40–50% for patients with genotype 1 [5, 6]. Several direct-acting antiviral agents (DAAs) for HCV infection have been clinically evaluated [7]. Telaprevir (VX-950/MP-424) is a novel peptidomimetic slow- and tight-binding inhibitor of HCV NS3-4A protease, which was discovered using a structure-based drug design approach [8]. As one of the most advanced DAAs against HCV, phase 3 clinical trials of telaprevir are on-going in the US, EU, and Japan. Recent clinical trials of telaprevir in combination with the standard treatment have indicated a promising advancement in therapy for treatment-naïve CHC patients as well as patients who did not respond previously to the standard treatment alone [9–11]. However, compared with the standard treatment alone, telaprevir is associated with an increased incidence of several side effects, such as anemia and skin rash.

The epidemiology of HCV in Japan is different from that in the US and EU; the majority of Japanese HCV carriers are of age >55 years, and three-fourths of Japanese HCV carriers are infected with genotype 1, which consists almost entirely of subtype 1b [12–14]. The dose reduction rate and the frequency of discontinuation of this treatment are high in elderly patients [15]. The SVR rate of the standard therapy is lower in females than males, especially in older patients in Japan [16]. In addition to the need for a therapy yielding higher SVR rates than the current standard therapy, there is also the need for a treatment regimen with a lower incidence of severe side effects because of the characteristics of HCV carriers in Japan.

Since our institution is a site of the phase 2a trial of telaprevir monotherapy among Japanese patients infected with HCV subtype 1b, our primary objective was to evaluate the safety, tolerability, and efficacy of telaprevir alone for up to 24 weeks. We also assessed the selection of HCV subtype 1b variants under prolonged telaprevir monotherapy and the susceptibility of these selected variants to the standard PEG-IFN and RBV therapy.

Patients and methods

Study design and organization

This single-arm, open-label study was conducted between January 2008 and September 2008 at Sapporo Kosei General Hospital, Sapporo, Japan, as a site of the telaprevir phase 2a monotherapy trial in Japan. The study was conducted in compliance with the Good Clinical Practice guidelines and the Declaration of Helsinki. Before the study, the protocol and informed consent form were approved by an institutional review board. Written informed consent was obtained from each patient after sufficient explanation before participation in the study.

All patients received telaprevir at a dose of 750 mg every 8 h orally for a maximum of 24 weeks, which was determined by the stopping rule of viral kinetics [2 $\log_{10}$ increase from the nadir or 3 $\log_{10}$ IU/ml if the nadir was below the lower limit of quantification (LOQ)]. Telaprevir was administered in the fed state. After the patients met the stopping rule of viral kinetics, the investigators recommended the patients to begin the standard treatment for HCV infection (weight-based PEG-IFN alpha-2b and RBV) in order to prevent them from the earlier treatment failure. This standard treatment was off-study. The dose of PEG-IFN alpha-2b was specified in the package insert. The doses of RBV were based on total body clearance (CL/F) calculated by the following equation:

$$\begin{aligned} \mathrm{CL}/F\,(\mathrm{L/h}) = {} & 3.23 \times \text{body weight (kg)} \\ & \times (1 - 0.0094 \times \text{age}) \times (1 - 0.42 \\ & \times \text{gender}) / \text{serum creatinine}\,(\mu\mathrm{mol/L}), \end{aligned}$$

where gender = 0 for male and 1 for female. The RBV dose was set for a targeted blood concentration of 2250 ng/ml.

Telaprevir was supplied as 250-mg tablets for oral administration provided by Mitsubishi Tanabe Pharma Corp., Osaka, Japan. PEG-IFN alpha-2b and ribavirin (Pegintron® and Rebetol®) were obtained from Schering-Plough, KK, Osaka, Japan.

Participants

Patients were enrolled in this study according to the following inclusion criteria: diagnosis of CHC; infection with HCV genotype 1b as determined by phylogenetic analysis on the NS5B region; no prior antiviral therapy for HCV; Japanese (Mongoloid) lineage; age 20–70 years at enrollment. Patients were excluded from the study if they met any of the following criteria: diagnosis of decompensated liver cirrhosis and/or hepatitis B surface antigen in serum; diagnosis or history of hepatocellular carcinoma; previous treatment for malignant neoplasm; diagnosis of autoimmune hepatitis, alcoholic liver disease, hemochromatosis, or chronic liver disease other than CHC; history of allergy to medication or anaphylactoid symptoms; women who were pregnant, breast feeding, or who planned to become pregnant.

Safety assessments

The safety and tolerability of the study treatments were assessed by clinical laboratory results, vital signs, physical examination results, and occurrence of adverse events. These safety parameters were recorded at regular intervals from day −28 through the follow-up visits. Adverse events

were classified according to the Medical Dictionary for Regulatory Activities (MedDRA), version 12.0.

HCV RNA measurement

The HCV subtype was determined by direct sequencing followed by phylogenetic analysis on the NS5B region [17]. The serum HCV RNA levels were determined using the COBAS TaqMan® HCV test (Roche Diagnostics, Tokyo, Japan). The linear dynamic range of the assay was from 1.2 to 7.8 $\log_{10}$ IU/ml. The LOQ of the assay was 1.2 $\log_{10}$ IU/ml, and the qualitative result below LOQ was also determined as positive (+) and negative (−). Blood samples in this study were collected on days −28, 1 (before the first dose), 3, 8, 15, 29, 43, 57, 71, 85, 99, 113, 127, 141, 155, and 169 of the study drug dosing period, at the 2-week follow-up, and on the days when the patients met the stopping rules. During the off-study treatment, blood samples were collected before the first injection, 1 and 2 weeks after the off-study treatment was initiated, and every 4 weeks thereafter.

Viral sequencing analysis

The HCV interferon sensitivity determining region (ISDR) on NS5A [18] and the core region [19] were analyzed by the direct sequencing method. The DNA fragment containing the 534-bp (181 amino acids) NS3 protease domain was amplified by the nested reverse transcription-polymerase chain reaction and cloned. At least 39 clones per specimen were sequenced and determined bidirectionally. The sequences of the NS3 protease domain registered in the public databases of the National Center for Biotechnology Information (NCBI), except the protease-resistant variants reported previously [20–23], were considered to be a naturally occurring variant and treated as a wild type in the analysis. The limit of detection for the sequencing analysis was approximately 3 $\log_{10}$ IU/ml.

Viral dynamics model analysis

The basic mathematical model for the analysis of HCV infection in vivo, which is a system of three ordinary differential equations for uninfected cells (T), productively infected cells (I), and free virus (V), has been reviewed elsewhere [24]. The solved Eq. 1 was fitted to the HCV RNA levels ($\log_{10}$ IU/ml) obtained in this study via nonlinear regression using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA). The positive and negative qualitative values below LOQ were treated as 1.0 and 0.5, respectively.

$$V(t) = V_0 + \log_{10}\left[e^{-ct} + \frac{(1-\varepsilon)c}{c-\delta}(e^{-\delta t} - e^{-ct})\right] \quad (1)$$

where c is the virion clearance rate from serum, δ is the clearance rate of infected cells, and ε is the effectiveness in blocking virion production.

Genetic variation near the IL28B gene

Analysis of genetic variation near IL28B gene was performed by use of Invader assay, TaqMan assay, or direct sequencing as described previously [25, 26]. In this study, a single nucleotide polymorphism (SNP) near IL28B gene (rs8099917), reported as one of the predictors of non-response to PEG-IFN and RBV therapy [27], was retrospectively checked.

Results

Patient characteristics

Four females at a median age of 54 years (range 48–58) were enrolled in the study. Patient baseline characteristics are summarized in Table 1. The mean baseline HCV RNA level was 6.1 $\log_{10}$ IU/ml (range 5.2–6.9). The amino acid (aa) sequences of the HCV core region at positions 70 and 91 and the ISDR were also analyzed. The substitution of arginine at core aa 70 was observed in 2 of the 4 patients, whereas the substitution of leucine at core aa 91 was not observed. The number of amino acid substitutions at ISDR aa 2209–2248 was 1 in 2 of the patients and 2 or more in 2 of the patients. We retrospectively checked rs8099917, which is the typical SNP near the IL28B locus associated with non-response to the standard therapy, and confirmed that all 4 study subjects possessed the major allele (T/T).

HCV RNA kinetics

Two of the 4 study patients completed the scheduled telaprevir dosing period of 24 weeks. Patient 2 showed an HCV RNA level below 1.2 $\log_{10}$ IU/ml at the end of treatment, whereas patient 1 showed a negative HCV RNA level at week 8 and had viral breakthrough at week 20 while receiving the study drug. The other 2 patients also showed a rapid decline in viral load to below 2 $\log_{10}$ IU/ml, but they met the stopping rule of viral breakthrough and ceased the study drug at weeks 15 and 6 (patients 3 and 4, respectively).

After the telaprevir monotherapy was stopped, each study patient agreed to enroll in the off-study treatment with PEG-IFN and RBV. By mutual agreement between the patients and investigators, the duration of the standard

Table 1 Baseline characteristics of enrolled patients

Factor	Patient			
	1	2	3	4
Age (years)	48	51	58	57
Sex	Female	Female	Female	Female
Height/body weight (cm/kg)	160.0/51.2	161.4/48.9	165.0/51.6	153.0/49.0
Body mass index (kg/m^2)	20.0	18.8	19.0	20.9
Subtype	1b	1b	1b	1b
Core aa 70/aa 91	R70H/wild	R70H/wild	Wild/wild	Wild/wild
ISDR substituted aa sites	1	2	1	3
IL28B SNP (rs8099917)[a]	T/T	T/T	T/T	T/T

[a] T/T is homozygote of the major allele

Table 2 Summary of the off-study treatment

	Patient			
	1	2	3	4
Baseline (TVR mono/off-study)				
Neutrophils (/μg)	3762/2142	2258/2995	2284/2503	1677/2013
Hemoglobin (g/dl)	14.6/10.8	13.4/10.9	12.9/10.7	12.3/11.7
Platelets ($\times 10^4$/μl)	22.4/16.8	28.1/25.9	12.4/14.3	15.5/17.6
ALT (IU/l)	20/11	28/11	40/18	66/91
HCV RNA ($\log_{10}$ IU/l)	6.2/3.7	5.9/3.3	6.9/5.1	5.2/5.0
Dosage				
PEG-IFN α-2b (μg)	80	80	80	80
RBV, max/min (mg)	400/–	600/400	600/200	600/200
Mean RBV (mg/kg/day)	7.8	8.8	8.3	7.2
Accumulated RBV, entire period (g/kg)	2.6	3.9	4.3	2.5
Outcome				
Time after the last TVR (days)	20	26	13	0
HCV RNA negativity (weeks)	2	13	8	8
Duration of treatment (weeks)	48	60	72	48
Treatment response	SVR	SVR	SVR	SVR

TVR telaprevir, *PEG-IFN* peginterferon, *RBV* ribavirin, *SVR* sustained virological response

treatment was decided according to gender, age, substitutions at core aa 70 and 91, the number of substitutions at the NS5A ISDR domain [28], and the time to HCV RNA becoming undetectable (Table 2). Patients 1 and 4 received the off-study treatment for 48 weeks. Patients 2 and 3 were treated beyond 48 weeks, and patient 3 completed 72 weeks of treatment. In patient 2, the off-study treatment was discontinued at week 60 because of the aggravation of subjective symptoms including malaise and insomnia. The HCV RNA levels became negative at 2, 13, 8, and 8 weeks in patients 1, 2, 3, and 4, respectively. SVR was attained in all patients after completion of the off-study treatment (Table 2). Viral kinetics during the 2 courses of treatment are shown in Fig. 1a.

Safety

During the telaprevir monotherapy, all subjects had at least one adverse event with mild to moderate severity. No serious adverse reactions occurred that caused the discontinuation of telaprevir. All patients exhibited a decrease in their hemoglobin levels. Other biochemical blood changes were found in one of each study patient (changes such as increased uric acid, decreased white blood cell count,

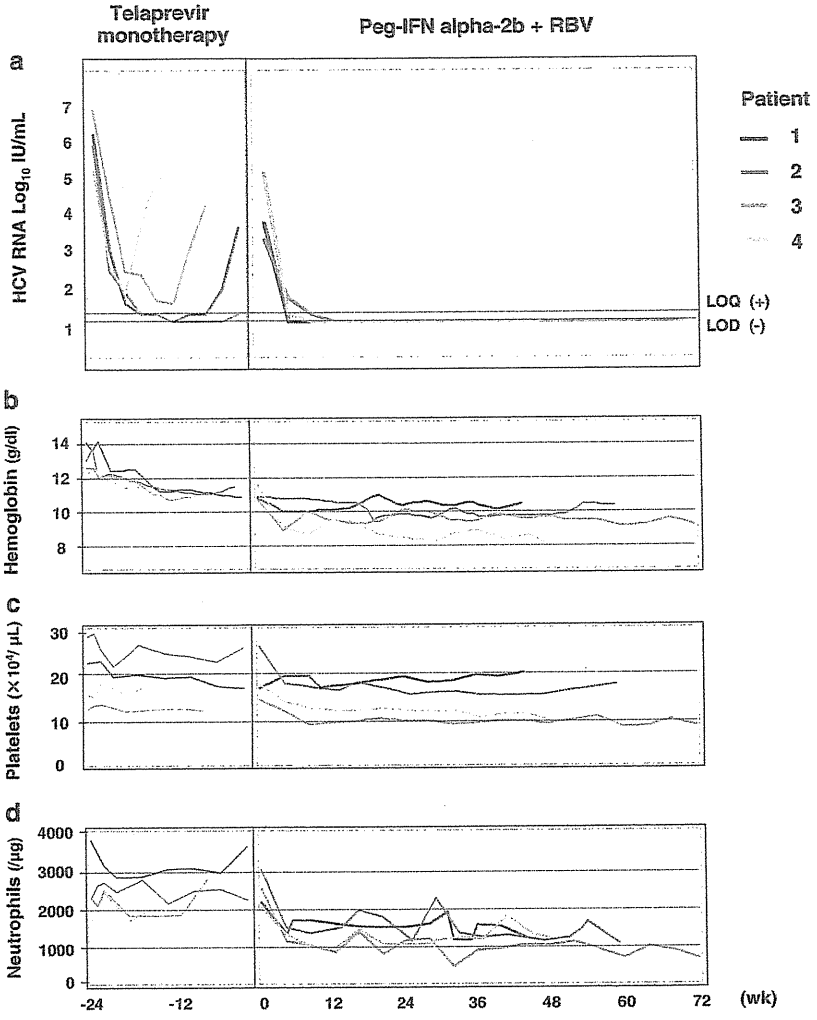

Fig. 1 Changes in plasma HCV RNA, hemoglobin, platelets, and neutrophils for individual patients during administration of telapreivir alone, or peginterferon alpha-2b and ribavirin. Panels on left are telaprevir alone, and those on right are peginterfron alpha-2b and ribavirin therapy. The HCV RNA levels were monitored by the COBAS TaqMan HCV test; limit of quantification (LOQ) is 1.2 $\log_{10}$ IU/ml, with qualitative values below LOQ a positive (+) and limit of detection (LOD) negative (−)

decreased platelet count, and increased serum creatinine level). The observed clinical symptoms were rash, headache, and gastrointestinal symptoms including nausea, stomach discomfort, and gastroesophageal reflux disease, peripheral edema, and pyrexia. No notable adverse event occurred during the off-study treatment with PEG-IFN and RBV except what is usually observed with the standard therapy (Table 3).

The median hemoglobin concentration at the beginning of this study was 13.2 g/dl (range 12.3–14.6) and decreased to 10.9 g/dl (range 10.7–11.7) at the beginning of the off-study treatment (Fig. 1b). No fixed tendency was observed for platelet count and neutrophil count during the course of telaprevir monotherapy, whereas these counts mildly decreased during the course of off-study treatment (Fig. 1c, d).

NS3 protease genotypic analysis

Clonal sequencing analysis on the NS3 protease domain was investigated (Fig. 2). Before the administration of telaprevir, only the wild-type variants were observed in all patients at two time points. Before viral breakthrough, a telaprevir-resistant variant (A156V) could be detected in only 1 patient (patient 3) on day 8 because of rapid viral decline below 3 $\log_{10}$ IU/ml. After emergence of A156V in this patient, the HCV RNA load was still suppressed under the telaprevir monotherapy until week 8; however, another double-substituted variant (T54A+I132L) was detected as the major variant after viral breakthrough. Although patient 4 showed a decrease in the HCV RNA level to 1.8 $\log_{10}$ IU/ml at week 1, viral breakthrough was observed at week 2, and there were two types of resistant variants (A156T and T54A). As the telaprevir treatment was prolonged, the major variant shifted to the double-substituted variant (T54S+A156T). Patient 1 completed the dosing schedule for 24 weeks, but experienced viral breakthrough at week 20. At the end of treatment, the novel substitution of A156F was observed as the major variant. After the withdrawal of telaprevir, other variants including A156Y and T54S+A156T emerged. However, the HCV

Table 3 Adverse events

	Telaprevir monotherapy *n* (%)	Peg-IFN α-2b+RBV *n* (%)
Anemia	4 (100)	4 (100)
Headache	2 (50)	
Rash	2 (50)	
Blood uric acid increased	1 (25)	
Pruritic rash	1 (25)	
Pruritus	1 (25)	
Nausea	1 (25)	
Stomach discomfort	1 (25)	
Gastroesophageal reflux disease	1 (25)	
Peripheral edema	1 (25)	
Pyrexia	1 (25)	
Musculoskeletal stiffness	1 (25)	
White blood cell count decreased	1 (25)	4 (100)
Platelet count decreased	1 (25)	2 (50)
Blood creatinine increased	1 (25)	1 (25)
General fatigue		2 (50)
Loss of appetite		2 (50)
Insomnia		1 (25)
Lack of concentration		1 (25)
Palpitations		1 (25)
Dyspnea		1 (25)

RNA levels remained lower than the baseline (around 4 $\log_{10}$ IU/ml) for 3 weeks, and the major variant further shifted to A156V+V158I just before initiation of the off-study treatment. Patient 2 completed the dosing schedule, with the HCV RNA level below 1.2 $\log_{10}$ IU/ml at the end of treatment. After completion, HCV RNA levels increased, and only the wild-type variant was observed at the 4-week follow-up.

Viral dynamics model analysis

In order to compare the viral dynamics in the initial phase of both treatments, the solved equation from the conventional mathematical model [24] was fitted to the observed values (Fig. 3). The best fit values are summarized in Table 4. At treatment initiation, the HCV RNA levels were equivalent or lower in the off-study treatment than in the telaprevir treatment. The first-phase clearing of telaprevir-resistant variants by the PEG-IFN+RBV treatment was comparable to that of the wild-type variants by telaprevir alone in 3 of the patients. However, in patient 2, the wild-type variants were less susceptible to PEG-IFN+RBV than telaprevir.

Discussion

In this study, 4 treatment-naïve patients with CHC participated in the phase 2a telaprevir monotherapy study in Japan. The subjects were all middle-aged to elderly females infected with HCV subtype 1b, the predominant subtype in Japan. The study patients possessed the baseline viral factors that suggest "difficult to treat" by the standard therapy [28]: the substitution at core aa 70 was observed in patients 1 and 2, and the number of aa substitutions at the NS5A ISDR domain was <1 in patients 1 and 3.

After the completion or discontinuation of the telaprevir monotherapy, PEG-IFN and RBV therapy was initiated as soon as possible to preserve the telaprevir-resistant variants as the majority of the viral population. The standard therapy was initiated soon because the in vivo viral fitness of the telaprevir-resistant variants was estimated to be lower than that of the wild type [20], and some select variants under the telaprevir treatment were susceptible to the PEG-IFN and RBV therapy [21]. Three patients who met viral breakthrough criteria during telaprevir monotherapy definitely had only the telaprevir-resistant variants, including novel substitutions of A156F and A156Y. In addition, the T54S and V158I substitutions, which were reported from the clinical trial of boceprevir [22, 23], were all observed to be a secondary mutation associated with A156 substitutions in this telaprevir trial. Moreover, the other patient (patient 2) had only the wild-type variants at 26 days after the completion of 24 weeks of telaprevir monotherapy. Although it is unclear whether the wild type arose as a reverse mutation, Suzuki et al. [29] recently reported a patient who achieved SVR in the same telaprevir monotherapy trial. These observations suggest a higher genetic barrier for telaprevir among Japanese patients with HCV subtype 1b than in patients observed previously in the EU and US [20, 21]. At least, the telaprevir-resistant variants observed in this study showed some susceptibility to the off-study treatment (Fig. 3).

Anemia has been described as a major adverse event caused by the triple combination therapy including telaprevir [9–11], but the onset mechanism of anemia has not been elucidated. In the phase 1b clinical trial of the triple combination regimen for 12 weeks in Japan, the discontinuation rate due to adverse events was 35% (7 of 20 patients); in 5 of these 7 patients, the triple therapy was discontinued because the hemoglobin decreased to <8.5 g/dl [30]. In the present study, all the patients developed mild anemia after the administration of telaprevir alone for up to 24 weeks (Fig. 1); the median baseline hemoglobin concentration of 13.2 g/dl had decreased to 10.9 g/dl at the initiation of the off-study treatment (Table 2). In 3 of the 4 study patients, the hemoglobin concentration further decreased with the

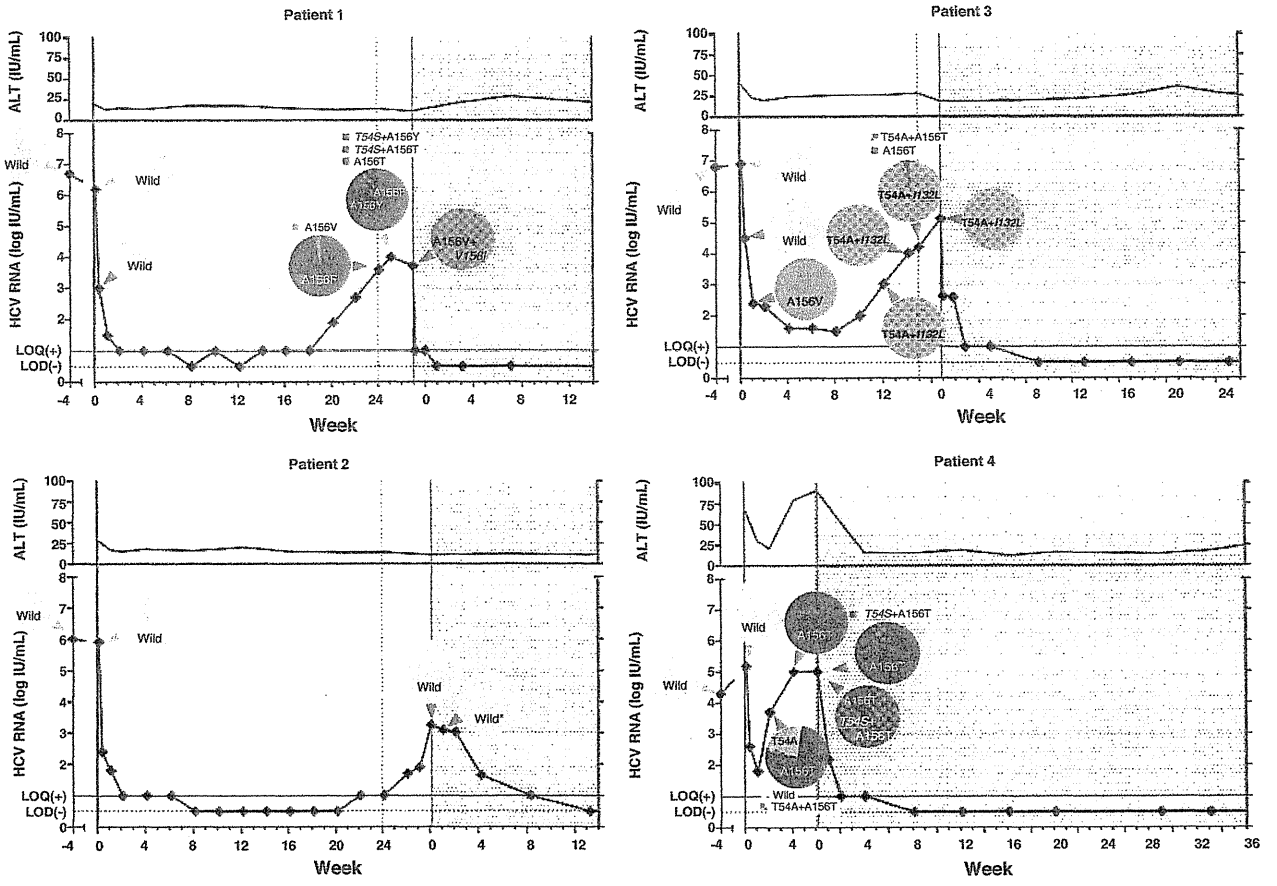

Fig. 2 Viral sequencing results and alanine aminotransferase (ALT) elevation after viral breakthrough for individual patients. *Shaded backgrounds* indicate the off-study treatment. *Circular charts* indicate the population of NS3 protease variants in >39 clones, except the chart indicated by an *asterisk* in 16 clones. *Arrowheads*: *aqua* pretreatment, *red* during treatment, *green* at initiation of off-study treatment

standard treatment; therefore, a dose reduction of RBV was required. Especially in patient 4, the dose of RBV was reduced to 200 mg from the initial dose of 600 mg to maintain the hemoglobin concentration above 8 g/dl. Thus, we managed the decrease in hemoglobin without using erythropoietin. No discontinuation due to anemia occurred during the off-study treatment. Hiramatsu et al. [31] reported that maintaining the RBV dose at >12 mg/kg/day was important even after complete early virological response to avoid relapse after the standard therapy. Although the RBV doses among our 4 study patients ranged from 7.2 to 8.8 mg/kg/day, and the accumulative RBV doses for 48 weeks in patients 1 and 4 were <3 g/kg, SVR was achieved in all cases. Besides relatively lower exposure to RBV, our patient demography of females at a median age of 54 years (range 48–58) is noteworthy. In their study on the standard therapy among Japanese patients infected with HCV subtype 1b, Sezaki et al. [16] reported SVR stratified rates as 53% in males and 22% in females in patients older than 50 years, and no significant gender difference was observed in patients younger than 50 years. However, a study performed in the US suggested higher SVR rates among females than males in patients infected with HCV subtypes 1a and 1b [32]. Although this controversy on gender difference may be attributed to different ethnic groups, the HCV subtypes 1a and 1b were considered to spread in a different epoch [33]. Therefore, we speculate that age distribution of HCV carriers in a certain geographic region exerts an impact on the response rates and severity of anemia with the standard therapy. The recent study on SNPs near the IL28B gene also confirmed that female gender and elderly age remain as factors related to non-virological response [27]. In conclusion, we can avoid treatment failure caused by anemia by carefully adjusting the RBV dosage in the standard therapy that

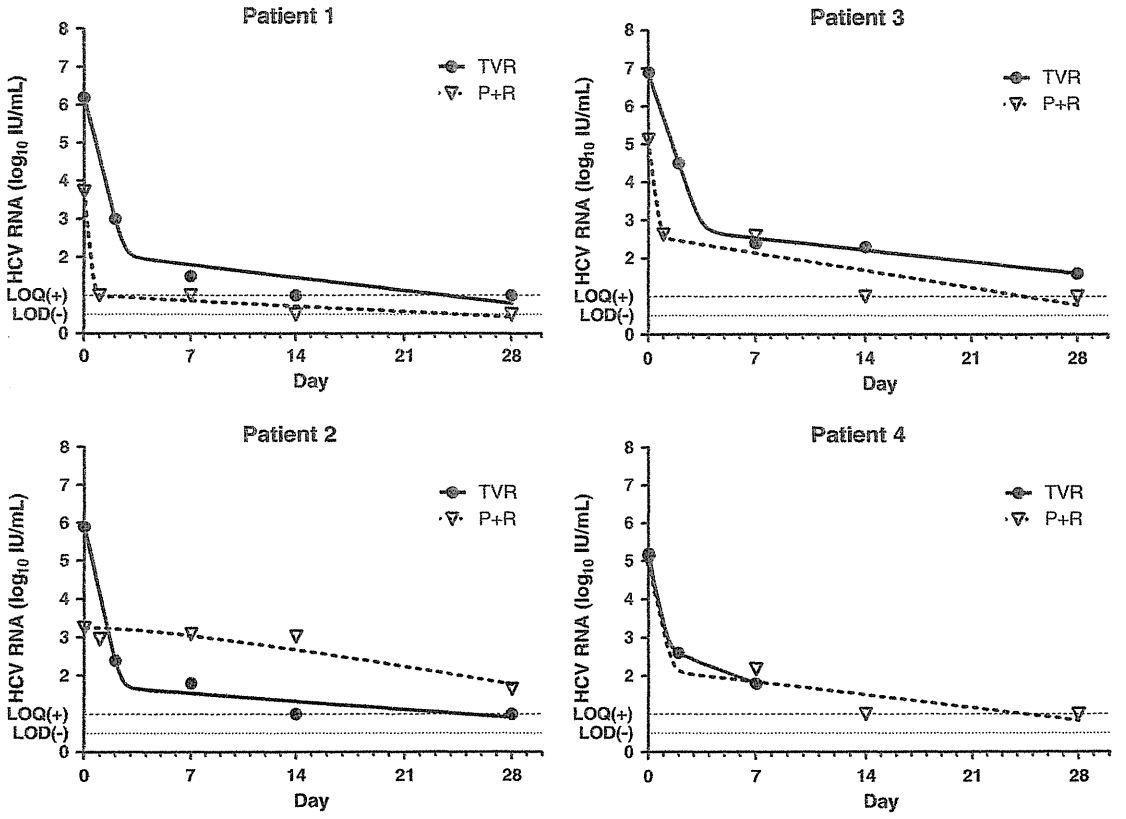

Fig. 3 Viral kinetics modeling on initial 4 weeks of telaprevir alone and peginterferon alpha-2b and ribavirin. *Solid lines* are telaprevir (TVR) alone and *dotted lines* are peginterferon alpha-2b and ribavirin (P+R)

Table 4 Estimates from the viral dynamics modeling analysis

Patient	Treatment	Baseline		Estimated parameters[a]		
		Viral load ($\log_{10}$ IU/ml)	NS3 aa substitution	ε	c (day^{-1})	δ (day^{-1})
1	Telaprevir mono	6.2	Wild	(0.9999)	(3.745)	0.1117
	PEG-IFN+RBV	3.7	A156V+V158I	0.9981	9.342	0.04699
2	Telaprevir mono	5.9	Wild	(0.9999)	(4.139)	0.07018
	PEG-IFN+RBV	3.3	Wild	$<10^{-11}$	0.1913	0.1828
3	Telaprevir mono	6.9	Wild	(0.9999)	(2.772)	0.1018
	PEG-IFN+RBV	5.1	T54A+I132L	0.9971	7.382	0.1494
4	Telaprevir mono[b]	5.2	Wild	(0.9954)	(4.572)	(0.3598)
	PEG-IFN+RBV	5.0	T54S+A156T	(0.9985)	(4.278)	0.1109

[a] ε is the effectiveness in blocking virion production, c is the virion clearance rate from serum, and δ is the clearance rate of infected cells. Software reported parenthetic values as ambiguous

[b] Estimated from days 0–7 because of viral breakthrough

follows telaprevir monotherapy. SVR was initially achieved in all cases. However, relapses occurred in patients who received telaprevir alone, suggesting that the current standard therapy remains important in this sequential regimen.

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Common Genetic Polymorphism of ITPA Gene Affects Ribavirin-Induced Anemia and Effect of Peg-Interferon Plus Ribavirin Therapy

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An association between a single nucleotide polymorphism (SNP) in the inosine triphosphate pyrophosphatase (ITPA) gene and reduction of hemoglobin during peg-interferon plus ribavirin combination therapy for patients with chronic hepatitis C virus (HCV) infection has been reported. However, the effect of the SNP on outcome of therapy has not been fully elucidated. Factors associated with anemia during combination therapy, including rs1127354 genotype, were analyzed in 1,002 treated patients. The effect of the SNP on outcome of therapy was analyzed in a subset of 830 patients with genotype 1. A rapid initial decrease in hemoglobin levels was observed in patients with rs1127354 genotype CC compared with a slow decrease in non-CC patients. Cumulative reduction of ribavirin was significantly more frequent in genotype CC patients than non-CC patients (odds ratio 1.928, $P = 8.6 \times 10^{-8}$). The frequency of patients who received at least the recommended 80% of scheduled ribavirin was significantly lower among genotype CC patients, especially among those who had pretreatment hemoglobin levels between 13.5 and 15 g/dl ($P < 0.03$), and the sustained viral response rate was significantly lower in this group of patients. Independent predictive factors for sustained virological response included a SNP in the IL28B locus (rs809991), age, fibrosis, ITPA SNP rs1127354 as well as pretreatment hemoglobin levels. Our data suggests that measures to prevent anemia should be considered for patients who have pretreatment hemoglobin levels less than 13.5 g/dl or who have rs1127354 genotype CC and pretreatment hemoglobin levels between 13.5 and 15 g/dl. ***J. Med. Virol. 83:1048–1057, 2011.*** © 2011 Wiley-Liss, Inc.

KEY WORDS: inosine triphosphate pyrophosphatase; single nucleotide polymorphism; peg-interferon; anemia; dose reduction

INTRODUCTION

Hepatitis C virus (HCV), a positive-strand RNA flavivirus, chronically infects 170 million people worldwide and is responsible for up to 300,000 deaths due

Abbreviations: HCV, hepatitis C virus; ITPA, inosine triphosphate pyrophosphatase; SNP, single nucleotide polymorphism.

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to progression to liver cirrhosis and hepatocellular carcinoma [Alter, 1995; Chevaliez and Pawlotsky, 2007]. Currently, peg-interferon plus ribavirin combination therapy (PEG-RBV) is the most effective treatment, but it is only effective in 50% of patients with genotype 1b, and the therapy has severe side effects often requiring dose modification or discontinuation [Hadziyannis et al., 2004]. However, there are several factors that may help predict outcome of therapy, including HCV genotype [Zeuzem et al., 1996], virus titer [Zeuzem et al., 1996; Dienstag and McHutchison, 2006], age, fibrosis of the liver, obesity, race, hepatic steatosis [Dienstag and McHutchison, 2006], LDL cholesterol, gamma-GTP [Akuta et al., 2007], insulin resistance [Romero-Gómez et al., 2005], amino acid substitutions at positions 70 and 91 of the HCV core protein and accumulation of substitutions in the interferon sensitivity determining region (ISDR) of the NS5A protein [Enomoto et al., 1995a; Akuta et al., 2005]. A series of recent studies have also identified common genetic variants in the IL28B locus on chromosome 19 [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009] that are strongly associated with outcome of combination therapy.

Ribavirin-induced anemia is a serious side effect of therapy which results in dose reduction of ribavirin and possibly of interferon as well. The precise mechanism of induction of anemia remains to be determined. Ribavirin-induced hemolytic anemia accompanied by an increase in reticulocyte counts has been reported to be associated with membrane oxidative damage as well as impairment of erythrocyte Na–K pump activity and increase in dithiotreitol-sensitive fraction, malondialdehyde, and methemoglobin levels [De Franceschi et al., 2000]. Treating patients with erythropoietin, which induces erythropoiesis and helps alleviate anemia, has been reported to be effective in preventing ribavirin dose reduction and leads to better therapy outcome [Dieterich et al., 2003].

Recently, single nucleotide polymorphisms (SNPs) in the inosine triphosphate pyrophosphatase (ITPA) locus have been found to be associated with anemia in patients treated with combination therapy [Fellay et al., 2010; Ochi et al., 2010; Thompson et al., 2010]. In Caucasian patients there are two SNPs that are associated with ITPA enzyme activity [Fellay et al., 2010; Thompson et al., 2010], although one of these SNPs appears to be absent in Japanese patients [Ochi et al., 2010]. Although the effect of the ITPA polymorphism on ribavirin-induced anemia has been clearly demonstrated by these studies, the effect of the SNP on outcome of therapy has not been fully explored. Our previous report suggested an association of the polymorphism with sustained virological response (SVR) [Ochi et al., 2010], whereas other reports found no association [Fellay et al., 2010; Thompson et al., 2010].

In the current study, 1,002 patients who were treated with peg-interferon 2b plus ribavirin combination therapy were analyzed to elucidate the precise effect of the ITPA SNP on hemoglobin reduction. A subset of 830 of the patients with genotype 1 were further examined to assess the effect of the SNP on therapy outcome. The results show that reduction of ribavirin was frequent among patients with low pretreatment hemoglobin levels (<13 g/dl) as well as those with the ribavirin-sensitive ITPA genotype (rs1127354 CC) and intermediate pretreatment hemoglobin levels (13.5–15 g/dl). Our results suggest that anemia-preventing measures, such as administration of erythropoietin, should be considered for patients likely to develop anemia.

MATERIALS AND METHODS

Patients

Data from 1,002 patients who were treated with peg-interferon alpha 2b and ribavirin combination therapy for chronic hepatitis C infection between December 2004 and January 2010 were collected from Toranomon Hospital (Tokyo) and hospitals belonging to the Hiroshima Liver Study Group (http://home.hiroshima-u.ac.jp/naika1/hepatology/english/study.html) in Hiroshima, Japan. Patient profiles are shown in Table I. All patients tested positive for HCV RNA for more than 6 months and were negative for hepatitis B and HIV and showed no evidence for other liver diseases including alcoholic hepatitis, hemochromatosis, Wilson's disease, and autoimmune hepatitis. Patients received weekly injections of peg-interferon-alpha-2b at 1.5 g/kg body weight for 48 weeks, and ribavirin was administered orally. The amount of ribavirin was adjusted based on body weight (600 mg for <60 kg, 800 mg for 60–80 kg, and 1,000 mg for >80 kg). Ribavirin dose was reduced when hemoglobin levels fell to 10 g/dl, and both peg-interferon and ribavirin were discontinued when hemoglobin levels dropped to <8.5 g/dl. Patients who remained positive for HCV RNA during the first 12 weeks of treatment but became negative by week 32 received extended administration of both drugs until 72 weeks. The successful endpoint of treatment was considered SVR, defined as undetectable HCV RNA levels 24 weeks after cessation of treatment. A subset of patients showed transient response (TR), in which HCV RNA dropped to undetectable levels but then later rebounded. The remaining patients in which HCV RNA never became undetectable were considered non-responders (NVR). Histopathological diagnosis was made by pathologists at each hospital according to the criteria of Desmet et al. [1994]. All subjects gave written informed consent to participate in the study according to the process approved by the ethical committee of each hospital and conforming to the ethical guidelines of the 1975 Declaration of Helsinki.

HCV RNA Levels

HCV RNA levels were measured throughout the course of therapy via RT-PCR using the original

TABLE I. Characteristics of Patients by ITPA rs1127354 SNP Genotype

	All patients	Patients with HCV genotype 1		
	Total (n = 1,002)	Total (n = 830)	CC (n = 628)	CA/AA (n = 202)
Age (years)	58 (51–64)	58 (51–64)	58 (52–64)	58 (51–64)
Sex (M/F)	539/463	448/382	328/300	120/82
Height (cm)	161 (154–168)	161 (154–168)	161 (154–168)	161 (155–168)
Weight (kg)	58.5 (52–67)	58.2 (52–66.2)	58.05 (51.8–66.45)	59 (52–65)
rs8099917 (TT/GT/GG)	720/253/25	585/222/20	437/174/15	148/48/5
rs12979860 (CC/CT/TT)	543/198/52	541/197/52	403/151/44	138/46/8
rs1127354 (CC/CA/AA)	753/227/22	628/183/19	628/0/0	0/183/19
Core70 (W/M/ND)	240/143/619	239/143/448	175/114/339	64/29/109
Core91 (W/M/ND)	217/168/617	216/168/446	168/123/337	48/45/109
ISDR (0–1/≥2/ND)	287/80/635	287/80/463	216/61/351	71/19/112
Fibrosis (1/2/3/4/ND)	252/191/124/29/401	252/190/124/29/230	194/138/90/23/179	58/52/34/6/51
Activity (0/1/2/3/ND)	9/252/280/42/419	9/251/280/42/248	6/187/213/31/191	3/64/67/11/57
WBC (/mm^3)	4,700 (3,900–5,600)	4,700 (3,900–5,600)	4,700 (3,900–5,530)	4,900 (4,000–5,942)
Plt ($\times 10^4$/mm^3)	15.6 (12.2–19.7)	15.4 (12.2–19.35)	15.3 (12.1–19.33)	15.9 (12.45–19.4)
Hb (g/dl)	14 (13.2–14.9)	14 (13.2–14.9)	14.1 (13.2–14.9)	14 (13.4–15)
AST (IU/L)	45 (34–66)	45 (34–66)	45 (34–67)	45.5 (34–64.5)
ALT (IU/L)	53 (36–85)	53 (36–85)	52 (36–84.5)	55 (34.5–85)
γGTP (IU/L)	40 (25–73)	40 (25–73)	39.5 (25–72)	43.5 (25.25–77.25)
Total cholesterol (mg/dl)	172 (151–193)	172 (151–193)	172 (150–194)	171 (154–190)
HDL cholesterol (mg/dl)	51 (40–64)	51 (40–64)	52 (40.25–64)	50 (38–63.75)
Fasting blood sugar (mg/dl)	98 (89–112.8)	98 (89–113)	99 (89–113)	95 (88–108)
Virus titer (log IU/ml)	6.5 (6–7)	6.5 (6–7)	6.5 (6–7)	6.5 (6.1–6.9)
Viral genotype (1b/1a/others)	814/9/179	814/9/7	618/6/4	196/3/3
RBV treatment period (weeks)	48 (37–59)	48 (37–59)	48 (34.75–57)	48 (47–64.75)
RBV reduction (no/yes/ND)	316/450/236	315/448/67	212/366/50	103/82/17
Weeks to first RBV reduction	16 (5–48)	16.5 (5–48)	12 (4–47)	44 (12–51.75)
Outcome of therapy (NR/TR/SVR)	154/157/283	154/156/281	125/120/202	29/36/79

ND, not determined or data unavailable.
Categorical variables are reported as counts, and continuous variables are reported as median and interquartile range.

Amplicor method, the high range method, or the TaqMan RT-PCR test. The measurement ranges of these assays were 0.5–850 KIU/ml, 5–5,000 KIU/ml, and 1.2–7.8 log IU, respectively. Samples exceeding the measurement range were diluted with PBS and reanalyzed. All values are reported as log IU/ml.

ISDR and Core aa Substitutions

Amino acid substitutions in the HVC core and ISDR regions were determined by direct sequencing of PCR products following extraction and reverse transcription of HCV RNA using serum samples kept frozen at −80°C. Core amino acid substitutions at positions 70 and 91 (core70 and core91, respectively) were determined according to Akuta et al. [2007, 2006], and the number of ISDR substitutions was established as in Enomoto et al. [1995b, 1996].

SNP Genotyping

Each patient was genotyped for two IL28B SNPs previously reported to be associated with therapy outcome: rs12979860 and rs8099917, and a SNP reported to be associated with ribavirin-induced anemia: rs1127354. Samples were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip, the Invader assay, or the TaqMan assay, as described previously [Ohnishi et al., 2001; Suzuki et al., 2003]. The two SNPs in the IL28B locus are in strong linkage disequilibrium, with a correlation coefficient of 0.99.

Statistical Analysis

The χ^2 and Mann–Whitney U-tests were applied to detect significant associations. Simple and multiple regression analyses were used to examine the association between treatment outcome and the values of other markers, using $P < 0.1$ as the criterion for inclusion in the multivariate model. All of the statistical analyses were two sided, and $P < 0.05$ was considered significant. All statistical analysis was performed using the PASW Statistics 18 program (SPSS, Inc., Chicago, IL).

RESULTS

Reduction of Hemoglobin Levels During Therapy by ITPA Genotype

Decrease in hemoglobin levels during therapy was analyzed by rs1127354 genotype (CC vs. non-CC). As shown in Figure 1, a rapid decrease in hemoglobin levels during the initial 4 weeks was observed in genotype CC patients. Hemoglobin levels in genotype

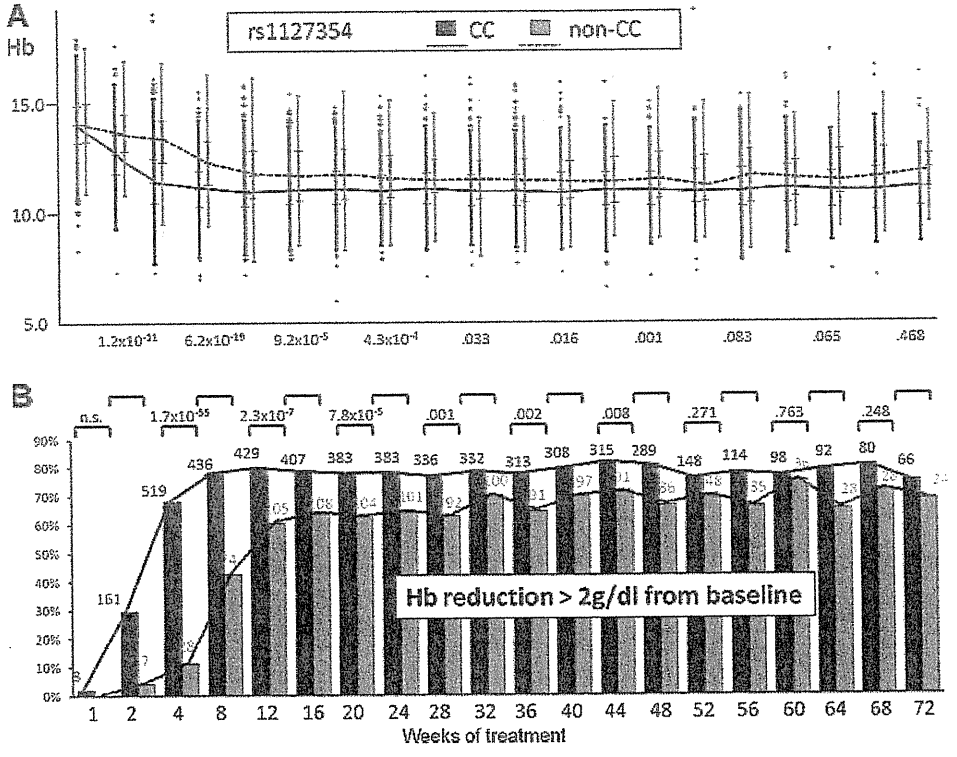

Fig. 1. Reduction of hemoglobin levels by ITPA polymorphism during peg-interferon plus ribavirin combination therapy. A: Hemoglobin levels in patients who were treated during the course of therapy. Patients were grouped by ITPA SNP rs1127354 genotype (CC or non-CC). Follow-up hemoglobin levels following cessation of therapy are not shown. B: Number of patients who showed >2 g/dl of hemoglobin. Statistical significance was assessed using the χ^2 and Mann–Whitney U-tests.

CC patients stabilized by week 8 and did not decrease further. In contrast, a slow but continuous decrease in hemoglobin level was observed in non-CC patients until week 48 (Fig. 1A). Reduction of hemoglobin by more than 2 g/dl was observed significantly more frequently in CC genotype patients than in non-CC patients (Fig. 1B). Differences between the two groups of patients were most pronounced between weeks 2 and 8 (Fig. 1B).

Ribavirin Dose Reduction by ITPA Genotype and Pretreatment Hemoglobin Levels

Decrease in hemoglobin levels resulted in ribavirin dose reduction. The frequency of hemoglobin decrease was higher in genotype CC patients compared with non-CC patients (Fig. 2A). Based on the assumption that initial hemoglobin levels influence incidence of ribavirin dose reduction, reduction frequency was analyzed by initial hemoglobin levels. As shown in Figure 2B–D, reduction of ribavirin was more frequent in genotype CC patients than non-CC patients in all three subsets of patients but was more prominent in patients with intermediate pretreatment hemoglobin levels between 13.5 and 15 g/dl (Fig. 2B–D).

Effect of ITPA Genotype and Pretreatment Hemoglobin Levels on Patients Receiving at Least 80% of Planned Ribavirin Administration

The reduction of ribavirin dosage during therapy resulted in reduction of the total amount of ribavirin given to each patient. As 80% of planned ribavirin administration appears to be a threshold associated with treatment outcome in patients with genotype 1b [McHutchison et al., 2002], the proportion of patients who received more than 80% of the initially planned dosage of ribavirin in 830 patients with genotype 1 and treated with the combination therapy (Table I) were analyzed. As shown in Figure 3, patients with non-CC genotypes tended to tolerate more than 80% of the predetermined dose of ribavirin compared with patients with CC. The difference was statistically significant only in patients whose pretreatment hemoglobin level was 13.5–15 g/dl, however (Fig. 3).

Factors Associated With Successful Administration of at Least 80% of Planned Ribavirin Dose

As it is possible that several factors including ITPA genotype and pretreatment hemoglobin levels are associated with dose reduction of ribavirin, the

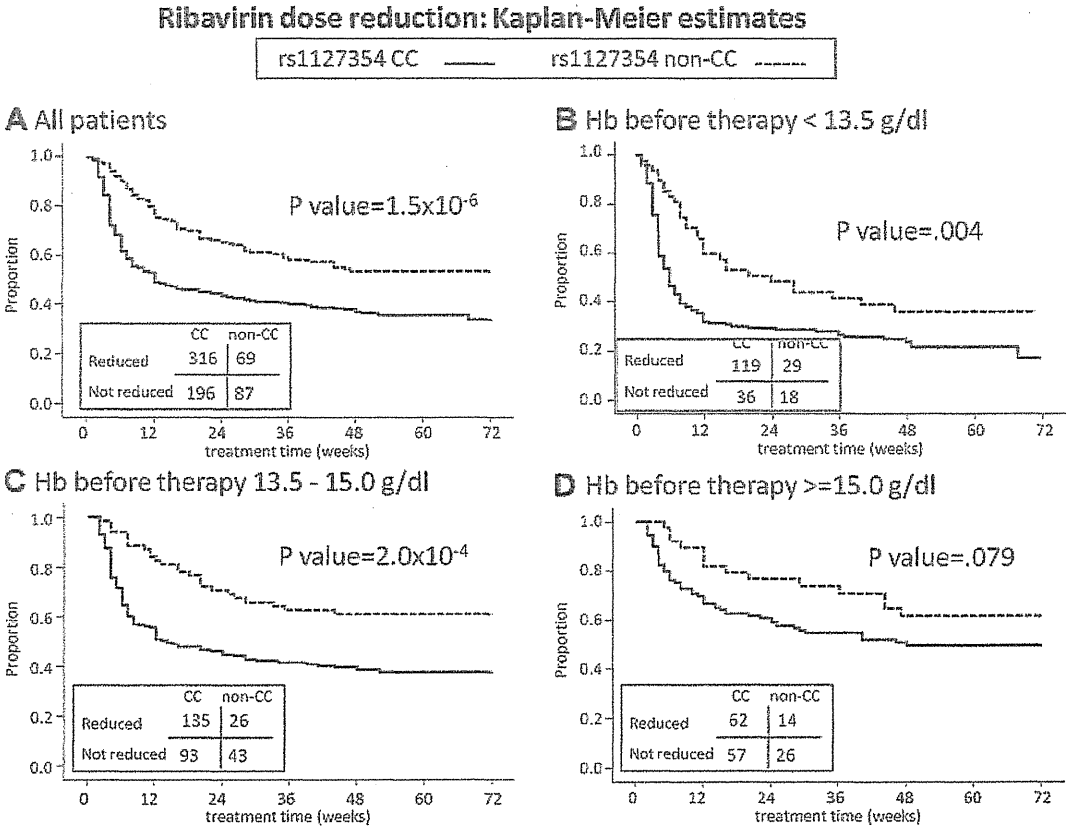

Fig. 2. Dose reduction of ribavirin in patients who were treated with combination therapy. Kaplan–Meier curves for dose reduction grouped by ITPA SNP rs1127354 genotype (solid line: CC, dashed-line: non-CC) among (A) all patients, (B) patients with low pretreatment hemoglobin levels (<13.5 g/dl), (C) patients with intermediate pretreatment hemoglobin levels (13.5–15.0 g/dl), and (D) patients with high pretreatment hemoglobin levels (≥15 g/dl).

effect of these factors as well as clinical factors were analyzed for dose reduction of ribavirin. As shown in Table II, univariate analysis identified ITPA SNP rs1127354 genotype, fibrosis stage and inflammatory activity of the liver, white blood cell count, platelet count, hemoglobin, ALT, age, and sex as factors associated with more than 80% ribavirin administration. Multivariate analysis identified age, hemoglobin, and rs1127354 genotype as independent predictive factors.

Effect of ITPA Genotype and Pretreatment Hemoglobin Levels on Outcome of Therapy

As the frequency of patients receiving more than 80% of planned ribavirin administration differed by pretreatment hemoglobin levels and ITPA genotype, treatment outcome might be expected to differ based on these factors. As expected, SVR rate was significantly higher in patients with non-CC genotypes with hemoglobin levels 13.5–15 g/dl, where the frequency of patients receiving 80% ribavirin administration differed most significantly between genotypes CC and non-CC (Fig. 4).

Predictive Factors of the Combination Therapy for SVR and NVR

Predictive factors for SVR and NVR were assessed, including baseline clinical factors, genotype of the recently reported IL28B SNP, and viral factors such as the number of substitutions in the ISDR, and substitutions at core amino acid 70 and 91. By univariate analysis, a number of factors were significantly associated with SVR, including IL28B SNP genotypes (rs8099917 and rs12979860), ITPA SNP rs1127354 genotype, core70 mutation, fibrosis of the liver, white blood cell count, platelet count, hemoglobin, ALT, fasting blood sugar, viral titer, age, sex, body mass index, and duration of the therapy (Table III). Multivariate analysis identified IL28B SNP rs8099917 genotype as the strongest independent predictor for SVR (OR 15.379, $P = 3.48E{-}07$), followed by hemoglobin level, ITPA SNP rs1127354 genotype, fibrosis of the liver, age, and body mass index (Table III). Significant independent predictive factors for NVR included IL28B SNP rs8099917 genotype fibrosis, and age (Table IV).

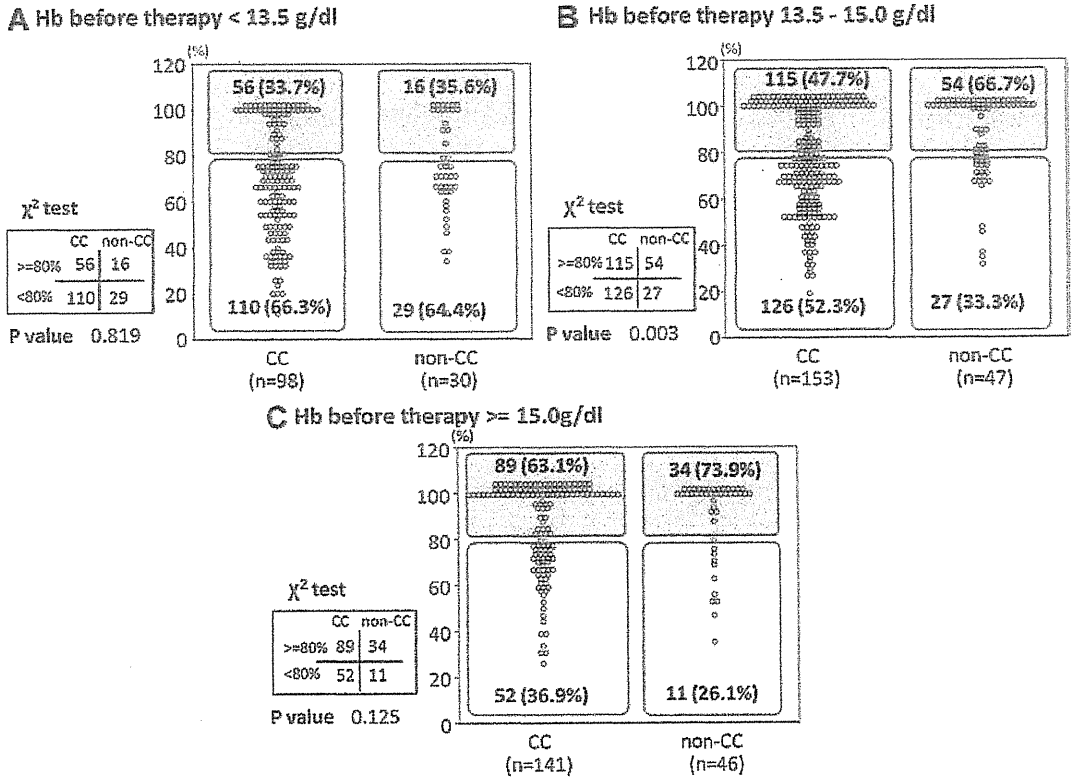

Fig. 3. Dose of ribavirin administered to patients with genotype 1 treated with combination therapy by ITPA rs1127354 genotype and pretreatment hemoglobin levels. Patients with genotype 1b and treated with ribavirin were divided into three groups based on their pretreatment hemoglobin levels: (A) <13.5 g/dl, (B) between 13.5 and 15.0 g/dl, and (C) ≥15 g/dl.

TABLE II. Factors Associated With Ribavirin Dose Reduction (80%) in Hepatitis C Virus Patients Determined by Logistic Regression Analysis

	Simple		Multiple		
Variable	OR	*P*-value	OR	95% CI	*P*-value
rs1127354 CC vs. CA/AA	0.580	0.002**	0.578	0.372–0.897	0.014*
Core70	1.007	0.974			
Core91	0.776	0.244			
ISDR 0/1 vs. >1	1.091	0.743			
BMI (kg/m^2)	1.008	0.740			
Fibrosis 1–2 vs. 3–4	1.676	0.009**	1.409	0.902–2.202	0.132
Activity 0–1 vs. 2–3	1.537	0.013*			
WBC (/mm^3)	1.000	1.2E−05**			
Plt (×10^4/mm^3)	1.070	5.2E−06**	1.000	1.000–1.000	0.178
Hb (g/dl)	1.485	1.7E−10**	1.244	1.066–1.453	0.006**
AST (IU/L)	1.001	0.769			
ALT (IU/L)	1.003	0.035*			
γGTP (IU/L)	1.001	0.362			
Albumin (g/dl)	1.549	0.460			
Total cholesterol (mg/dl)	0.997	0.175			
Triglycerides (mg/dl)	1.000	0.935			
HDL cholesterol (mg/dl)	0.989	0.066			
LDL cholesterol (mg/dl)	0.995	0.503			
Fasting blood sugar (mg/dl)	1.001	0.585			
Virus titer (log IU/ml)	1.047	0.567			
Age	0.936	2.1E−15**	0.934	0.914–0.954	3.5E−10**
Sex	0.586	3.9E−04**			

**$P < 0.01$.
*$P < 0.05$.

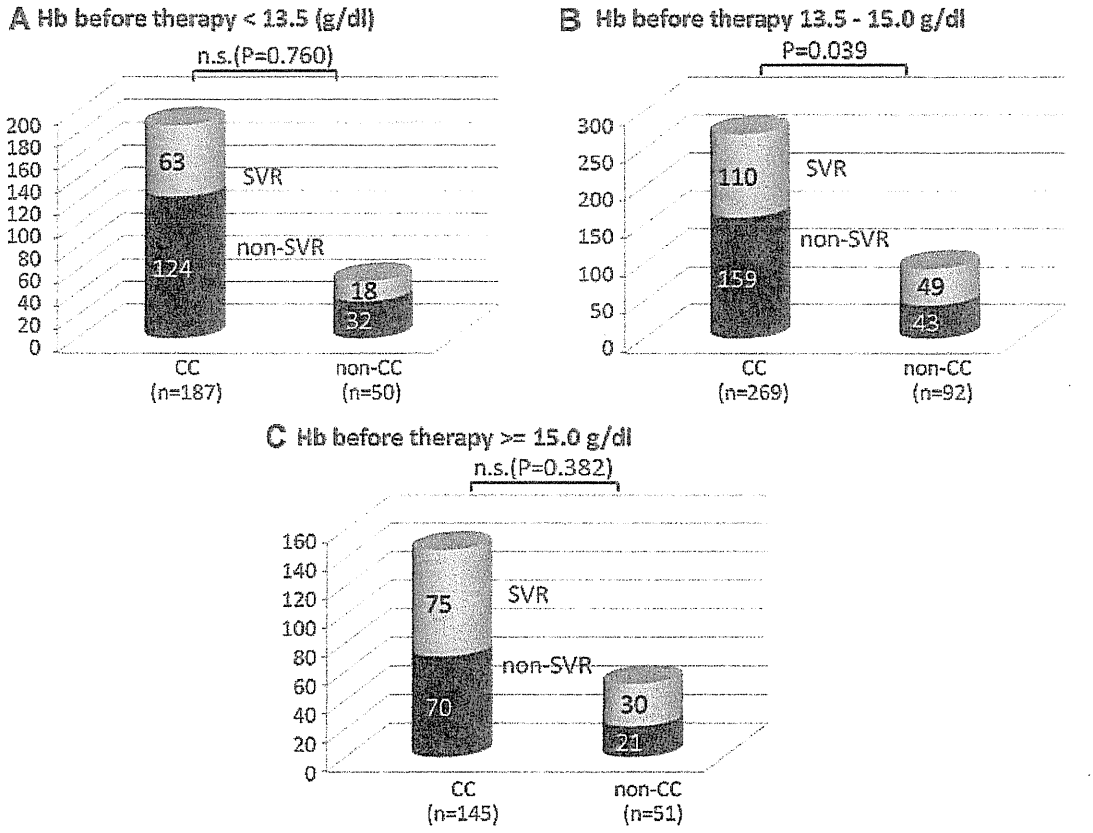

Fig. 4. Effect of combination therapy in patients with genotype 1b by ITPA rs1127354 genotype and pretreatment hemoglobin levels. Patients with genotype 1b and treated with ribavirin were divided into three groups based on their pretreatment hemoglobin levels: (A) <13.5 g/dl, (B) between 13.5 and 15.0 g/dl, and (C) ≥15 g/dl.

DISCUSSION

Ribavirin-induced anemia is one of the most serious side effects resulting from combination therapy [De Franceschi et al., 2000], but a polymorphism within the ITPA gene has recently been shown to affect incidence of this form of anemia [Fellay et al., 2010; Ochi et al., 2010; Thompson et al., 2010]. This study showed that hemoglobin decrease is faster and more severe, especially in the first 12 weeks of treatment, in patients with the anemia-susceptible ITPA rs1127354 CC genotype (Fig. 1). The rapid reduction of hemoglobin observed in genotype CC patients persisted to the end of therapy and was associated with early reduction of ribavirin dosage (Fig. 2), resulting in lower total ribavirin administration. The linear and continuous decrease in hemoglobin seen in non-CC patients also contributed to the reduction of ribavirin administration but not as drastically as in patients with the CC genotype (Fig. 2). The other significant ITPA SNP, rs7270101, is associated with splicing variant formation and reduced activity of the ITPA enzyme in patients of European and African ancestry, but this SNP is absent in the Japanese population [Ochi et al., 2010]. Therefore, only the missense SNP rs1127354, which results in a P32T amino acid change and reduced enzyme activity, was analyzed. Thompson et al. [2010] divided patients into four groups (−, +, ++, +++) based on the genotypes of these two SNPs. According to their classification, CC and non-CC genotypes in this study are almost comparable to "−" and "++" in their study because there are no patients with the rs1127354 AA genotype, and there were only two "+++" patients present in their study. Hemoglobin decrease was slightly milder in the this study compared to Thompson et al. [2010], probably due to early reduction in ribavirin dose in Japanese patients resulting from lower pretreatment hemoglobin levels.

Initial hemoglobin levels indeed had a strong influence on reduction of ribavirin dose. As shown in Figure 3, ITPA genotype did not have a significant influence on patients with <80% ribavirin administration when pretreatment hemoglobin levels were <13.5 or >15 g/dl. Accordingly, because reduction of ribavirin to <80% results in decreased rate of SVR [McHutchison et al., 2002], patients with pretreatment hemoglobin levels below 13.5 g/dl or patients with pretreatment hemoglobin levels between 13.5 and 15 g/dl who have the ITPA anemia-susceptible genotype should receive treatment with drugs such as erythropoietin to prevent reduction of ribavirin.

TABLE III. Predictive Factors Associated With Sustained Viral Response in Hepatitis C Virus Patients Determined by Logistic Regression Analysis

	Simple		Multiple		
Variable	OR	*P*-value	OR	95% CI	*P*-value
rs8099917 TT vs. TG/GG	3.614	1.85E−16**	15.358	5.371–43.919	3.48E−07**
rs12979860 CC vs. CT/TT	4.271	8.87E−16**			
rs1127354 CC vs. CA/AA	0.660	0.006**	0.368	0.161–0.838	0.017*
Core70	1.891	0.005**			
Core91	1.503	.059			
ISDR 0/1 vs. >1	0.660	0.106			
BMI (kg/m^2)	0.944	0.007**	0.865	0.758–0.987	0.032*
Fibrosis 1–2 vs. 3–4	2.290	5.83E−05**	4.540	1.618–12.734	0.004**
Activity 0–1 vs. 2–3	0.869	0.412			
WBC (/mm^3)	1.000	0.008**			
Plt ($\times 10^4$/mm^3)	1.072	4.68E−08**	1.055	0.976–1.141	0.176
Hb (g/dl)	1.172	0.001**	1.505	1.106–2.048	0.009**
AST (IU/L)	1.000	0.824			
ALT (IU/L)	1.003	0.027*			
γGTP (IU/L)	0.998	0.118			
Albumin (g/dl)	2.802	0.089			
Total cholesterol (mg/dl)	1.002	0.345			
Triglyceride (mg/dl)	0.997	0.094			
HDL cholesterol (mg/dl)	1.003	0.670			
LDL cholesterol (mg/dl)	0.999	0.922			
Fasting blood sugar (mg/dl)	0.989	0.001**	0.991	0.977–1.005	0.197
Virus titer (log IU/ml)	0.722	1.83E−04**	0.798	0.567–1.124	0.196
Age	0.960	5.13E−11**	0.957	0.919–0.995	0.028*
Sex	0.713	0.009**			
RBV treatment period (weeks)	1.012	3.86E−04**			

**$P < 0.01$.
*$P < 0.05$.

TABLE IV. Predictive Factors Associated With NVR in Chronic Hepatitis C Virus Patients Treated With Peg-Interferon Plus Ribavirin Combination Therapy

	Simple		Multiple		
Variable	OR	*P*-value	OR	95% CI	*P*-value
rs8099917 TT vs. TG/GG	6.663	6.00E−32**	7.157	3.592–14.262	2.21E−08**
rs12979860 CC vs. CT/TT	7.589	1.07E−30**			
rs1127354 CC vs. CA/AA	0.673	0.027*			
Core70	2.531	5.25E−05**			
Core91	1.951	0.003**	1.604	0.849–3.033	0.146
ISDR 0/1 vs. >1	0.569	0.053			
BMI (kg/m^2)	0.969	0.189**	0.910	0.822–1.008	0.070
Fibrosis 1–2 vs. 3–4	1.826	0.002**	2.941	1.404–6.162	0.004**
Activity 0–1 vs. 2–3	0.866	0.424			
WBC (/mm^3)	1.000	0.052			
Plt ($\times 10^4$/mm^3)	1.048	0.001**			
Hb (g/dl)	1.112	0.046*			
AST (IU/L)	0.999	0.608			
ALT (IU/L)	1.001	0.651			
γGTP (IU/L)	0.996	0.007**			
Albumin (g/dl)	1.534	0.479			
Total cholesterol (mg/dl)	1.005	0.058			
Triglyceride (mg/dl)	0.998	0.100			
HDL cholesterol (mg/dl)	1.003	0.669			
LDL cholesterol (mg/dl)	0.997	0.664			
Fasting blood sugar (mg/dl)	0.998	0.461			
Virus titer (log IU/ml)	0.753	0.006**	0.744	0.534–1.036	0.080
Age	0.977	0.001**	0.958	0.927–0.99	0.010**
Sex	0.830	0.202			
RBV treatment period (weeks)	1.021	1.86E−07**	1.012	0.996–1.027	0.135

Results of simple and multiple logistic regression are shown. The multivariate model was constructed using stepwise selection of significant univariate terms.
**$P < 0.01$.
*$P < 0.05$.

Although the ITPA polymorphism was significantly associated with ribavirin-induced anemia [Fellay et al., 2010; Thompson et al., 2010], no effect on outcome of therapy was found in the two previous studies on ITPA polymorphism from the United States. In contrast, Ochi et al. [2010] reported a possible association between ITPA genotype and outcome of therapy in Japan. Similarly, results of this study suggest an association between ITPA genotype and outcome of combination therapy for HCV genotype 1 in Japanese patients (Table II). There are several potential reasons for the different effects of ITPA genotype among these studies. First, the incidence of anemia-protective (rs1127354 non-CC) genotypes is higher in Japanese patients (20%) compared with patients with European (16.7%) and Sub-Saharan African (6.7%) ancestry [Olivier, 2003], suggesting a lack of power to detect the association in studies based on these populations. Secondly, the age of treated patients is higher in Japan than in the US (50–55 vs. 45) [Kainuma et al., 2010], which may lead to a higher incidence of ribavirin dose reduction during therapy [Hung et al., 2006]. Similarly, lower pretreatment levels of hemoglobin in Japanese patients compared with US patients (13.0 g/dl vs. 14.9 g/dl) [Fellay et al., 2010; Ochi et al., 2010] might result in a greater incidence of ribavirin reduction in Japanese patients and enhance the effects of the ITPA SNP on treatment outcome.

This study showed that a significantly larger number of patients ultimately received <80% of planned ribavirin administration when their hemoglobin levels were either <13.5 g/dl or between 13.5 and 15 g/dl in ribavirin-sensitive patients (ITPA rs1127354 genotype CC) (Fig. 4). As reported previously, administration of <80% of planned ribavirin is associated with poor outcome of therapy, and this study confirmed that reduction of ribavirin is significantly associated with SVR ($P < 0.009$, data not shown). Treatment of these patients with erythropoietin may therefore help prevent ribavirin dose reduction and improve SVR rate. However, in Japan erythropoietin is not available to treat this condition. As erythropoietin has been shown to improve anemia and treatment outcome of combination therapy, administration should be considered, at least for patients matching the criteria in this study, to improve the outcome of therapy.

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Safety, pharmacokinetics and resistant variants of telaprevir alone for 12 weeks in hepatitis C virus genotype 1b infection

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SUMMARY. *Background:* Telaprevir in combination with peginterferon and ribavirin is a promising advancement in chronic hepatitis C treatment. However, the safety, tolerability, pharmacokinetics and antiviral profiles of telaprevir alone beyond 2 weeks have not been studied. *Methods:* In a phase 1b study in Japan, 10 treatment-naïve patients infected with hepatitis C virus genotype 1b with high viral load (>5 $\log_{10}$ IU/mL) received telaprevir 750 mg every 8 h (q8h) for 12 weeks. We examined the safety, tolerability, pharmacokinetics, hepatitis C virus (HCV) RNA levels and resistant variants of telaprevir. *Results:* Neither serious adverse events nor discontinuations of study drug owing to an adverse event occurred. The most common adverse drug reactions were rash (80%) and anaemia (70%). Telaprevir concentration reached its steady state within 2 days after the first administration without abnormal accumulation. Telaprevir alone provided potent antiviral activity: a median $\log_{10}$ decrease of 2.325 at 16 h and 5.175 on Day 14. During the treatment, HCV RNA levels at the nadir were below the limit of the quantification in seven patients and undetectable in three of 10 patients. Viral breakthrough associated with mainly Ala^{156}-substituted variants occurred in eight patients, and only one patient showed end-of-treatment response. The selected variants reverted to the wild-type during the 24-week follow-up period. *Conclusion:* Telaprevir alone was well tolerated at 750 mg q8h for up to 12 weeks. The safety profile and emergence of resistant variants of genotype 1b under telaprevir monotherapy for 12 weeks will become increasingly important in evaluating an oral combination of telaprevir with other direct-acting antiviral agents.

Keywords: genotype 1b, pharmacokinetics, resistant variants, telaprevir monotherapy, tolerability.

INTRODUCTION

Hepatitis C virus (HCV) infection often causes chronic hepatitis (CHC) that may result in life-threatening complications including cirrhosis and hepatocellular carcinoma (HCC) [1,2]. Thus, the development of medical agents or therapies that are highly effective against HCV has been eagerly sought for a long time. The current standard of care (SOC) for patients with hepatitis C, the concomitant administration of peginterferon (PEG-IFN) with ribavirin (RBV) for 48 weeks, is one such therapy, but it results in sustained virological response (SVR) in only about 45% of patients with genotype 1 HCV infection [3–5]. In addition to this low rate of SVR, another large problem of the SOC is that its practical use has been often interrupted or discontinued with several side effects including flu-like symptoms, depression, neutropenia and anaemia, and some patients are also excluded from SOC. Patients not eligible for SOC include many with comorbid conditions that often accompany HCV, including decompensated liver disease and renal failure. Thus, there is an unmet need for CHC therapies that are more effective and are better tolerated than what is presently available. Telaprevir, which is a novel peptidemimetic slow and tight-binding inhibitor of the HCV NS3-4A protease discovered using a structure-based drug design approach [6], has been intensively developed in the world as a member of a new class of direct-acting antivirals (DAAs) to improve SVR rates for genotype 1. In the first, phase 1 trial (VX04-950-101) in CHC patients, telaprevir was well tolerated and reduced HCV RNA in plasma by 2 $\log_{10}$ or greater after its consecutive administration for 14 days [7]. In a subsequent

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHC, chronic hepatitis C; DAA, direct acting antiviral; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; LLOQ, lower limit of quantification; LOD, limit of detection; PEG-IFN, peginterferon; q8h, every 8 h; RBV, ribavirin; SOC, standard of care; SVR, sustained virological response.

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phase 1 clinical trial (VX05-950-103), all eight patients given telaprevir alone had an initial, rapid and profound antiviral response, but the four patients with genotype 1a infection experienced a viral breakthrough, whereas the other four patients with genotype 1b infection had a continuous decline in viral load [8]. Because genotype 1b infection accounts for 70% of patients and genotype 1a is rarely met with in Japan [9], viral kinetics and emergence of resistant variants from telaprevir use alone beyond 2 weeks remain to be evaluated among patients with genotype 1b infection. Besides virological reasons, a safer therapy without concomitant administration of PEG-IFN or RBV is desirable if possible, because the majority of HCV carriers are of age >55 years whose tolerability is of concern in Japan [10]. Therefore, the purpose of this trial is to examine the safety, tolerability, antiviral effects and pharmacokinetics of monotherapy with telaprevir in 10 Japanese patients with genotype 1b infection for up to 12 weeks.

PATIENTS AND METHODS

Study design and organization

This single-arm, open-label study was conducted from December 2007 to October 2008 at the Department of Hepatology in the Toranomon Hospital in Metropolitan Tokyo in full compliance with the guideline of Good Clinical Practice and the Declaration of Helsinki (ClinicalTrials.gov Identifier: NCT00591214). Before the study started, the protocol and informed consent forms were reviewed and approved by the institutional review board. Informed consent was obtained from all patients in writing after sufficient explanation was given and before they participated in the study. For 12 consecutive weeks, all 10 patients received 750 mg telaprevir q8h under feeding conditions. Telaprevir was supplied as a 250-mg tablet.

Patients

Patients enrolled in this study were treatment-naïve, HCV-infected male or female participants with characteristics shown in Table 1, who met the following inclusion criteria: diagnosed with chronic hepatitis C; infected with HCV genotype 1b proved by phylogenetic analysis in the NS5B region; not received any prior antiviral therapy for hepatitis C; had HCV RNA level of 5 $\log_{10}$ IU/mL or more determined by the Roche COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan); belonged to Japanese race (Mongoloid) aged from 20 to 65 years at entry; and agreed birth control from the time of obtaining informed consent to 24 weeks after the completion of administration of the study drug. Patients were excluded from the study if they met any of the following criteria: diagnosed with decompensated liver cirrhosis and/or presence of hepatitis B surface antigen in serum; diagnosed with HCC or its history; previously treated for malignant neoplasm; diagnosed with autoimmune hepatitis, alcoholic liver disease, haemochromatosis, or chronic liver disease other than chronic hepatitis C; women who were pregnant, were breast-feeding, or who could become pregnant; had a history of alcohol addiction; and had complications of heart, kidney and lung disease.

Hepatitis C virus RNA measurement

Antiviral effects of telaprevir on HCV were assessed by measuring the serum HCV RNA levels using the COBAS

Table 1 Patient characterstics, treatment duration, and viral response

	Sex	Age	BMI (kg/m^2)	Baseline hepatitis C virus (HCV) RNA ($Log_{10}IU/mL$)	Treatment duration (day)	HCV RNA Nadir ($Log_{10}IU/mL$)	Viral response
1	M	31	29.1	7.10	58*	1.6	Breakthrough
2	M	64	30.7	6.70	50*	<1.2 detectable	Breakthrough
3	M	48	25.7	5.10	63*	Undetectable	Breakthrough
4	M	49	22.7	6.60	45*	3.0	Breakthrough
5	F	64	24.2	6.95	85 (completed)	1.2	Partial responder†
6	M	58	19.7	6.50	63*	<1.2 detectable	Breakthrough
7	F	63	22.8	6.40	58*	<1.2 detectable	Breakthrough
8	M	49	22.6	5.50	87 (completed)	Undetectable	Relapser
9	M	59	21.2	6.35	85 (completed)	Undetectable	Breakthrough
10	F	55	19.0	6.25	51*	<1.2 detectable	Breakthrough

Subjects whose viral level increased by 2 $Log_{10}IU/mL$ from nadir or more than 3 $Log_{10}IU/mL$ after reaching undetectable levels during treatment phase are defined to show breakthrough. *Subjects discontinued telaprevir due to viral breakthrough. †Subject who did not meet both criteria of breakthrough and relapse.