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Utility of Detection of Telaprevir-Resistant Variants for Prediction of Efficacy of Treatment of Hepatitis C Virus Genotype 1 Infection

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The clinical usefulness of detecting telaprevir-resistant variants is unclear. Two hundred fifty-two Japanese patients infected with hepatitis C virus (HCV) genotype 1b received triple therapy with telaprevir–peginterferon (PEG-IFN)–ribavirin and were evaluated for telaprevir-resistant variants by direct sequencing at baseline and at the time of reelevation of the viral load. An analysis of the entire group indicated that 76% achieved a sustained virological response. Multivariate analysis identified a PEG-IFN dose of <1.3 μg/kg of body weight, an *IL28B* rs8099917 genotype (genotype non-TT), detection of telaprevir-resistant variants of amino acid (aa) 54 at baseline, nonresponse to prior treatment, and a leukocyte count of <5,000/mm^3 as significant pretreatment factors for detection of telaprevir-resistant variants at the time of reelevation of the viral load. In 63 patients who showed nonresponse to prior treatment, a higher proportion of patients with no detected telaprevir-resistant variants at baseline (54%) achieved a sustained virological response than did patients with detected telaprevir-resistant variants at baseline (0%). Furthermore, 2 patients who did not have a sustained virological response from the first course of triple therapy with telaprevir received a second course of triple therapy with telaprevir. These patients achieved a sustained virological response by the second course despite the persistence of very-high-frequency variants (98.1% for V36C) or a history of the emergence of variants (0.2% for R155Q and 0.2% for A156T) by ultradeep sequencing. In conclusion, this study indicates that the presence of telaprevir-resistant variants at the time of reelevation of viral load can be predicted by a combination of host, viral, and treatment factors. The presence of resistant variants at baseline might partly affect treatment efficacy, especially in those with nonresponse to prior treatment.

New strategies have been introduced recently for the treatment of chronic hepatitis C virus (HCV) infection based on the inhibition of protease in the nonstructural 3 (NS3)/NS4 region of the HCV polyprotein. Of the new agents currently available, telaprevir (VX-950) is used for the treatment of chronic HCV infection (1). Three studies (PROVE1, PROVE2, and a Japanese study [2–4]) showed that a 24-week regimen of triple therapy (telaprevir, peginterferon [PEG-IFN], and ribavirin) for 12 weeks followed by dual therapy (PEG-IFN and ribavirin) for 12 weeks (also called the T12PR24 regimen) achieved sustained virological response (SVR) (negative for HCV RNA for >24 weeks after the withdrawal of treatment) rates of 61%, 69%, and 73%, respectively, in patients infected with HCV genotype 1 (HCV-1). However, another study (PROVE3) found lower SVR rates to the T12PR24 regimen (39%) in nonresponders to previous PEG-IFN–ribavirin therapy infected with HCV-1 who did not achieve HCV RNA negativity during or at the end of the initial triple therapy course (5).

Telaprevir-based therapy is reported to induce resistant variants of HCV (6, 7). A recent report indicated that resistant variants are observed in most patients after failure to achieve an SVR by telaprevir-based treatment and that they tend to be replaced with wild-type viruses over time, presumably due to the lower fitness of those variants (8). However, the clinical usefulness of detecting telaprevir-resistant variants is still unclear. First of all, pretreatment factors associated with the detection of telaprevir-resistant variants at the time of reelevation of viral load have not been investigated. Furthermore, it is not clear at this stage whether the detection of telaprevir-resistant variants at baseline is useful for predicting the efficacy of telaprevir-based treatment and whether a history of the emergence of telaprevir-resistant variants affects treatment efficacy with the second course of telaprevir-based treatment.

Based on the above background, there is a need to investigate the clinical usefulness of detecting telaprevir-resistant variants. The aim of this study was to determine the pretreatment factors associated with the subsequent detection of telaprevir-resistant variants at the time of reelevation of viral load and the importance of telaprevir-resistant variants for predicting the efficacy of telaprevir-based treatment in patients infected with HCV-1b.

MATERIALS AND METHODS

Study population. From May 2008 through August 2013, 340 consecutive patients infected with HCV were selected for triple therapy with telaprevir (MP-424 or Telavic; Mitsubishi Tanabe Pharma, Osaka, Japan), PEG-IFN-α2b (PegIntron; MSD, Tokyo, Japan), and ribavirin (Rebetol; MSD, Tokyo) at the Department of Hepatology, Toranomon Hospital (located in metropolitan Tokyo, Japan). Subsequently, 252 of these patients received the triple therapy based on the following inclusion and exclusion criteria: (i) diagnosis of chronic hepatitis C, (ii) HCV-1b confirmed by sequence analysis, (iii) HCV RNA level of ≥5.0 log IU/ml as determined

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by the Cobas TaqMan HCV test (Roche Diagnostics, Tokyo, Japan), (iv) follow-up duration of ≥24 weeks after the completion of triple therapy, (v) no history of treatment with NS3/4A protease inhibitors, (vi) absence of decompensated liver cirrhosis and hepatocellular carcinoma (HCC), (vii) negative for hepatitis B surface antigen (HBsAg), (viii) no evidence of human immunodeficiency virus infection, (ix) negative history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, and chronic liver disease other than chronic hepatitis C, (x) negative history of depression, schizophrenia, or suicide attempts, angina pectoris, cardiac insufficiency, myocardial infarction, severe arrhythmia, uncontrolled hypertension, uncontrolled diabetes, chronic renal dysfunction, cerebrovascular disorders, thyroidal dysfunction uncontrolled by medical treatment, chronic pulmonary disease, allergy to medication, or anaphylaxis at baseline, and (xi) pregnant or breastfeeding women or those willing to become pregnant during the study and men with a pregnant partner were excluded. The study protocol was in compliance with the guidelines for good clinical practice and the 1975 Declaration of Helsinki and was approved by the institutional review board of the Toranomon Hospital. Each patient received ample information about the goals and potential side effects of the treatment and their right to withdraw from the study at any time. Each patient provided a signed consent form before participating in this trial.

The efficacy of treatment was evaluated by the presence or absence of an HCV RNA-negative result at 24 weeks after the completion of therapy (i.e., SVR), as determined by the Cobas TaqMan HCV test (Roche Diagnostics). Furthermore, failure to achieve an SVR was classified as nonresponse (HCV RNA detected during or at the end of treatment) or relapse (at the time of reelevation of viral load after the end of treatment, even when HCV RNA result was negative at the end of treatment).

Twenty patients (8%) were assigned to a 12-week regimen of triple therapy (the T12PR12 group) and were randomly divided into two groups (10 patients each) treated with either 1,500 mg/day or 2,250 mg/day of telaprevir to evaluate the treatment efficacy during 12 weeks on treatment. Sixty patients (24%) were allocated to a 24-week regimen of the same triple therapy described above followed by dual therapy of PEG-IFN and ribavirin for another 12 weeks (the T12PR24 group) to evaluate treatment efficacy according to the response to prior treatment, and they were treated with 2,250 mg/day of telaprevir. Another group of 172 patients (68%) was treated as described above for the T12PR24 group except for the dosages of telaprevir; this group was divided into two groups treated with either 1,500 mg/day (111 patients) or 2,250 mg/day (61 patients) of telaprevir, as selected by the attending physician. Table 1 summarizes the profiles and laboratory data of the entire group of 252 patients at the commencement of treatment. They included 155 males and 97 females 21 to 73 years of age (median, 58 years). At the start of treatment, telaprevir was administered at a median dose of 30.8 mg/kg of body weight (range, 14.1 to 59.2 mg/kg) daily. One hundred thirty-one patients (52%) were treated with 2,250 mg/day of telaprevir, while the other 121 patients (48%) were treated with 1,500 mg/day of telaprevir. PEG-IFN-α2b was injected subcutaneously at a median dose of 1.5 μg/kg (range, 0.7 to 1.8 μg/kg) once a week. Ribavirin was administered at a median dose of 10.9 mg/kg (range, 4.3 to 15.8 mg/kg) daily. Each drug was discontinued or its dose reduced as required per the judgment of the attending physician, in response to a fall in hemoglobin level, leukocyte count, neutrophil count, or platelet count, or the appearance of side effects. The triple therapy was discontinued when the leukocyte count decreased to $<1{,}000/mm^3$, the neutrophil count decreased to $<500/mm^3$, the platelet count decreased to $<5.0 \times 10^4/mm^3$, or when hemoglobin decreased to <8.5 g/dl.

Follow-up. Clinical and laboratory assessments were performed at least once every month before, during, and after treatment. They were performed every week in the initial 12 weeks of treatment. Adverse effects were monitored clinically by careful interviews and a medical examination at least once every month. Compliance with treatment was evaluated by a questionnaire.

TABLE 1 Profile and laboratory data at commencement of telaprevir, peginterferon, and ribavirin triple therapy in patients infected with HCV genotype 1b

Variable	Patient data
Patient demographics	
No. of patients	252
Sex (no. of males/no. of females)	155/97
Median age (yr) (range)	58 (21–73)
Median body mass index (kg/m^2) (range)	22.8 (16.0–36.7)
Laboratory data (median [range])	
Level of viremia (log IU/ml)	6.7 (5.0–7.8)
Aspartate aminotransferase (IU/liter)	37 (15–624)
Alanine aminotransferase (IU/liter)	42 (11–525)
Albumin (g/dl)	3.9 (2.5–4.7)
Gamma-glutamyl transpeptidase (IU/liter)	34 (3–319)
Leukocyte count ($/mm^3$)	4,700 (2,000–8,400)
Hemoglobin (g/dl)	14.3 (12.1–17.6)
Platelet count ($10^4/mm^3$)	16.5 (8.5–33.8)
Treatment	
Median PEG-IFN-α2b dose (μg/kg) (range)	1.5 (0.7–1.8)
Median ribavirin dose (mg/kg) (range)	10.9 (4.3–15.8)
Median telaprevir dose (mg/kg) (range)	30.8 (14.1–59.2)
No. of patients with telaprevir dose of 1,500/2,250 mg/day	121/131
No. of patients on T12PR12/T12PR24 treatment regimen	20/232
Response to prior treatment	
No. of treatment-naive patients/no. of patients with relapse to prior treatment/no. of patients with nonresponse to prior treatment (IFN monotherapy/ribavirin combination therapy)/unknown	79/109/63 (16/47)/1
Amino acid substitutions in HCV genotype 1b	
Core aa 70 (arginine/glutamine [histidine]/ND[a])	162/88/2
Core aa 91 (leucine/methionine/ND)	139/111/2
ISDR of NS5A (wild type/non-wild type/ND)	199/24/29
IRRDR of NS5A (≤5/≥6/ND)	180/69/3
V3 of NS5A (≤2/≥3/ND)	64/185/3
IL28B genotype	
rs8099917 genotype (TT/non-TT/ND)	181/69/2
ITPA genotype	
rs112735 genotype (CC/non-CC)	186/65/1
NS3/4A protease inhibitor-resistant variants by direct sequencing[b]	
V36/T54/Q80/R155/A156/D168/V170	1/7/55/1/2/26/0

[a] ND, not determined.

[b] The NS3/4A protease inhibitor-resistant variants detected by direct sequencing included V36A/C/M/L/G, T54A/S, Q80K/R/H/G/L, R155K/T/I/M/G/L/S/Q, A156V/T/S/I/G, D168A/V/E/G/N/T/Y/H/I, and V170A (19, 20).

Measurement of HCV RNA. The antiviral effects of the triple therapy on HCV were assessed by measuring blood plasma HCV RNA levels. In this study, HCV RNA levels during treatment were evaluated at least once every month before, during, and after therapy. HCV RNA concentrations were determined using the Cobas TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2 to 7.8 log IU/ml, and undetectable samples were defined as negative.

Determination of *IL28B* and *ITPA* genotypes. The *IL28B* rs8099917 and *ITPA* rs112735 genotypes have been reported as predictors of treatment efficacy and side effects to PEG-IFN–ribavirin dual therapy, and they were genotyped by using the Invader assay, TaqMan assay, or direct sequencing, as described previously (9–13).

Detection of amino acid substitutions in core and NS5A regions of HCV-1b. With the use of HCV-J (GenBank accession no. D90208) as a reference type (14), the sequence of amino acids (aa) 1 to 191 in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed in a previous study to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and at aa 91 of leucine (Leu91) or methionine (Met91) (15). The sequence of aa 2209 to 2248 in the NS5A of HCV-1b (the interferon sensitivity-determining region [ISDR]) reported by Enomoto and coworkers (16) was determined, and the number of amino acid substitutions in the ISDR was defined as wild type ($\leq$1) or non-wild type ($\geq$2) compared to that of HCV-J. Furthermore, the sequence of aa 2334 to 2379 in the NS5A region of HCV-1b (the IFN/ribavirin resistance-determining region [IRRDR]) reported by El-Shamy and coworkers (17), including the sequence of aa 2356 to 2379 referred to as the variable region 3 (V3), was determined and then compared with the consensus sequence constructed in a previous study. The numbers of amino acid substitutions in the IRRDR and V3 regions were divided into two groups for analysis (those with $\leq$5 and $\geq$6 aa substitutions in the IRRDR, and those with $\leq$2 and $\geq$3 aa substitutions in the V3). In the present study, the amino acid substitutions of the core region and the NS5A-ISDR/IRRDR/V3 of HCV-1b were analyzed by direct sequencing.

Assessment of NS3/4A protease inhibitor-resistant variants. The genome sequence of 609 nucleotides (203 amino acids) in the N terminal of the NS3 region of HCV isolates from the patients was examined. HCV RNA was extracted from 100 μl of blood serum sample, and the nucleotide sequences were determined by direct sequencing and deep sequencing. The primers used to amplify the NS3 region were NS3-F1 (5′-ACA CCG CGG CGT GTG GGG ACA T-3′, nucleotides 3295 to 3316) and NS3-AS2 (5′-GCT CTT GCC GCT GCC AGT GGG A-3′, nucleotides 4040 to 4019) as the first (outer) primer pair and NS3-F3 (5′-CAG GGG TGG CGG CTC CTT-3′, nucleotides 3390 to 3407) and NS3-AS2 as the second (inner) primer pair (18). Thirty-five cycles of first and second amplifications were performed as follows: denaturation for 30 s at 95°C, annealing of primers for 1 min at 63°C, extension for 1 min at 72°C, and final extension at 72°C for 7 min. The PCR-amplified DNA was purified after agarose gel electrophoresis and then used for direct sequencing and ultradeep sequencing.

Patients were examined for NS3/4A protease inhibitor-resistant variants by direct sequencing at baseline and at the time of reelevation of viral loads. Furthermore, patients who did not have an SVR with the first course of triple therapy with telaprevir and received the second course of the triple therapy with telaprevir were analyzed for telaprevir-resistant variants by ultradeep sequencing at baseline and at the time of reelevation of viral loads. NS3/4A protease inhibitor-resistant variants included V36A/C/M/L/G, T54A/S, Q80K/R/H/G/L, R155K/T/I/M/G/L/S/Q, A156V/T/S/I/G, D168A/V/E/G/N/T/Y/H/I, and V170A. Telaprevir-resistant variants (at aa 36, aa 54, aa 155, aa 156, and aa 170) and TMC435-resistant variants (at aa 80, aa 155, and aa 168) were evaluated (19, 20).

Direct sequencing was analyzed by the Dye Terminator method. Dideoxynucleotide termination sequencing was performed with the BigDye deoxy terminator version 1.1 cycle sequencing kit (Life Technologies, Carlsbad, CA) (18). The sequence data were deposited in GenBank. Also, ultradeep sequencing was performed using the Ion Personal Genome Machine (PGM) sequencer (Life Technologies). An Ion Torrent adapter-ligated library was prepared using an Ion Xpress Plus fragment library kit (Life Technologies). Briefly, 100 ng of fragmented genomic DNA was ligated to the Ion Torrent adapters P1 and A. The adapter-ligated products were nick translated and PCR amplified for a total of 8 cycles. Subsequently, the library was purified using AMPure beads (Beckman Coulter, Brea, CA) and the concentration determined using the StepOnePlus real-time PCR (Life Technologies) and Ion Library quantitation kit, according to the instructions provided by the manufacturers. Emulsion PCR was performed using the Ion OneTouch (Life Technologies) in conjunction with the Ion OneTouch 200 template kit version 2 (Life Technologies). Enrichment for templated Ion Sphere particles (ISPs) was performed using the Ion OneTouch enrichment system (Life Technologies) according to the instructions provided by the manufacturer. Templated ISPs were loaded onto an Ion 314 chip and subsequently sequenced using 130 sequencing cycles according to the Ion PGM 200 sequencing kit user guide. The total output read length per run was >10 Mb (0.5 million tags, 200-base read) (21). The results were analyzed with the CLC Genomics Workbench software (CLC bio, Aarhus, Denmark) (22).

We also included a control experiment to validate the error rates in ultradeep sequencing of the viral genome. In this study, the amplification products of the second-round PCR were ligated with a plasmid and transformed in *Escherichia coli* by using a cloning kit (TA Cloning; Invitrogen, Carlsbad, CA). A plasmid-derived NS3 sequence was determined as the template, in a control experiment. The fold coverages evaluated per position for aa 36, aa 54, aa 155, aa 156, and aa 170 in the NS3 region were 359,379×, 473,716×, 106,435×, 105,979×, and 49,058×, respectively. Thus, using the control experiment based on a plasmid carrying the HCV NS3 sequence, amino acid mutations were defined as amino acid substitutions at a frequency of >0.2% among the total coverage. This frequency ruled out putative errors caused by the ultradeep sequence platform used in this study (23).

Statistical analysis. Nonparametric variables were compared between the groups by the chi-square and Fisher's exact probability tests. Univariate and multivariate analyses for factors affecting the presence of telaprevir-resistant variants by direct sequencing at the reelevation of viral load were performed by the chi-square test and logistic regression, respectively. Patients who achieved an SVR were said to have no detection of resistant variants at the reelevation of viral load. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated to determine the reliability of the predictors of the response to therapy.

Nucleotide sequence accession numbers. The N-terminal sequences of the NS3 regions of the telaprevir-resistant variant isolates were deposited in GenBank under accession numbers AB709241, AB709263, AB709264, AB709276, AB709279, AB709283, AB709286, AB709289, AB709295, AB709296, AB709300, AB709303, AB709307, AB709310, AB709311, AB709312, AB709317, AB709319, AB709321, AB709322, AB709345, AB709348, AB709352, AB709353, AB709354, AB709356, AB709357, AB709358, AB709360, AB709370, AB709377, AB709382, AB709383, AB709384, AB709388, AB709390, AB709392, AB709396, AB709398, AB709399, AB709401, AB709405, AB709409, AB709410, AB709414, AB709418, AB709422, AB709426, AB709437, AB709444, AB709445, AB709451, AB709456, AB709461, AB709474, AB709476, AB709481, AB709484, AB709485, AB709486, AB709488, AB709489, AB709490, AB709491, AB709492, AB709493, AB709502, AB709507, AB709508, AB709514, AB709515, AB709525, AB709526, AB709527, and AB826566 to AB826684.

RESULTS

Virological response to therapy. An analysis of the entire group showed that 76% (192 of 252 patients) achieved an SVR. According to the treatment regimen, an SVR was achieved by 45% (9 of 20 patients) and 79% (183 of 232 patients) of the T12PR12 and T12PR24 groups, respectively. Taking into consideration the response to prior treatment, an SVR was achieved by 86% (68 of 79 patients), 84% (91 of 109 patients), and 35% (32 of 63 patients) of the treatment-naive patients, patients who showed relapse following prior treatment, and nonresponders to prior treatment, respectively. In the 231 patients of the T12PR24 group, an SVR was achieved by 88% (61 of 69 patients), 85% (89 of 105 patients), and

TABLE 2 Frequencies of the subjects in whom NS3/4A protease inhibitor-resistant variants were detected by direct sequencing at baseline and at the time of reelevation of viral loads[a]

Time of variant detection	% (*n*) by aa position[b]:						
	36	54	80	155	156	168	170
Baseline	0.4 (1)	3 (7)	22 (55)	0.4 (1)	0.8 (2)	10 (26)	0 (0)
Reelevation of viral load	7 (18)	12 (30)	5 (11)	0.4 (1)	4 (10)	1.2 (3)	0.4 (1)

[a] NS3/4A protease inhibitor-resistant variants included V36A/C/M/L/G, T54A/S, Q80K/R/H/G/L, R155K/T/I/M/G/L/S/Q, A156V/T/S/I/G, D168A/V/E/G/N/T/Y/H/I, and V170A (19, 20).

[b] The data represent the percentages (*n*) of patients in whom NS3/4A protease inhibitor-resistant variants were detected by direct sequencing. Patients who achieved a sustained virological response were said to have no detection of resistant variants by direct sequencing at the time of reelevation of the viral load.

56% (32 of 57 patients) of the treatment-naive patients, patients who showed relapse following prior treatment, and nonresponders to prior treatment, respectively. Furthermore, an SVR was achieved by 86% (12 of 14 patients) and 47% (20 of 43 patients) of the nonresponders to prior IFN monotherapy and ribavirin combination therapy, respectively.

NS3/4A protease inhibitor-resistant variants detected by direct sequencing at baseline and at the time of reelevation of viral loads. All of the 252 patients were evaluated for resistant variants by direct sequencing at baseline. Sixty patients who did not achieve an SVR were also analyzed for resistant variants by direct sequencing at the time of reelevation of viral load. One hundred ninety-two patients who achieved SVR were said to have no detection of resistant variants as determined by direct sequencing at the reelevation of viral load.

As a whole, the frequency of the subjects in whom telaprevir-resistant variants were detected increased from 5% (12 of 252 patients) at baseline to 18% (45 of 252 patients) at the time of reelevation of viral load. On the other hand, the frequency of the subjects in whom TMC435-resistant variants were detected decreased from 31% (78 of 252 patients) at baseline to 6% (14 of 252 patients) at the time of reelevation of viral load. Table 2 shows the frequencies of subjects in whom resistant variants were detected at baseline and at the time of reelevation of viral load per position for aa 36, aa 54, aa 80, aa 155, aa 156, aa 168, and aa 170 in the NS3 region.

Pretreatment factors associated with detection of telaprevir-resistant variants by direct sequencing at the time of reelevation of viral load. Univariate analysis of the data of the entire group identified eight pretreatment factors that were significantly associated with the detection of telaprevir-resistant variants by direct sequencing at the time of reelevation of viral load: *IL28B* rs8099917 genotype (genotype non-TT) ($P < 0.001$), nonresponse to prior treatment ($P < 0.001$), PEG-IFN dose of <1.3 μg/kg ($P = 0.001$), detection of variants at aa 54 at baseline ($P = 0.002$), Gln70/His70 substitution of aa 70 ($P = 0.003$), gamma-glutamyl transpeptidase (GGT) level of ≥50 IU/liter ($P = 0.006$), leukocyte count of <5,000/mm^3 ($P = 0.026$), and ribavirin dose of <8.0 mg/kg ($P = 0.026$). Multivariate analysis that included the above variables identified five pretreatment factors that were independently associated with the detection of telaprevir-resistant variants at the time of reelevation of viral load: PEG-IFN dose of <1.3 μg/kg (odds ratio [OR], 9.71; $P < 0.001$), *IL28B* rs8099917 genotype (genotype non-TT) (OR, 8.61; $P < 0.001$), detection of variants at aa 54 at baseline (OR, 33.4; $P = 0.002$), nonresponse to prior treatment (OR, 2.66, $P = 0.018$), and leukocyte count of <5,000/mm^3 (OR, 2.46; $P = 0.042$) (Table 3).

Prediction of treatment efficacy by the combination of response to prior treatment and presence of telaprevir-resistant variants by direct sequencing at baseline. The SVR rates based on the combination of response to prior treatment and the presence of telaprevir-resistant variants by direct sequencing at baseline are shown in Fig. 1. In 79 treatment-naive patients, the SVR rates were not different between those patients in whom there were no detected telaprevir-resistant variants (86% [65 of 76 patients]) and those in whom variants were detected (67% [2 of 3 patients]). In 109 patients who showed relapse following prior treatment, the SVR rates were not different between those patients in whom there were no detected variants (83% [86 of 104 patients]) and those in whom variants were detected (100% [5 of 5 patients]). In contrast, in 63 patients who showed nonresponse to prior treatment, a higher proportion of patients with undetected telaprevir-resistant variants (54% [32 of 59 patients]) achieved an SVR than did patients in whom telaprevir-resistant variants were detected (0% [0 of 4 patients]) ($P = 0.053$). Thus, with the combination of nonresponse to prior treatment and detection of telaprevir-resistant variants, the sensitivity, specificity, PPV, and NPV for those with non-SVR were 7% (4 of 60 patients), 100% (191 of 191 patients), 100% (4 of 4 patients), and 77% (191 of 247 patients), respectively. These results indicated that the use of the combination of the above two factors has high specificity and PPV for the prediction of a non-SVR.

TABLE 3 Multivariate analysis of factors associated with detection of telaprevir-resistant variants by direct sequencing at the reelevation of viral load, to telaprevir, peginterferon, and ribavirin triple therapy in patients infected with HCV genotype 1b

Detection factors	Category	Odds ratio (95% CI[a])	*P*[b]
PEG-IFN-α2b dose (μg/kg)	≥1.3	1	
	<1.3	9.71 (3.23–29.4)	<0.001
IL28B rs8099917 genotype	TT genotype	1	
	Non-TT genotype	8.61 (3.48–21.3)	<0.001
Variants of aa 54 at baseline	No detection	1	
	Detection	33.4 (3.77–295)	0.002
Response to treatment	Naive or relapse	1	
	Nonresponse	2.66 (1.18–5.96)	0.018
Leukocyte count (/mm^3)	≥5,000	1	
	<5,000	2.46 (1.03–5.85)	0.042

[a] CI, confidence interval.

[b] Only variables that achieved statistical significance ($P < 0.05$) on multivariate logistic regression analysis are shown.

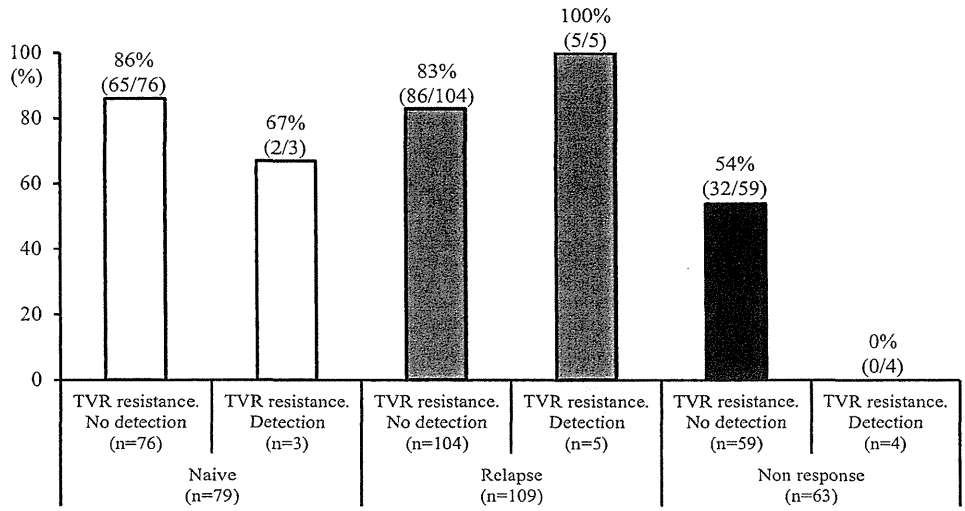

FIG 1 The rates of sustained virological response by the combination of response to prior treatment and presence of telaprevir (TVR)-resistant variants by direct sequencing at baseline are shown. Of those who showed nonresponse to prior treatment, a higher proportion of patients with undetected TVR-resistant variants (54%) achieved a sustained virological response than patients with detected TVR-resistant variants (0%) ($P = 0.053$).

Table 4 summarizes the profiles of 4 patients with nonresponse to prior treatment and in whom telaprevir-resistant variants were detected by direct sequencing at baseline. All of these 4 patients did not achieve an SVR with triple therapy. Interestingly, both T54S as a telaprevir-resistant variant and Q80L as a TMC435-resistant variant (19) were detected by direct sequencing at baseline.

Evolution of telaprevir-resistant variants over time as investigated by ultradeep sequencing in patients who received the second course of triple therapy. Two of 60 patients who did not achieve an SVR with the first course of triple therapy with telaprevir received the second course of triple therapy with telaprevir. They were analyzed for telaprevir-resistant variants by ultradeep sequencing at baseline and at the time of reelevation of viral loads.

Figure 2A shows the clinical course of case 1. In the first course of triple therapy with telaprevir (T12PR24) in a 57-year-old, V36C (0% of 32,413× coverage) was not detected by ultradeep sequencing at baseline of the first course, but very-high-frequency variants of V36C (97.2% of 36,757× coverage) were detected at the time of reelevation of viral loads. In the second course of triple therapy with telaprevir (T12PR54) when the patient was 59 years old, very-high-frequency variants of V36C (98.1% of 94,547× coverage) persisted at baseline of the second course, despite the passing of 2 years after cessation of the first therapy course. Case 1 achieved HCV RNA-negative status at 20 weeks after the start of the second course (late virological response), so PEG-IFN and ribavirin therapy was extended to 54 weeks. In conclusion, case 1 achieved an SVR after the second course of triple therapy with telaprevir, despite the persistence of very-high-frequency variants.

Figure 2B shows the clinical course of case 2. In the first course of triple therapy with telaprevir (T12PR24) in a 61-year-old patient, R155Q (0% of 23,751× coverage) and A156T (0% of 16,040× coverage) were not detected by ultradeep sequencing at baseline of the first course, but very-low-frequency variants of R155Q (0.2% of 11,572× coverage) and A156T (0.2% of 16,040× coverage) were detected at the time of reelevation of viral loads. In the second course of triple therapy with telaprevir (T12PR20) when the patient was 64 years old, R155Q (0% of 80,572× coverage) and A156T (0% of 87,686× coverage) were not detected by ultradeep sequencing at baseline of the second course, which was 2 years after cessation of the first course. In conclusion, case 2 achieved an SVR by the second course of triple therapy with telaprevir, despite the history of the emergence of variants.

TABLE 4 Profiles of 4 patients with nonresponse to prior treatment and detection of telaprevir-resistant variants by direct sequencing at baseline

Case no.	Sex	Age (yr)	Response to prior treatment[a]	Amino acid detected at aa position:							Time of HCV RNA-negative result during treatment (wks)	Efficacy of triple therapy
				36	54	80	155	156	168	170		
1	Male	70	Nonresponse to IFN monotherapy	V	S	L	R	A	D	I	2	Non-SVR
2	Male	47	Nonresponse to IFN monotherapy	V	S	L	R	A	D	I	4	Non-SVR
3	Male	61	Nonresponse to RBV combination therapy	V	S	L	R	A	D	I	3	Non-SVR
4	Female	60	Nonresponse to RBV combination therapy	V	S	L	R	A	D	I	4	Non-SVR

[a] RBV, ribavirin.

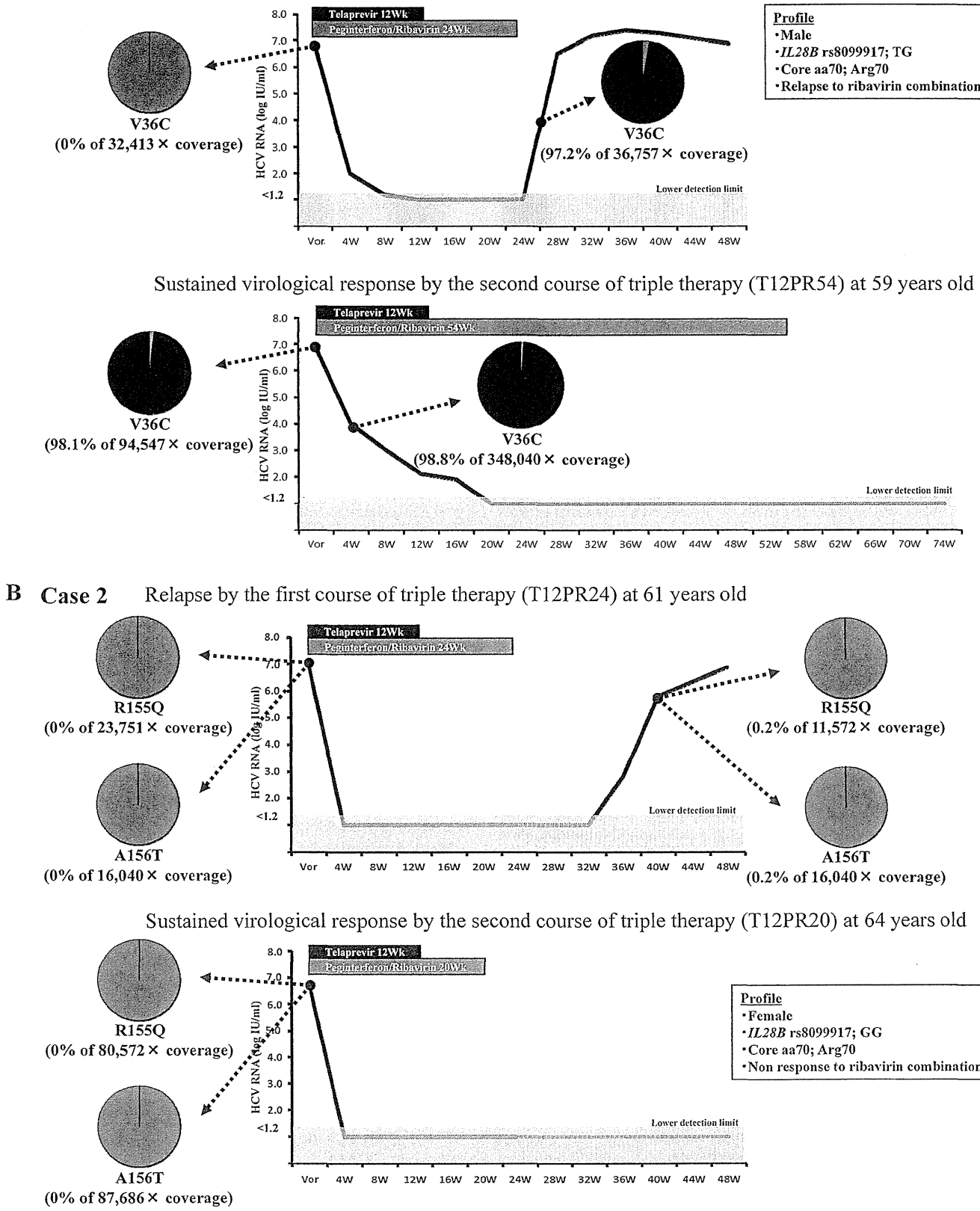

FIG 2 Two patients who did not achieve a sustained virological response with the first course of triple therapy with telaprevir received the second course of the triple therapy with telaprevir. They were analyzed for telaprevir-resistant variants by ultradeep sequencing at baseline and at the time of reelevation of viral loads. (A) Case 1 achieved a sustained virological response with the second course of therapy despite the persistence of very-high-frequency variants. (B) Case 2 achieved a sustained virological response with the second course of therapy despite the history of the emergence of variants.

DISCUSSION

Patients who fail to achieve an SVR to triple therapy need to be identified to avoid unnecessary side effects, high costs, and the emergence of telaprevir-resistant variants. Host genetic factors (e.g., *IL28B* genotype), and viral factors (e.g., amino acid substitutions in the core/NS5A region) have often been used as pretreatment predictors of poor virological response to PEG-IFN–ribavirin dual therapy (9–11, 15, 17) and telaprevir–PEG-IFN–ribavirin triple therapy (24–26). However, the pretreatment factors associated with the detection of telaprevir-resistant variants at the time of reelevation of viral load are still unknown. The present study identified that the detection of telaprevir-resistant variants at the time of reelevation of viral load can be predicted by a combination of host (*IL28B* rs8099917 genotype and leukocyte count), viral (variants of aa 54 at baseline), and treatment factors (PEG-IFN dose). All of the 4 patients with nonresponse to prior treatment and in whom telaprevir-resistant variants were detected at baseline did not achieve an SVR with triple therapy, and the use of the combination of nonresponse to prior treatment and the detection of telaprevir-resistant variants at baseline had high specificity and PPV for the prediction of a non-SVR. This finding suggests that there is a complex relationship between host susceptibility to IFN and viral sensitivity to NS3/4A protease inhibitors in determining treatment efficacy. Interestingly, in all of the 4 patients, both T54S as a telaprevir-resistant variant and Q80L as a TMC435-resistant variant (19) were detected by direct sequencing at baseline. This result suggests that patients with the above two factors should be carefully introduced to NS3/4A protease inhibitors besides telaprevir because of the high risk of the emergence of resistant variants. However, the present study was performed with a small number of patients, so further studies based on a larger number of patients should be performed.

In the present study employing ultradeep sequencing technology, 2 patients who did not achieve an SVR with the first course of triple therapy with telaprevir received the second course of the triple therapy with telaprevir. They achieved an SVR with the second course, despite the persistence of very-high-frequency variants (case 1, 98.1% for V36C) or a history of the emergence of variants (case 2, 0.2% for R155Q and 0.2% for A156T) as determined by ultradeep sequencing. This finding may be due to one or more reasons. One reason is probably related to the high susceptibility of telaprevir-resistant variants to IFN. One previous study indicated that mice infected with the resistant strain (A156F [99.9%]) developed only low-level viremia, and the virus was successfully eliminated with IFN therapy (27). In the other clinical report, telaprevir-resistant variants that emerged during 24-week telaprevir monotherapy were eliminated by the combination therapy of PEG-IFN plus ribavirin (28). Furthermore, this finding probably suggests that a small number of mutant-type viral RNAs may be incomplete or defective, since a large proportion of viral genomes are thought to be defective due to their high replication and mutation rates (29). Further studies employing ultradeep sequencing should be performed to evaluate whether a history of the emergence of NS3/4A protease inhibitor-resistant variants, besides telaprevir-resistant variants, affects the efficacy of a second course of NS3/4A protease inhibitor-based treatment.

The results of the present study should be interpreted with caution, since the study was performed with a small number of Japanese patients infected with HCV-1b. Any generalization of the results should await confirmation by a multicenter randomized trial based on a larger number of patients, including patients of other races and those infected with HCV-1a. Furthermore, the other limitation of the present study is that the loss of telaprevir-resistant variants was not investigated long after the cessation of therapy. Further large-scale studies should be performed to investigate the impacts of telaprevir-resistant variants on the response to treatment using new drugs, including direct-acting antiviral agents.

In conclusion, this study based on Japanese patients infected with HCV-1b indicates that telaprevir-resistant variants at the time of reelevation of viral load can be predicted by a combination of host, viral, and treatment factors. In those patients with no response to prior treatment, the present results suggest that telaprevir-resistant variants at baseline might partly affect the efficacy of triple therapy treatment. This finding indicates the clinical utility of detecting telaprevir-resistant variants to predict treatment efficacy, and it suggests a complex relationship between host susceptibility to IFN and viral sensitivity to NS3/4A protease inhibitors in determining treatment efficacy. Further large-scale prospective studies are needed to investigate the clinical usefulness of telaprevir-resistant variants and to develop more effective therapeutic regimens in patients infected with HCV-1.

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Antiviral Therapy

Telaprevir is effective given every 12 hours at 750 mg with peginterferon-alfa-2b and ribavirin to Japanese patients with HCV-1b IL28B rs8099917 TT

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Original article

Telaprevir is effective given every 12 hours at 750 mg with peginterferon-alfa-2b and ribavirin to Japanese patients with HCV-1b IL28B rs8099917 TT

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Abstract

Background: The aim of this study is to explore the efficacy, safety and pharmacokinetics of 750mg telaprevir (TVR) given at 8 or 12 hour intervals during triple therapy with peg-interferon-alfa-2b (PEG-IFN) and ribavirin (RBV) for patients with chronic hepatitis C virus (HCV) infection.

Methods: 52 patients with high viral loads of genotype 1b who were expected to respond well to therapy (rs8099917 TT genotype or relapse to previous therapy) were randomly assigned to two groups who were given 750mg TVR at either 8 or 12 hour intervals (q8h or q12h) in combination with PEG-IFN and RBV for 12 weeks, followed by an 12 additional weeks of treatment with PEG-IFN and RBV alone. The primary end point of the study was undetectable HCV RNA at 12 weeks after the end of treatment (SVR_{12}).

Results: SVR_{12} rates were 92.3% (24/26) for both q8h and q12h. The changes in mean log_{10} HCV RNA levels and viral response were also similar in q8h compared to q12h, whereas pharmacokinetic properties such as C_{max}, AUC0-24h and C_{trough} of TVR were slightly higher in q8h than in q12h (P>0.2). The frequency of TVR discontinuation due to anemia or renal damage was significantly higher in q12h than in q8h (6/26(23%) vs. 0/20, respectively; P=0.02).

Conclusions: TVR given at 12 hour intervals should be considered for patients with lower body weight, especially patients with prior relapse and with IL28B polymorphisms at rs8099917 TT (interferon lambda 4 ss469415590 polymorphism TT/TT) genotype in patients with genotype 1b HCV infection.

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Running head: Trial of 750 mg of telaprevir given at 8 versus 12 hour intervals

Introduction

There are estimated to be 170 million hepatitis C virus (HCV) carriers worldwide [1,2]. About 30% of carriers develop serious liver diseases, such as decompensated cirrhosis and hepatocellular carcinoma [3,4]. Eradication of the virus is necessary to prevent the development of severe liver damage in these patients.

Telaprevir (TVR), an HCV NS3/4A serine protease inhibitor, has recently been approved in the United States (US), Canada, the European Union (EU) and Japan for treatment of patients with chronic HCV genotype 1 infection. In Phase 3 studies, sustained viral response (SVR) rates increased significantly in both treatment-naïve as well as previously treated patients when TVR was administered in combination with pegylated interferon (PEG-IFN) and ribavirin (RBV) compared to PEG-IFN and RBV alone [5–7]. High SVR rates were also observed in Phase 3 studies in Japan [8,9]; however, side effects of triple therapy in the Japanese studies were so severe that many patients were forced to discontinue therapy due to adverse events, such as anemia and fatigue [5–9]. Anemia, in particular, is commonly associated with triple therapy. The frequency of anemia ranged from 15% to 19% [5,6] in patients treated with PEG-IFN and RBV alone, whereas in patients treated with triple therapy, the frequency of anemia increased to between 30% and 37% [5,6]. In addition, RBV dose-reduction rates and discontinuation rates of TVR treatment due to severe adverse events are higher in Japan than in the USA and EU [5–9]. The higher discontinuation rate may result from taking the same standard prescription dosage of TVR in spite of the lighter body weight of Japanese patients compared with patients in other countries. Japanese patients also tend to be relatively older, and may therefore be at greater risk of severe side effects due to poorer drug metabolism rates. The aim of this study is thus to compare effects and safety of triple therapy with TVR administered at 12 hour intervals compared with the standard 8 hour interval regimen. We also studied pharmacokinetics of TVR in both group of patients to see how the reduction of TVR affects the concentration of TVR.

Patients and Methods

Patients

We enrolled patients at Hiroshima University Hospital, Toranomon Hospital and Sapporo Kosei General Hospital. Patients were enrolled from August 2012, and the last patient completed follow-up in May 2013. Criteria for inclusion were age between 20 and 70 years, chronic infection with HCV genotype 1b, and plasma HCV RNA level of 100,000 IU per ml or greater. We selected patients who were expected to respond well to triple therapy based on one of the following criteria: 1) patients with the treatment-

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favorable rs8099917 TT genotype in the IFN lambda 3 (IL28B) locus, or 2) patients who experienced relapse during prior treatment with PEG-IFN and RBV combination therapy. In order to avoid poor response to reduction of TVR, we excluded patients who were expected to have poor response to the therapy, including prior non-responders to PEG-IFN and RBV therapy (i.e., patients who failed to become negative for HCV RNA) and patients with rs8099917 T/G or G/G genotypes. Exclusion criteria also included liver disease due to other causes, decompensated cirrhosis, presence of liver cancer, HBV or HIV infection, renal insufficiency, history of heart disease or cerebral infarction, and pregnancy or current breastfeeding. IL28B rs8099917, IFN lambda 4 (IFNL4) ss469415590 and inosine triphosphate pyrophosphatase (ITPA) polymorphism (rs1127354) were genotyped using the Invader assay, TaqMan assay or by direct sequencing, as described elsewhere [10–12]. Amino acid substitutions in the HCV core were determined using direct sequencing of polymerase chain reaction products after extraction and reverse transcription of HCV RNA. Core amino acid substitutions at positions 70 and 91 (core 70 and core 91, respectively) were determined as in Akuta et al [13,14]. The demographic and baseline characteristics of patients are shown in Table 1. Median body weight was 62.3 kg, and 25 patients (48%) had body weight lower than 60 kg. IFNL4 ss469415590 and IL28B rs8099917 genotypes were completely linked, except in one patient (Supplementary Table 1).

Study design and randomization

This was an exploratory, prospective, multicenter, randomized study. Experimental procedures were approved by the institutional review boards at participating hospitals, and informed consent was obtained from all participants. Sample size was not based on hypothesis testing other than the precision estimate of SVR. If we assume that 80%, 85%, and 90% of subjects will have undetectable HCV RNA 12 weeks after the end of therapy (SVR_{12}), then 25 subjects per arm would yield two-sided 95% confidence intervals of 64.3 to 95.7%, 71.0 to 99.0% and 78.0 to 100%, respectively. The study was conducted in accordance with the Declaration of Helsinki, and the trial was registered with UMIN Clinical Trials (UMIN000006758). Randomization was stratified according to the combination of prior treatment experience and amino acid substitution at HCV core amino acid 70 (treatment-naïve and wild type, naïve and mutant, transient response and wild, transient response and mutant, non-response and wild or non-response and mutant), age (<60 or ≧60), gender (male or female) and baseline Hb level (<13 or ≧13 g/dl). As shown in Table 1, the demographic and baseline characteristics were well balanced in the two groups of patients.

Mythos (Osaka, Japan), a third party institute that was not involved in the conduct of the study, randomly allocated the two groups of patients to different doses of TVR by means of computer-generated randomization codes.

Study procedures

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TVR was administrated at a randomized dose of 750mg after meals at q8h or q12h intervals. PEG-IFN alfa-2b (PegIntron; MSD, Tokyo, Japan) was administered subcutaneously at a dose of 1.5 μg per kilogram of body weight once weekly, and oral RBV (Rebetol; MSD) was administered at a total dose of 600 to 1200 mg per day based on body weight. Patients received 12 weeks of treatment with TVR plus PEG-IFN/RBV followed by PEG-IFN/RBV alone for an additional12 weeks. Follow-up observation was performed for 24 weeks. RBV dosage was reduced or discontinued as required, based on reduction of hemoglobin levels or the development of adverse events. When hemoglobin decreased below 10 g/dL, the daily dose of RBV was reduced from 600 to 400 mg, from 800 to 600 mg and from 1000 to 600 mg, depending on the initial dose of each patient. RBV was withdrawn when hemoglobin decreased below 8.5 g/dL. Decrease of TVR dose was not permitted, but administration was stopped if necessary due to the development of adverse events.

Efficacy assessments

Serum HCV RNA levels were measured using COBAS TaqMan HCV RNA 2.0 assay (Roche Diagnostics), with a lower limit of quantification of 25IU/ml and a lower limit of detection of 10 IU/ml. The lower limit of detection was used in the determination of undetectable HCV RNA at week 4. HCV RNA levels were measured on day 1 and at the following times: weeks 2, 4, 8, 12, 16, 20, and 24 and every 4 weeks until the end of treatment; and every 4 weeks after the end of treatment until 12 weeks after the end of treatment.

End points

The primary end point was the proportion of patients who had undetectable plasma HCV RNA 12 weeks after the end of treatment (SVR_{12}). The secondary end point was the rate of discontinuation of the therapy due to adverse events.

Pharmacokinetic assessments

Blood samples were collected immediately prior to administering the morning dose, and at week 2 at 1, 2.5, 4, 6, 8 and 12h after the first dose to determine the concentration of TVR (750mg q8h or 750mg q12h) in the plasma. Plasma concentrations of TVR were determined using a high-performance liquid chromatographic apparatus fitted with a mass spectrometer. AUC24h was calculated by multiplying AUC8h by 3 or AUC8h by 2. The maximum plasma concentration (C_{max}) and trough plasma concentration (C_{trough}) were directly determined from the observed values at week 2. Ribavirin concentration was measured prior to the morning dose at week 2.

Safety assessments

Safety assessments including physical examinations, clinical laboratory tests and evaluation of adverse events were performed at each hospital visit during and after treatment at least every 4 weeks until 12 weeks after cessation of the therapy.

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Statistical analysis

Analysis was performed on the intention-to-treatment population, defined as all randomly assigned patients who received one dose of the study medication. Categorical variables between groups were compared using Fisher's exact test and continuous variables using the Mann-Whitney test. All analyses were performed using R version 2.15.3.

Results

Efficacy

SVR12 rates were 92.3% (24/26) for both q8h and q12h. The percentage of patients with undetectable HCV RNA at weeks 2, 4, 12, 24, end of treatment (EOT) and at 12 weeks after EOT (SVR12) was not statistically different between the two groups of patients (Supplementary Fig. 3). Similar decreases in mean log10 HCV RNA levels were observed in both groups of patients (Fig. 1). The SVR12 rate did not differ when the patients were divided by response to previous therapy, age, gender and platelet count (Supplementary Table 2). These results show that the anti-viral effect of triple therapy is nearly equivalent between the two patient groups.

Four patients did not achieve SVR_{12}.The patient characteristics (age [range], gender [male/female], viral load [range] and platelet count [range]) of four patients were 64 years [62-65], 3/1, 6.9 log IU/mL [5.8-7.2] and$17x10^4$/μL [12–22], respectively.

Pharmacokinetics

Mean pharmacokinetics parameters of TVR are shown in Table 2. Trough plasma concentration (C_{trough}) was slightly lower in the q12h group than in the q8h group. AUC24h was also slightly higher in the q8h group than in the q12h group. However, these differences were not statistically significant. The maximum plasma concentration (C_{max}) was similar in both groups of patients.

The mean (±SD) of ribavirin concentration (C_{trough}) at week 2 in the q8h and q12h groups was 1706 (±221) and 1562 (±222) ng/mL, respectively. Although the concentration was slightly higher in the q8h group than in the q12h group, the difference was not statistically significant (P=0.515).

Safety

There were no deaths or serious adverse effects. Adverse events with a frequency of more than 5% in total patients are listed in Table 3. The overall safety profile was similar in both groups of patients except for the frequency of renal damage. The ratios of discontinuation of all treatment due to adverse events were 12% (3/26) in the q8h group and 15% (4/26) in the q12h group (Supplementary Table 3a). Discontinuation of TVR occurred in 42.3% (11/26) of patients in the q8h group and 21.4% (6/28) of patients in the q12h group. Frequency of TVR discontinuation due to anemia or renal damage was

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significantly higher in q12h than in q8h (6/26(23%) vs. 0/20, respectively; P=0.02)(Supplementary Table 3b).

For anemia, decreases of mean hemoglobin levels were similar during the initial 6 weeks. Although mean hemoglobin levels continued to decrease in the q8h group, hemoglobin levels stopped decreasing in the q12h group after week 6 (Fig. 2a). Low hemoglobinemia (<8.5 g/dL) occurred in 8 patients (30.8%) in the q8h group and 6 patients (23.1%) in the q12h group. The genotype of the ITPA SNP had no significant effect on the frequency of anemia. In terms of renal damage, during the 12 weeks of the triple therapy, eGFR decreased significantly more in the q8h group than in the q12h group (Fig. 2b).

Adherence to pegylated IFN and ribavirin treatment was higher in the q12h group, although the difference was not statistically significant (Supplementary Table 4).

Discussion

With the introduction of TVR, the eradication rate of HCV has improved significantly [5–7]. However, severe adverse effects associated with TVR have also been reported, some or which occur more frequently in Japanese patients [8,9]. The dose of TVR for use in triple therapy was determined based on a dose-finding study conducted in the United States and Europe [15], which found that the q8h dosage regimen achieved the greatest reduction of HCV RNA. However, body weights of Japanese patients who were treated with TVR, peginterferon alfa-2b and ribavirin [9] were 61-63 kg compared to 79-91kg among American and European patients who were treated with boceprevir, peginterferon alfa-2b and ribavirin combination therapy [16]. As the dose of TVR is the same among countries where triple therapy is approved, we considered the possibility that the dose of TVR might be too high for smaller Japanese patients and could be reduced. Suzuki et al. previously reported that the anti-viral effect of triple therapy was similar when patients were given TVR at 1500mg/day (every 8 hours at 500mg) compared with those given at 2250mg/day (every 8 hours at 750mg) in the Japanese patients [17], suggesting that reduction of TVR might be possible. However, the treatment period of their study was only 12 weeks, and the study was a non-randomized controlled study with a small number of patients. Therefore, we conducted a randomized controlled trial to confirm that the dose reduction is as effective as the approved regimen. Therefore, we also attempted to test if TVR is as effective when administered at 12 hour intervals instead of 8 hour intervals, based on a pharmacokinetics study in which Marcellin et al. found no difference in viral response and safety profiles between patients treated with the triple therapy with TVR2250 mg (q12h) and TVR2250 mg (q8h) [18]. Furthermore, Buti et al. reported that the effectiveness and safety were similar between patients treated with triple therapy with 2250 mg TVR (q12h) and 2250 mg TVR (q8h) in the OPTIMIZE trial (Phase3b) [19].

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We showed in this study that the effect of TVR given every12 hours at 750mg with PEG-IFN alfa-2b and RBV is the same as TVR given every 8 hours among Japanese chronic hepatitis C patients. However, four patients failed to achieve SVR_{12}, and all treatment was discontinuation within four weeks in these patients. Safety profiles were similar except for differences in the frequency of anemia and renal damage. Hemoglobin levels continued to decline only in patients who received the larger 2,250mg dose, whereas hemoglobin levels plateaued by week 6 in patients who received the 1,500mg dose. We also found that the 1,500mg dosage was also accompanied with a lower frequency of renal damage (Fig. 2b). Incidence of TVR discontinuation was significantly less frequent in patients treated with the 1,500mg regimen. These results suggest that reduction of TVR to 1,500mg and administration of the drug every 12 hours is as effective as the approved 2,250mg dose and is less likely to result in premature termination of TVR therapy (Supplementary Table 3).

We assessed the effect of reduced TVR only in patients who relapsed under previous PEG-IFN/RBV therapy or had the IL28B SNP rs8099917 TT genotype that is associated with a good response to interferon therapy. Patients who had relapsed during previous PEG-IFN/RBV therapy have been reported to respond well to triple therapy [9]. The majority of patients with the rs8099917 TT genotype have also been reported to successfully eradicate the virus with triple therapy [20,21]. The effect of TVR reduction on patients who are expected to be difficult to treat should be further explored in a different trial.

Until recently it was unknown why SNPs near the IL28B locus, such as rs8099917 and rs12979860, are associated with the outcome of interferon therapy. However, the recent characterization of IFNL4 and its association with polymorphism ss469415590 (TT or ΔG) has shed light on this issue [22]. Genotype ss469415590 TT, which fails to express functional IFNL4, is associated with both eradication of HCV by peg-interferon plus ribavirin combination therapy as well as spontaneous clearance of the virus [22]. As this polymorphism is in strong linkage disequilibrium with rs8099917 and rs12979860 in Asian populations [22], it is assumed that in the majority of patients the IL28B and IFNL4 ss469415590 genotypes are in complete linkage disequilibrium, and in fact, there was only one patient who had a discrepancy between ss469415590 and rs8099917 genotypes (Supplementary Table 1). Taken together, patients with ss469415990 genotype TT/TT are expected to be successfully treated with the 1,500mg regimen.

Our results were obtained from Japanese patients with body weights between 61 and 63 kg in each group of patients (Table 1). Results obtained here should be confirmed in patients with a larger body weight. Alternatively, administration of TVR based on body weight should be considered in order to maintain high eradication rates while reducing the risk of adverse effects. However, it should be noted that the limitations of the study are the relatively small patient numbers and enrolling two main groups including prior relapsers and treatment-naïve patients with favorable INFL4 genotypes. A more comprehensive study is essential in the future.

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Disclosure statement

Kawakami Y., Suzuki F., Karino Y., Toyota J ., Kumada H. and Chayama K. are none to declare.

Figure Legends

Figure 1. Decrease of HCV RNA during the therapy.

Data are shown as mean (SD).

Figure 2a. Time course of hemoglobin levels during the triple therapy.

Change from base line hemoglobin concentrations are noted as mean (SD).

Figure 2b. Time course of eGFR levels from baseline during triple therapy.

Asterisks indicate statistically significant differences between patients treated with 1,500mg versus 2,250mg treated. *;P<0.05, **; P<0.01.

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Table 1. Baseline characteristics of patients

Characteristic	750mg q8h group (n=26)	750mg q12h group (n=26)	P-value
Male/Female	17/9	18/8	0.77
Age (years)	61 (24-68)	61 (37-70)	0.99
Body weight (kg)	61.4 (39-82)	63.3 (40-81)	0.76
Body mass index (kg/m^2)	23.5 (16.8-32.0)	22.5 (17.8-27.7)	0.61
White blood cell count ($/mm^3$)	4890 (3500-8920)	4995 (2970-11830)	0.67
Hemoglobin (g/dL)	14.2 (12.2-16.5)	15.2 (11.4-17.4)	0.17
Platelet count ($\times 10^4/\mu L$)	15.9 (5.7-25.3)	16.9 (5.2-25.6)	0.74
ALT (IU/L)	36 (16-292)	40 (14-117)	0.62
γGTP (IU/L)	26 (13-125)	20 (10-192)	0.25
eGFR (mL/min)	80 (62-105)	80 (60-120)	0.61
HCV-RNA (log IU/mL)	6.8 (5.3-7.4)	6.9 (5.2-7.8)	0.26
Previous IFN therapy (naive/relapse/non-response)	14/9/3	11/11/4	0.42
rs8099917 (TT/TG)	25/1	26/0	0.32
ss469415590 (TT/TT/TT/ΔG)	24/2	26/0	0.49
rs1127354 (CC/non-CC/ND)	18/8	20/5/1	0.39
HCVcore70 (wild/mutant/ND)	17/6/3	20/4/2	0.67

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Table 2. Pharmacokinetic Parameters of Telaprevir at Week 2.

Pharmacokinetic parameter	750mg q8h group (n=10)	750mg q12h group(n=10)	P-value
C_{trough} (μ g/ml)	2.80±1.33	2.00±0.59	0.243
1hr(μ g/ml)	2.93±1.35	3.07±0.81	0.661
2.5hr(μ g/ml)	3.60±1.66	3.24±1.22	0.842
4hr(μ g/ml)	3.42±1.40	3.03±1.02	0.661
6hr(μ g/ml)	3.02±1.41	2.51±0.97	0.549
8hr(μ g/ml)	2.48±1.37	1.98±0.77	0.549
12hr(μ g/ml)	3.42±1.47	1.36±0.70	<0.001
C_{max} (μ g/ml)	3.90±1.50	3.74±0.99	0.720
AUC24h (μ g*h/ml)	74.91±32.91	57.16±18.12	0.243

All values are expressed as mean ± SD.
AUC24h calculated by multiplying AUC8h by 3 or AUC12h by 2.