) and genetic variation in rs8099917 (genotype TT; OR 9.00, p = 0.048). Two factors were identified by multivariate analysis as independent parameters that either significantly or marginally influenced loss of HCV RNA at 12 weeks (male sex; OR 11.0, p = 0.036, and rs8099917 genotype TT; OR 10.3, p = 0.042) (table 2).

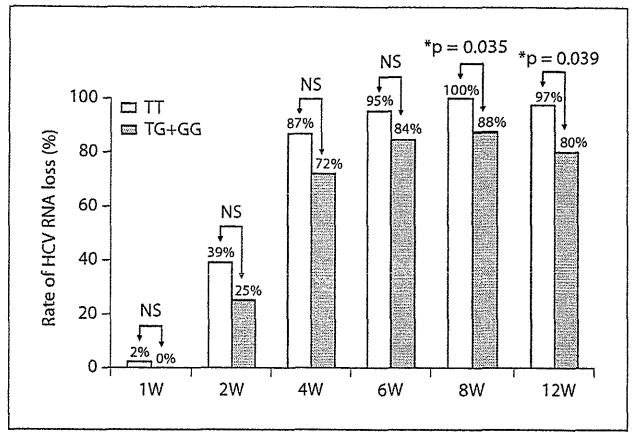


Fig. 2. Rates of loss of HCV RNA according to genetic variation near the *IL28B* gene. According to genetic variation near the *IL28B* gene, the rate of HCV RNA loss at 1, 2, 4 and 6 weeks was not significantly different between rs8099917 genotype TT and non-TT (TG and GG). However, at 8 and 12 weeks, the rate of HCV RNA loss of patients with genotype TT was significantly higher than that of patients with genotype non-TT.

Predictive Factors Associated with Sustained Virological Response

Univariate analysis identified three parameters that correlated with sustained virological response significantly: substitution of aa 70 (Arg70; OR 3.51, p = 0.011), and genetic variation in rs8099917 (genotype TT; OR 11.1, $p < 0.001$) and rs12979860 (genotype CC; OR 10.2, $p < 0.001$). Two factors were identified by multivariate analysis as independent parameters that either significantly or marginally influenced sustained virological response (rs8099917 genotype TT; OR 9.94, $p<0.001$, and Arg70; OR 3.15, p = 0.055) (table 2).

Comparison of Factors Associated with Each Treatment Efficacy Identified by Multivariate Analysis

Table 3 shows independent parameters that either significantly or marginally influenced multivariate logistic regression for each evaluation of treatment efficacy. Multivariate analysis identified substitution of aa 70 and 91 as a predictor of ≥3.0 log fall in HCV RNA level at 24 h (Arg70 and Leu91) and sustained virological response (Arg70), and rs8099917 (TT) as a predictor of HCV RNA loss at 12 weeks and sustained virological response. Thus, genetic variation near *IL28B* gene and aa substitution of the core region affect viral dynamics of different phases during triple therapy.

Table 2. Factors associated with treatment efficacy of telaprevir, peginterferon and ribavirin triple therapy in Japanese patients infected with HCV-1b, identified by univariate and multivariate analysis

	Factor	Category	Univariate logistic regression		Multivariate logistic regression	
			OR (95% CI)	p	OR (95% CI)	p
A	≥3.0 log fall in HCV RNA at 24 h					
	Substitution of aa 70 and 91	1: Gln70 (His70) and/or Met91	1		1	
		2: Arg70 and Leu91	4.94 (1.70–14.4)	0.003	3.99 (1.31–12.2)	0.015
	Body mass index	1: <25.0	1		1	
		2: ≥25.0	3.92 (1.22–12.6)	0.022	3.24 (0.95–11.1)	0.061
B	HCV RNA loss at 2 weeks					
	Platelet count, $\times 10^4/mm^3$	1: <15.0	1		1	
		2: ≥15.0	6.99 (1.49–32.8)	0.014	6.99 (1.49–32.8)	0.014
	Level of viremia, log IU/ml	1: ≥7.0	1		–	–
		2: <7.0	3.13 (1.02–9.52)	0.045	–	–
C	HCV RNA loss at 4 weeks					
	History of blood transfusion	1: presence	1		1	
		2: absence	5.71 (1.66–19.6)	0.006	4.29 (1.86–15.6)	0.026
	Body mass index	1: <20.0	1		1	
		2: ≥20.0	4.29 (1.26–14.5)	0.019	3.47 (0.91–13.3)	0.069
D	HCV RNA loss at 12 weeks					
	Sex	1: female	1		1	
		2: male	9.52 (1.08–83.3)	0.043	11.0 (1.16–100)	0.036
	rs8099917 genotype	1: TG+GG	1		1	
		2: TT	9.00 (1.02–79.5)	0.048	10.3 (1.08–98.0)	0.042
E	Sustained virological response					
	rs8099917 genotype	1: TG+GG	1		1	
		2: TT	11.1 (3.68–33.5)	<0.001	9.94 (3.05–32.4)	<0.001
	Substitution of aa 70	1: Gln70 (His70)	1		1	
		2: Arg70	3.51 (1.33–9.26)	0.011	3.15 (0.97–10.2)	0.055
	rs12979860 genotype	1: CT+TT	1		–	–
		2: CC	10.2 (3.33–3.13)	<0.001	–	–

Variables that achieved statistical significance ($p < 0.05$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent predictive factors.

The other significant predictors of HCV RNA loss were platelet count (≥15.0 × $10^4/mm^3$) at 2 weeks, history of blood transfusion (absence) at 4 weeks, and sex (male) at 12 weeks.

Discussion

Thompson et al. [27] reported that genetic variation near *IL28B* gene was also associated with increased on-treatment and sustained virological response and effectively predicted treatment outcome in treatment-naive HCV-1 patients treated with PEG-IFN plus ribavirin. However, HCV RNA loss at 4 weeks (rapid virological response) was a strong predictor of sustained virological response regardless of genetic variation near the *IL28B* gene. This phenomenon probably explains why it might be important to identify the pretreatment factors that could predict viral dynamics during treatment. The present study is the first to identify the pretreatment factors that could predict viral dynamics during triple therapy in patients infected with HCV-1. These results should be interpreted with caution since races other than Japanese and the patients infected with HCV-1a were not included. Any generalization of the results should await confirmation by studies including patients of other races and with HCV-1a to explore whether genetic variation near *IL28B* gene and aa substitution

Table 3. Comparison of factors associated with treatment efficacy of telaprevir, peginterferon and ribavirin triple therapy in Japanese patients infected with HCV-1b identified by multivariate analysis

Factor	≥3.0 log fall in HCV RNA (at 24 h)	HCV RNA loss (at 2 weeks)	HCV RNA loss (at 4 weeks)	HCV RNA loss (at 12 weeks)	Sustained virological response
Core aa 70 and 91	Arg70 and Leu91 p = 0.015 3.99 (1.31–12.2)*				Arg70 p = 0.055 3.15 (0.97–10.2)*
IL28B rs8099917				genotype TT p = 0.042 10.3 (1.08–98.0)*	genotype TT p < 0.001 9.94 (3.05–32.4)*
Others	body mass index p = 0.061 3.24 (0.95–11.1)*	platelet count p = 0.014 6.99 (1.49–32.8)*	body mass index p = 0.069 3.47 (0.91–13.3)* history of blood transfusion p = 0.026 4.29 (1.86–15.6)*	sex p = 0.036 11.0 (1.16–100)*	

Only variables that achieved statistical significance ($p < 0.05$) or marginal significance ($p < 0.10$) on multivariate logistic regression are shown. * OR (95% CI).

of core region also affect viral dynamics during triple therapy.

Two studies showed that aa substitution of the core region and genetic variation near *IL28B* gene affected viral dynamics during treatment, and sustained virological response to 48-week PEG-IFN plus ribavirin therapy in patients infected with HCV-1 [27, 28]. Furthermore, a recent report also showed that aa substitutions of core region might be used to predict very early dynamics (within 48 h) after the start of triple therapy of telaprevir with PEG-IFN and ribavirin [29]. In the present study, multivariate analysis identified substitution of aa 70 and 91 as a predictor of ≥3.0 log fall in HCV RNA level at 24 hours (i.e. viral dynamics of very early phase) and sustained virological response, and rs8099917 as a predictor of HCV RNA loss at 12 weeks (i.e. viral dynamics of later phase) and sustained virological response. This study is the first to report that genetic variation near *IL28B* gene and aa substitution of the core region affect viral dynamics of different phases during triple therapy, and probably explains why the combination of these independent factors is very useful as pretreatment predictors of sustained virological response by triple therapy [22]. The underlying mechanisms of the different viral dynamics to treatment are still unclear, and further studies based on a larger number of patients are necessary to investigate the present results.

Previous data indicated that absence of advanced liver fibrosis and male gender were positive predictors of virological response to 48-week PEG-IFN plus ribavirin therapy [13, 28]. The present study also showed that higher levels of platelet count at 2 weeks, as a surrogate marker of milder liver fibrosis, and male gender at 12 weeks were significant positive predictors of HCV RNA loss during triple therapy. The other positive predictors were absence of history of blood transfusion at 4 weeks and higher levels of body mass index at 24 h and 4 weeks, but the underlying mechanisms are still unclear. Thus, this report identified the pretreatment factors that could predict viral dynamics during triple therapy, but this study, based on a small number of patients, might provide misleading results (e.g. possible type error). Further studies of a larger number of patients are required to explore predictors, including viral- and host-related factors.

The limitations of the present study were that aa substitutions in areas other than the core region and NS5A-ISDR of the HCV genome, such as the interferon/ribavirin resistance determining region (IRRDR) [30], were not examined. Furthermore, HCV mutants with aa conversions for resistance to telaprevir during triple therapy, such as the 156S mutation [31], were also not investigated. In this regard, telaprevir-resistant HCV mutants were reported to be susceptible to IFN in both in vivo and in vitro studies [32, 33]. Thus, viral factors before and during triple therapy should be investigated in

future studies, and identification of these factors should facilitate the development of more effective therapeutic regimens.

In conclusion, this study identified genetic variation near *IL28B* gene and aa substitution of the core region as predictors of viral dynamics during triple therapy of telaprevir/PEG-IFN/ribavirin in Japanese patients infected with HCV-1b. Further large-scale prospective studies are necessary to investigate whether the present results relate to the efficacy of the triple therapy, and further understanding of the complex interaction between virus- and host-related factors should facilitate the development of more effective therapeutic regimens.

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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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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

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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Potential conflict of interest: Nothing to report.

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