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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.

TaqMan HCV test (Roche Diagnostics). Blood samples were collected on Day-28, before dosing (0 h) and 2.5, 4, 8, 16 and 24 h after the first dosing on Day 1 and before dosing on Days 3, 8, 14, 29, 43, 57 and 86 and at Weeks 1, 2, 4, 8, 12, 16, 20 and 24 after the end of treatment. The linear dynamic range of the assay was 1.2 to 7.8 $\log_{10}$ IU/mL. The qualitative result below the lower limit of quantification (LLOQ) was also determined as positive (1.0) and negative (0.5).

Sequence analysis of the hepatitis C virus NS3 protease domain

Hepatitis C virus RNA was isolated from serum samples collected on Day-28, and Days 1, 3, 8, 14, 29, 43, 57 and 86 and at Weeks 1, 2, 4, 8, 12, 16, 20 and 24 after the end of treatment. The DNA fragment of 534 bp in length (181 amino acids) encompassing the NS3 protease domain was amplified by nested RT-PCR and cloned. At least 39 clones per specimen were sequenced bidirectionally. The limit of detection (LOD) for sequencing analysis was around 3 $\log_{10}$ IU/mL.

Safety assessments

Safety and tolerability of study treatments were assessed by clinical laboratory results, vital signs, 12-lead electrocardiograms (ECGs) and occurrence of adverse events. These safety parameters were recorded at regular intervals from Day-28 through the follow-up visits.

Determination of pharmacokinetic parameters

Blood samples were collected immediately before dosing (0 h) and 1, 2.5, 4, 6, 8, 12, 16 and 24 h after the first dosing on Days 1, 14 and 85 and before dosing on Days 3, 8, 29, 43 and 57. Plasma concentrations of telaprevir were determined using a high-performance liquid chromatographic apparatus fitted with mass spectrometry. Plasma concentrations and actual plasma-sampling times were used to calculate the area under the plasma concentration–time curve from 0 to 8 h ($AUC_{0-8\ h}$) and terminal half-life ($t_{1/2}$) by the noncompartmental method using WinNonlin software version 5.2.1. The maximum plasma concentration (C_{max}) and time to reach C_{max} (t_{max}) were directly determined from the observed values on Days 1, 14 and 85.

Statistical analysis

From the plasma concentrations of telaprevir, descriptive statistics were calculated. The number of patients with adverse events was summarized by MedDRA (version 11.1.) system organ class, preferred term, severity and relationship to study drug. All statistical analyses were performed using the validated version 9.1.3 of the SAS® System (SAS Institute Inc., Cary, NC, USA).

RESULTS

Baseline characteristics

A total of 10 Japanese patients, whose background characteristics are shown in Table 1, were enrolled in this study. Their median age was 56.5 years (range, 31–64), and 7 (70.0%) and 3 (30.0%) were men and women, respectively. Baseline HCV RNA levels of each subject were similar in the range 5.10 $\log_{10}$–7.10 $\log_{10}$ IU/mL (median: 6.450).

Safety and tolerability

There were neither serious adverse events nor discontinuations owing to an adverse event. In the present study, 75 adverse events and 66 adverse drug reactions, respectively, developed in nine of 10 patients (90.0%). An incidence of adverse events that developed in two or more patients by the preferred terms is shown in Table 2. The adverse events with the incidence of 30% or higher were rash developing in eight patients (80.0%) (if pruritic rash is included in rash, nine patients [90.0%]), anaemia in seven patients (70.0%), blood uric acid increased in five patients (50.0%), low-density lipoprotein increased in five patients (50.0%), stomach discomfort in four patients (40.0%), peripheral oedema was present in three patients (30.0%), blood triglycerides increased in three patients (30.0%), and pruritus was seen in

Table 2 Incidence of adverse events that occurred in two or more patients

	N = 10			
	Mild	Moderate	Severe	Total
	N (%)	*N* (%)	*N* (%)	*N* (%)
Subjects with adverse events	9 (90.0)	5 (50.0)	0 (0.0)	9 (90.0)
Rash	7 (70.0)	1 (10.0)	0 (0.0)	8 (80.0)
Anaemia	7 (70.0)	0 (0.0)	0 (0.0)	7 (70.0)
Blood uric acid increase	4 (40.0)	1 (10.0)	0 (0.0)	5 (50.0)
Low-density lipoprotein increase	4 (40.0)	1 (10.0)	0 (0.0)	5 (50.0)
Stomach discomfort	4 (40.0)	0 (0.0)	0 (0.0)	4 (40.0)
Blood triglycerides increase	3 (30.0)	0 (0.0)	0 (0.0)	3 (30.0)
Pruritus	3 (30.0)	0 (0.0)	0 (0.0)	3 (30.0)
Peripheral Oedema	2 (20.0)	1 (10.0)	0 (0.0)	3 (30.0)
Malaise	2 (20.0)	0 (0.0)	0 (0.0)	2 (20.0)
Pyrexia	2 (20.0)	0 (0.0)	0 (0.0)	2 (20.0)
Nasopharyngitis	1 (10.0)	1 (10.0)	0 (0.0)	2 (20.0)

three patients (30.0%). The moderate adverse events (one each) that developed in five patients were vertigo, peripheral oedema, nasopharyngitis, increase in blood uric acid and in low density lipoprotein, facial palsy and rash, whereas all other adverse events were mild. It is notable that although seven patients discontinued the therapy, none did so owing to adverse events.

Antiviral activity

Telaprevir rapidly decreased serum HCV RNA level in all patients enrolled in this study. The median serum HCV RNA level changed from 6.45 $\log_{10}$ IU/mL (range: 5.1–7.1) just before administration to 4.00 $\log_{10}$ IU/mL (range: 3.0–4.7) at 16 h after administration and 1.10 $\log_{10}$ IU/mL (range: 0.5–3.3) on Day 14 (Fig. 1). Telaprevir showed potent antiviral activity: a median $\log_{10}$ decrease of 2.325 at 16 h and 5.175 on Day 14. During the administration period of 12 weeks, HCV RNA levels decreased to less than the LLOQ of 1.2 $\log_{10}$ IU/mL in seven patients, and three patients achieved HCV RNA negativity on Day 14 or Day 29. After the decrease in serum HCV RNA, breakthrough occurred in eight patients, and seven of those patients discontinued the trial during the dosing period (from Day 45 to Day 63, Table 1). In addition, one of the remaining three patients who completed the administration of the study drug achieved virus negativity by the end of administration (Day 86), but relapsed 1 week after completion of drug therapy.

Hepatocyte injury markers

As shown in Fig. 2a, the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels decreased during and after telaprevir treatment. Median changes from baseline (Day 1) in ALT and AST were −26.5 IU/L (range: −217–5, N = 10) and −8.5 IU/L (range: −118–2, N = 10) on Day 29, respectively. Fig. 2b shows total bilirubin levels. No clinically significant change in bilirubin was observed in all patients. These data indicate that long-term exposure to telaprevir caused neither damage nor injury in the liver.

Sequence analysis of hepatitis C virus NS3

Amino acid substitutions in the NS3 protease domain, which were selected by telaprevir administration, were examined in 39 clones or more for each sample (Table 3). The predominant variants detected during the early time points after administration (on Days 3 and 8) were V36G, T54A and A156V. Subsequently, these variants decreased below the LOD in nine patients, and the predominant variants detected at viral breakthrough after Week 6 of administration (Day 43–86) were single-substituted variants of A156F/T/V and multiple-substituted variants of T54S+A156T and

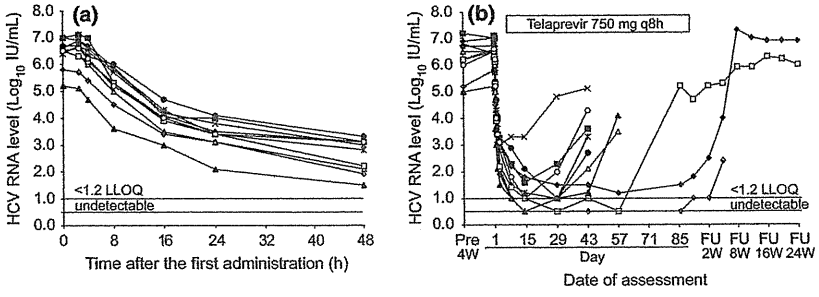

Fig. 1 Changes in patient hepatitis C virus (HCV) RNA level. For 12 consecutive weeks, all 10 patients received 750 mg telaprevir q8h under feeding conditions. <1.2 LLOQ, below lower limit of quantification of 1.2 $\log_{10}$ IU/mL; FU, follow-up.

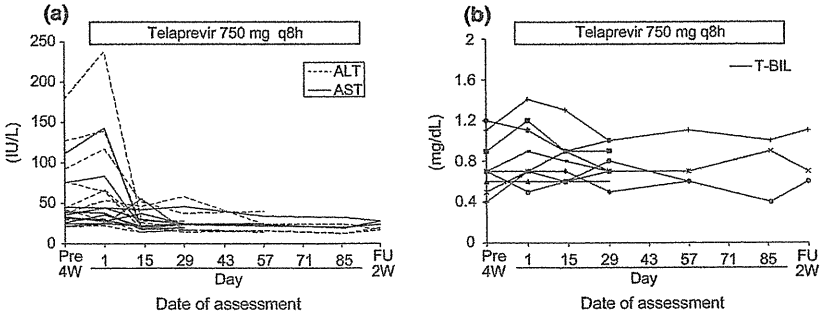

Fig. 2 Change in alanine aminotransferase, aspartate aminotransferase (a) and total bilirubin (b) levels. FU, follow-up.

Table 3 Means of hepatitis C virus (HCV) RNA levels and representation rates of variants in all subjects during and after telaprevir treatment

	Pre	Day 3	Day 8	Day 14	Day 29	Day 43	Day 50–60	Day 86	FU2W	FU4W	FU8W	FU12W	FU24W
N	10	10	10	10	10	10	9	3	3	3	2	2	2
Mean of HCV RNA level (log_{10} IU/mL)	6.35	2.61	1.84	1.45	1.56	2.53	3.22	2.40	2.90	4.13	6.60	6.45	6.45
HCV NS3 variants (%)													
Wild	100.0	40.0	0.2	–	0.2	0.1	–	–	–	18.3	86.4	51.8	97.8
V36A	–	–	–	–	–	–	0.3	–	–	23.8	3.4	38.5	1.1
V36G	–	10.0	0.4	–	2.4	0.8	–	–	–	–	–	–	–
T54A	–	–	9.4	9.5	4.7	0.1	–	–	–	17.9	1.1	5.0	–
A156F	–	–	–	–	–	10.0	25.5	0.8	–	–	–	–	–
A156T	–	–	–	0.5	–	7.5	16.6	31.1	16.3	–	–	1.2	–
A156V	–	–	30.0	–	1.1	15.9	2.3	–	–	–	–	–	–
T54S+A156S	–	–	–	–	–	–	–	–	–	19.2	3.4	1.3	–
T54S+A156T	–	–	–	–	–	9.6	11.4	1.5	16.3	15.0	–	–	–
A156T+V158I	–	–	–	–	–	3.3	10.1	–	–	–	–	–	–

–, not detected; FU, follow-up. Minor substitutions (maximum occupancy in a specimen was less than 10%): T54S, R155G, R155L, A156S, V36A+T54A, V36A+A156S, V36G+A156V, T54A+R155L, T54A+A156S, T54A+A156V, T54S+R155L, T54S+A156V, T54A+V132L, A156S+V132L, T54A+V163I, T54S+A156T+V158I, V36A+T54A+A156S

A156T+V158I; no wild-type virus was detected. In the three patients who completed the administration of telaprevir for 12 weeks, V36A, T54A and T54S+A156S/T were detectable after treatment. In the two patients followed up for 24 weeks, gradual enrichment of the wild-type viruses was observed.

Pharmacokinetics

The plasma concentration *vs* time curves on Days 1, 14 and 85 are shown in Fig. 3a and the C_{trough} on Days 1, 2, 3, 8, 14, 15, 29, 43, 57 and 85 in Fig. 3b. The pharmacokinetic parameters of telaprevir on Days 1, 14 and 85 are given in Table 4.

As the t_{max} were similar on Days 1, 14 and 85 with medians of 2.50, 2.49 and 2.72 h, respectively, the repeated administration under the present conditions was unlikely to cause any change in absorption. The pharmacokinetic parameters of C_{max}, $AUC_{0-8\ h}$ and C_{trough} were lower on Day 1 than those on Days 14 and 85; thus, on Days 1, 14 and 85, the mean values of C_{max} were respectively 2.24, 3.34 and 3.68 μg/mL, the mean values of $AUC_{0-8\ h}$ were respectively 11.60, 22.31 and 23.98 μg·h/mL, and the mean values of C_{trough} at 8 h after the first administration were respectively 1.462, 2.239 and 2.312 μg/mL. The plasma concentration of telaprevir reached steady state on Day 2.

DISCUSSION

During the past decade, the combined use of PEG-IFN and RBV has provided a significant therapeutic advance for

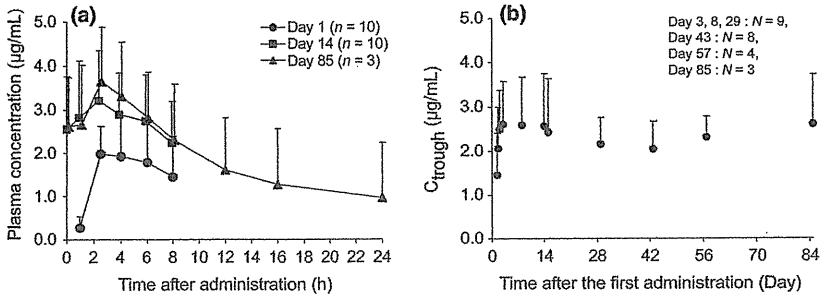

Fig. 3 Time course of plasma concentration (a) and C_{trough} (b) of telaprevir. Symbols and error bars indicate mean values and SD, respectively.

Table 4 Pharmacokinetic parameters of plasma telaprevir

	N	C_{max} (μg/mL)	t_{max} (h)*	$AUC_{0-8\ h}$ (μg·h/mL)	C_{trough} (μg/mL) †	$t_{1/2}$ (h)
Day 1	10	2.24 ± 0.93	2.50 (2.30–7.92)	11.60 ± 4.74	1.462 ± 0.949	5.57 ± 2.67‡,§
Day 14	10	3.34 ± 1.11	2.49 (0.98–5.97)	22.31 ± 8.29	2.239 ± 0.953	9.64 ± 6.14‡,¶
Day 85	3	3.68 ± 1.29	2.72 (2.68–4.00)	23.98 ± 9.45	2.312 ± 1.265	18.35 ± 22.91**

Mean value ± SD. *Median (minimum value to maximum value). †C_{trough} at 8 h after the first administration. ‡Calculated from measured values at 8 h after the first administration. §$N = 7$. ¶$N = 8$. **Calculated from measured values at 24 h after the first administration.

patients with CHC. Approximately 50% of patients infected with genotype 1 HCV do not, however, achieve SVR with this SOC [3–5]. On the contrary, the treatment with telaprevir-based triple regimen significantly improved SVR rates in patients with genotype 1 HCV. The PROVE 1 and 2 studies of telaprevir use with PEG-IFN and RBV in treatment-naïve patients achieved SVR rates of 61% and 69% (placebo: 41–46%) [11,12]. The Japanese study of the telaprevir-based triple regimen also showed high SVR rates [13–15]. However, the key safety concerns with the telaprevir-based triple regimen were anaemia, rash and IFN-induced systemic symptoms, all of which were most likely caused by the PEG-IFN/RBV treatment. In Japan, there are currently a large number of aged people with genotype 1b HCV and high viral loads, which is one of the most intractable HCV genotypes. As a result of advanced age, many subjects could not tolerate the adverse drug reactions in the telaprevir-based regimen, which was also observed with PEG-IFN/RBV therapies [13,15]. This observation prompted us to re-examine the safety profiles and pharmacokinetics of monotherapy with telaprevir for 12 weeks in Japanese patients.

In this study, 10 treatment-naïve patients with genotype 1b CHC and a high median viral load of 6.45 $\log_{10}$ IU/mL (range: 5.10–7.10) (Table 1) took 750 mg of telaprevir q8h for 12 weeks under feeding conditions. The plasma concentrations of telaprevir reached steady state within 2 days after the initiation of administration in the 750-mg q8h regimen as is shown by the constant C_{trough} from Day 2 to Day 85; hence, all the patients enrolled in this study were sufficiently exposed to telaprevir during treatment (Fig. 3b). These results demonstrate that the plasma concentrations of telaprevir were manageable even during the long-term repeated administration. There were no clinically significant events, although the incidence of some events exceeded 20.0% (Table 2). Notably, mild anaemia developed in seven patients (70%) and its occurrence was consistent with the decrease in haemoglobin values, although gradual, during the first 29 days after administration of telaprevir. The incidence of rash, which is reported to develop with a high incidence and high severity in the clinical trials of co-administration of telaprevir with PEG-IFN and RBV [11,12], was also high but its severity was mild in this study. Although exposure to telaprevir was sufficient to eliminate the virus, neither serious adverse events nor discontinuations because of adverse events occurred during the study period. The results confirmed the high tolerability of telaprevir alone after long-term administration. Although there has been no direct comparison of telaprevir monotherapy and telaprevir-based triple therapy, based on these results, the severe adverse drug reactions reported for telaprevir-based triple therapy including anaemia and rash were likely to be ascribed to the synergistic and/or additive effects of the three drugs, i.e., telaprevir, PEG-IFN, and RBV. The safety information under telaprevir monotherapy described here is very important to understand the aspects of adverse drug reactions, especially anaemia and rash, in telaprevir-based triple therapy. In addition, compared to baseline, the ALT and AST levels were significantly lower during the treatment in all patients, indicating that telaprevir was unlikely to cause direct liver damage or injury even after long-term use.

Although there is a report on HCV RNA mutation after monotherapy with a protease inhibitor for 14 days [16], no information about the selective pressure of such protease inhibitors administered alone for a longer period is available at present. During the treatment period in this study, HCV RNA levels were below the LLOQ in seven patients and undetectable in three patients. Importantly, one patient showed an end-of-treatment response. Viral breakthrough resulting from the selection of Ala^{156}-substituted variants with high-level resistance to telaprevir [16] occurred in eight patients. It has been reported that high-level resistance was absent, low-level resistance was minimized, and the majority of the viral population reverted to the wild-type by 3–7 months after telaprevir dosing for 14 days [16]. In the two patients who were studied up to the last visit, enrichment of the wild-type viruses was observed at Week 24 of the follow-up period. It is thus clear that the variants that appeared during prolonged administration of telaprevir for 12 weeks could be replaced by or could revert to the wild-type viruses. This study also provides new knowledge about a selective pathway of the NS3 protease domain of HCV genotype 1b during long-term telaprevir administration (Table 3). It is notable that the wild-type viruses were eliminated promptly by Day 3 of telaprevir monotherapy in all cases, but variants with amino acid substitutions such as V36G, A156V and T54A still remained on Days 3 and 8.

From Day 50 to Day 99, A156T was the predominant variant after viral breakthrough. On Day 43, several substitutions that are rarely reported were found: a single substitution of A156F and multiple substitutions of T54S+A156T and A156T+V158I. In the clonal sequencing analysis in this trial, the observed T54S and V158I substitutions were mostly associated with the A156S/T substitution, and enrichment of multiple-substituted variants was observed under prolonged telaprevir treatment (Fig. S1). A phenotypic enzyme assay suggested that the solo T54S substitution did not change the inhibitory concentration of telaprevir (data not shown). It has also been reported that the T54S and V158I substitutions were also positively selected in the clinical trials of boceprevir, but the solo V158I substitution did not confer telaprevir resistance [17]. Therefore, these two substitutions may be a secondary resistance-associated variant of genotype 1b. Moreover, we could speculate that these variants are susceptible to PEG-IFN and RBV, because the viral variants emerging after the longer selective pressure with telaprevir monotherapy were decreased rapidly by switching the treatment with telaprevir to that with PEG-IFN and RBV [18]. Although it was reported that one patient with low viral load achieved SVR in the treatment regimen in which 750 mg telaprevir was administered q8h for 24 weeks [19], no patients with high viral load achieved SVR in this study. As discussed earlier, PEG-IFN and RBV-free therapy is an unmet and strong medical need in Japan. Therefore, an oral cocktail therapy for HCV genotype 1b infection using telaprevir and different types of DAAs, for example HCV NS5A or NS5B polymerase inhibitors, would be warranted to improve efficacy and reduce adverse drug reactions of the telaprevir, PEG-IFN and RBV triple therapy.

In conclusion, the results of this study indicate that telaprevir is well tolerated at 750 mg q8h for 12 weeks in Japanese patients with HCV genotype 1b infection. The data obtained in this study on telaprevir monotherapy demonstrate that the severe side effects, rash and anaemia observed in the telaprevir-based triple regimen were likely to be attributable to the additive and/or synergistic effect of telaprevir, PEG-IFN and RBV, and this consideration has encouraged us to evaluate telaprevir in a combination therapy with a different class of DAAs in future.

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DISCLOSURE

The others have nothing to declare.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1: Pie chart of variant occupation at each time point in the typical two cases. Proposed secondary resistant associated substitutions are underlined.

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Amino Acid Substitution in HCV Core/NS5A Region and Genetic Variation Near *IL28B* Gene Affect Treatment Efficacy to Interferon plus Ribavirin Combination Therapy

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Key Words
Hepatitis C virus · Interferon · Ribavirin · Core region · NS5A region · ISDR · IRRDR · *IL28B*

Abstract
Objective: To evaluate predictive factors of treatment efficacy to interferon (IFN)/ribavirin in patients infected with HCV genotype 1b (HCV-1b). ***Methods:*** This study investigated pretreatment predictors, including viral- (aa substitutions in core aa 70/91 and NS5A-ISDR/IRRDR) and host-related factors (genetic variation near *IL28B* gene), to 48-week IFN/ribavirin in 490 Japanese adults infected with HCV-1b. ***Results:*** The proportion of patients who showed end-of-treatment response (ETR), sustained virological response (SVR), and SVR after ETR was 76, 54, and 76%, respectively. There was a significant positive correlation between the number of aa substitutions in ISDR and those in IRRDR. Concerning the substitution of core aa 91, the number of aa substitutions in ISDR/IRRDR of patients with Leu91 was significantly higher than that of patients with Met91. Furthermore, levels of viremia were influenced by aa substitutions in core aa 91 and ISDR/IRRDR. By multivariate analysis, rs8099917 genotype was an important predictor of ETR and SVR. With regard to viral factors, core aa 70/91 was an important predictor of ETR, and SVR after ETR. ISDR was an important predictor of SVR, and SVR after ETR. ***Conclusion:*** aa substitution in core/NS5A region and genetic variation near *IL28B* were important predictors of treatment efficacy to IFN/ribavirin.

Introduction

Treatment of chronic hepatitis C virus (HCV) infection with interferon (IFN) combined with ribavirin carries potential serious side effects and is costly, especially when used long enough to achieve a high sustained virological response (SVR) in patients infected with HCV genotype 1b (HCV-1b) and high viral loads. For these rea-

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sons, those patients who do not achieve SVR need to be identified, so as to free them of unnecessary side effects and reduce costs, preferably before the start of the combination therapy.

Viral- and host-related factors are useful as predictors of treatment efficacy to 48-week IFN/ribavirin combination therapy. With regard to viral factors, amino acid (aa) substitutions at position 70 and/or 91 in the core region of HCV-1b are pretreatment predictors of virological response to combination therapy [1–4], and also affect clinical outcome, including hepatocarcinogenesis [5, 6]. Furthermore, the NS5A region of HCV-1b, including IFN-sensitivity-determining region (ISDR) [7, 8] and IFN/ribavirin resistance-determining region (IRRDR) [9, 10], are also useful as pretreatment predictors of virological response to combination therapy [11, 12]. With regard to host factors, genetic variations near *IL28B* gene (rs8099917, rs12979860) on chromosome 19, which encodes IFN-λ-3, are pretreatment predictors of virological response to combination therapy in individuals infected with HCV-1 [13–16], and also affect clinical outcome, including spontaneous clearance of HCV [17]. A recent report identified genetic variation near *IL28B* gene and aa substitution of the core region as predictors of SVR to triple therapy of telaprevir/pegylated (PEG)-IFN/ribavirin in Japanese patients infected with HCV-1b [18]. However, to our knowledge, there are no previous reports of IFN/ribavirin combination therapy based on multivariate analysis to investigate pretreatment predictors, including all of aa substitutions in core aa 70/91 and NS5A-ISDR/IRRDR, and genetic variation near *IL28B* gene.

The aim of the present study was to investigate predictive factors of treatment efficacy, including viral- (aa substitutions in core aa 70/91 and NS5A-ISDR/IRRDR) and host-related factors (genetic variation near *IL28B* gene), to 48-week IFN/ribavirin in Japanese adults infected with HCV-1b.

Patients and Methods

Study Population

A total of 1,249 HCV-1b-infected Japanese adult patients were consecutively recruited into the study protocol of combination therapy with IFN (PEG-IFNα-2b or IFNα-2b) plus ribavirin between December 2001 and January 2009 at Toranomon Hospital, Tokyo, Japan. Among these, 490 patients, who could complete a total of 48 weeks of combination therapy, were enrolled in this retrospective study, and fulfilled the following criteria: (1) negativity for hepatitis B surface antigen (HBsAg) in serum; (2) HCV-1b only confirmed by sequence analysis; (3) HCV-RNA levels of ≥5.0 log IU/ml determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan) within the preceding 2 months of enrolment; (4) no hepatocellular carcinoma; (5) body weight >40 kg; (6) lack of coinfection with human immunodeficiency virus; (7) no previous treatment with antiviral or immunosuppressive agents within the preceding 3 months of enrolment; (8) none was an alcoholic; lifetime cumulative alcohol intake was <500 kg; (9) none had other forms of liver diseases, such as hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, or autoimmune liver disease, and (10) none of the females was pregnant or breastfeeding.

The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the institutional review board. Each patient gave their informed consent before participating in this trial.

The treatment efficacy was evaluated in terms of HCV-RNA negativity at the end of treatment (end-of-treatment response (ETR)) and 24 weeks after the completion of therapy (SVR), based on the COBAS TaqMan HCV test (Roche Diagnostics). SVR in patients who achieved ETR was defined as SVR after ETR. ETR, SVR, and SVR after ETR could be evaluated in 487 (99%), 448 (91%), and 321 (66%) of 490 patients, respectively.

422 (86%) patients received PEG-IFNα-2b at a median dose of 1.4 μg/kg (range 0.7–1.9) subcutaneously each week plus oral ribavirin at a median dose of 11.1 mg/kg (range 3.7–15.1) daily for 48 weeks. The remaining 68 (14%) patients received 6 million units of IFNα-2b intramuscularly each day for 48 weeks (daily for the initial 2 weeks, followed by three times per week for 46 weeks), and oral ribavirin at a median dose of 11.3 mg/kg (range 6.8–13.4) daily for 48 weeks.

Table 1 summarizes the profiles and laboratory data of the 490 patients at the commencement of treatment. They included 310 males and 180 females aged 20–75 years (median 54).

Measurement of HCV RNA

The antiviral effects of treatment on HCV were assessed by measuring plasma HCV-RNA levels. In this study, HCV-RNA levels 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 1.2–7.8 log IU/ml, and the undetectable samples were defined as negative.

Detection of aa Substitutions in Core, and NS5A Regions of HCV-1b

With the use of HCV-J (accession No. D90208) as a reference [19], the sequence of 1–191 aa in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed on the previous study to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [1]. The sequence of 2,209–2,248 aa in the NS5A of HCV-1b (ISDR) reported by Enomoto et al. [7, 8] was determined, and the number of aa substitutions in ISDR was defined as wild-type (WT) (0, 1) or non-wild-type (non-WT) (≥2) in comparison with HCV-J. Furthermore, the sequence of 2,334–2,379 aa in the NS5A of HCV-1b (IRRDR) reported by El-Shamy et al. [9, 10] was determined and then compared with the consensus sequence constructed on the previous study. In the present study, aa substitutions of the core region and NS5A-ISDR/IRRDR of HCV-1b were analyzed by direct sequencing [10, 18].

Genetic Variation near IL28B Gene

Samples for genome-wide association survey were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip. Genotyping data were subjected to quality control before the data analysis. Genotyping for replication and fine mapping was performed by use of Invader assay, TaqMan assay, or direct sequencing as described previously [20, 21].

In this study, genetic variations near *IL28B* gene (rs8099917), reported as the pretreatment predictors of treatment efficacy in Japanese patients [14, 18], were investigated.

Statistical Analysis

Non-parametric tests (Mann-Whitney U test, χ^2 test and Fisher's exact probability test) were used to compare the characteristics of the groups. Correlation analysis was evaluated by the Spearman rank correlation test. Uni- and multivariate logistic regression analyses were used to determine those factors that significantly contributed to ETR, SVR, and SVR after ETR. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. All p values <0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance ($p < 0.05$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent predictive factors. Each variable was transformed into categorical data consisting of two simple ordinal numbers for uni- and multivariate analyses. Potential predictive factors associated with ETR, SVR, and SVR after ETR included the following variables: sex, age, history of blood transfusion, familial history of liver disease, body mass index, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, γ-glutamyl transpeptidase (GGT), leukocyte count, hemoglobin, platelet count, level of viremia, α-fetoprotein, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, uric acid, ribavirin dose/body weight, genetic variation near *IL28B* gene, and aa substitution in the core region, and NS5A-ISDR/IRRDR. Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, Ill., USA).

Table 1. Patient profile and laboratory data at commencement of the 48-week combination therapy of IFN + ribavirin in 490 patients infected with HCV-1b

Demographic data	
Number of patients	490
Male/female	310/180
Age, years	54 (20–75)
History of blood transfusion	169 (34%)
Family history of liver disease	96 (20%)
Body mass index, kg/m^2	22.6 (15.7–34.7)
Laboratory data	
Level of viremia, log IU/ml	6.4 (2.2–7.7)
Serum AST, IU/l	50 (16–296)
Serum ALT, IU/l	67 (12–836)
Serum albumin, g/dl	3.9 (3.1–4.7)
GGT, IU/l	44 (10–592)
Leukocyte count, n/mm^3	4,700 (1,200–10,900)
Hemoglobin, g/dl	14.4 (10.6–18.1)
Platelet count, $\times 10^4$/mm^3	16.7 (6.4–37.5)
α-Fetoprotein, μg/l	5 (1–459)
Total cholesterol, mg/dl	170 (96–284)
High-density lipoprotein cholesterol, mg/dl	46 (13–95)
Low-density lipoprotein cholesterol, mg/dl	100 (32–190)
Triglycerides, mg/dl	90 (33–416)
Uric acid, mg/dl	5.5 (2.3–9.4)
Treatment	
PEG-IFNα-2b/IFNα-2b	422/68
Ribavirin dose, mg/kg	11.2 (3.7–15.1)
aa substitutions in the HCV-1b	
Core aa 70, arginine/glutamine (histidine)	266/151
Core aa 91, leucine/methionine	246/169
ISDR of NS5A, 0/1/≥2	227/95/84
IRRDR of NS5A, ≤3/4–5/≥6	73/67/60
Genetic variation near IL28B *gene*	
rs8099917 genotype, TT/TG/GG	150/65/4

Data represent number of patients with percentages in parentheses, or median (range) values.

Results

Response to Therapy

ETR was achieved by 372 of 487 (76%) patients, SVR by 244 of 448 (54%), and SVR after ETR by 244 of 321 (76%).

Number of aa Substitutions in NS5A-ISDR and NS5A-IRRDR

As a whole, 0, 1, and ≥2 aa substitutions in ISDR were found in 56% (227 of 406), 23% (95 of 406), and 21% (84 of 406) of patients, respectively. Thus, the percentage of patients with ≤1 aa substitution in ISDR (WT) was 79% (322 of 406). Furthermore, ≤3, 4–5, and ≥6 aa substitutions in IRRDR were found in 36% (73 of 200), 34% (67 of 200), and 30% (60 of 200) of patients, respectively (fig. 1).

The correlation between ISDR and IRRDR was analyzed. There was a significant positive correlation between the number of aa substitutions in ISDR and those in IRRDR ($r = 0.308$, $p < 0.001$) (fig. 2).

aa Substitutions in the Core Region and NS5A-ISDR/IRRDR

Concerning the substitution of core aa 70, the number of aa substitutions in ISDR of 256 patients with Arg70 (median 0) was not significantly different from that of 146 patients with Gln70 (His70) (median 0) (fig. 3a). Fur-

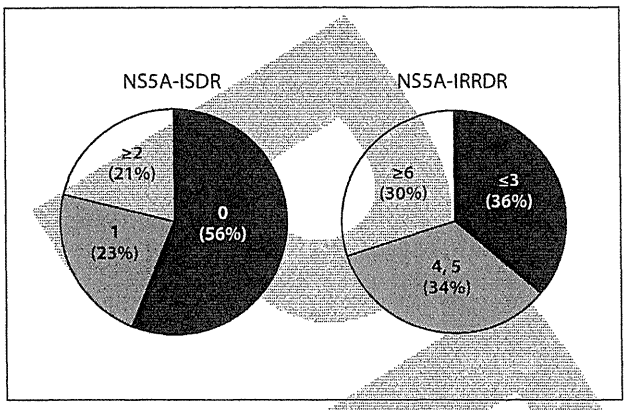

Fig. 1. The number of aa substitutions in NS5A-ISDR and NS5A-IRRDR. The percentage of patients with ≤1 aa substitution in ISDR (WT) was 79%.

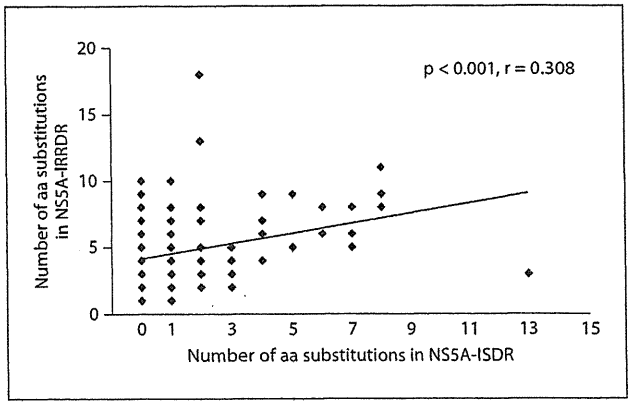

Fig. 2. Correlation between NS5A-ISDR and NS5A-IRRDR. There was a significant positive correlation between the number of aa substitutions in ISDR and that in IRRDR ($r = 0.308$, $p < 0.001$).

thermore, the number of aa substitutions in IRRDR of 123 patients with Arg70 (median 5) was also not significantly different from that of 77 patients with Gln70 (His70) (median 4) (fig. 3b).

Concerning the substitution of core aa 91, the number of aa substitutions in ISDR of 240 patients with Leu91 (median 1) was significantly higher than that of 161 patients with Met91 (median 0) ($p < 0.001$) (fig. 3c). Furthermore, the number of aa substitutions in IRRDR of 111 patients with Leu91 (median 5) was significantly higher than that of 89 patients with Met91 (median 3) ($p < 0.001$) (fig. 3d).

Viremia Level and aa Substitutions in Core Region/ISDR/IRRDR

Concerning the number of substitutions in ISDR, viremia levels of 321 patients with WT (median 6.5) were significantly higher than those of 84 patients with non-WT (median 5.7) ($p < 0.001$) (fig 4a).

Concerning the number of substitutions in IRRDR, viremia levels of 140 patients with ≤5 substitutions (median 6.4) were significantly higher than those of 60 patients with ≥6 (median 6.1) ($p = 0.027$) (fig. 4b).

Concerning the substitution of core aa 70, viremia levels of 265 patients with Arg70 (median 6.4) were not significantly different from those of 151 patients with Gln70 (His70) (median 6.3) (fig. 4c).

Concerning the substitution of core aa 91, viremia levels of 169 patients with Met91 (median 6.5) were significantly higher than those of 245 patients with Leu91 (median 6.2) ($p = 0.028$) (fig. 4d).

Thus, levels of viremia were influenced by aa substitutions in core aa 91 and ISDR/IRRDR.

Treatment Response according to the Number of aa Substitutions in IRRDR

Concerning the number of aa substitutions in IRRDR, a significantly higher proportion of patients with ≥4 aa substitutions (58%) showed SVR compared to patients with ≤3 (42%) ($p = 0.039$). In contrast, the SVR rate was not significantly different between patients with ≤4 (49%) and those with ≥5 (57%) aa substitutions. Likewise, the SVR rate was not significantly different between patients with ≤5 (51%) and those with ≥6 (55%) aa substitutions (fig. 5a).

The ETR rate was not significantly different between patients with ≤3 (74%) and those with ≥4 (82%) aa substitutions, nor between patients with ≤4 (76%) and those with ≥5 (83%). Likewise, the ETR rate was not significantly different between those with ≤5 (79%) and those with ≥6 (80%) aa substitutions (fig. 5b).

The SVR rate after ETR was not significantly different between patients with ≤3 (61%) and those with ≥4 (74%) aa substitutions, nor between patients with ≤4 (67%) and those with ≥5 (72%). Likewise, they were not significantly different between patients with ≤5 (67%) and those with ≥6 (75%) aa substitutions (fig. 5c).

Thus, it was useful as predictor of SVR to categorize into two groups of ≤4 and ≥5 aa substitutions by univariate analysis. However, the ETR and SVR after ETR rates were not significantly different according to the number of aa substitutions in IRRDR.

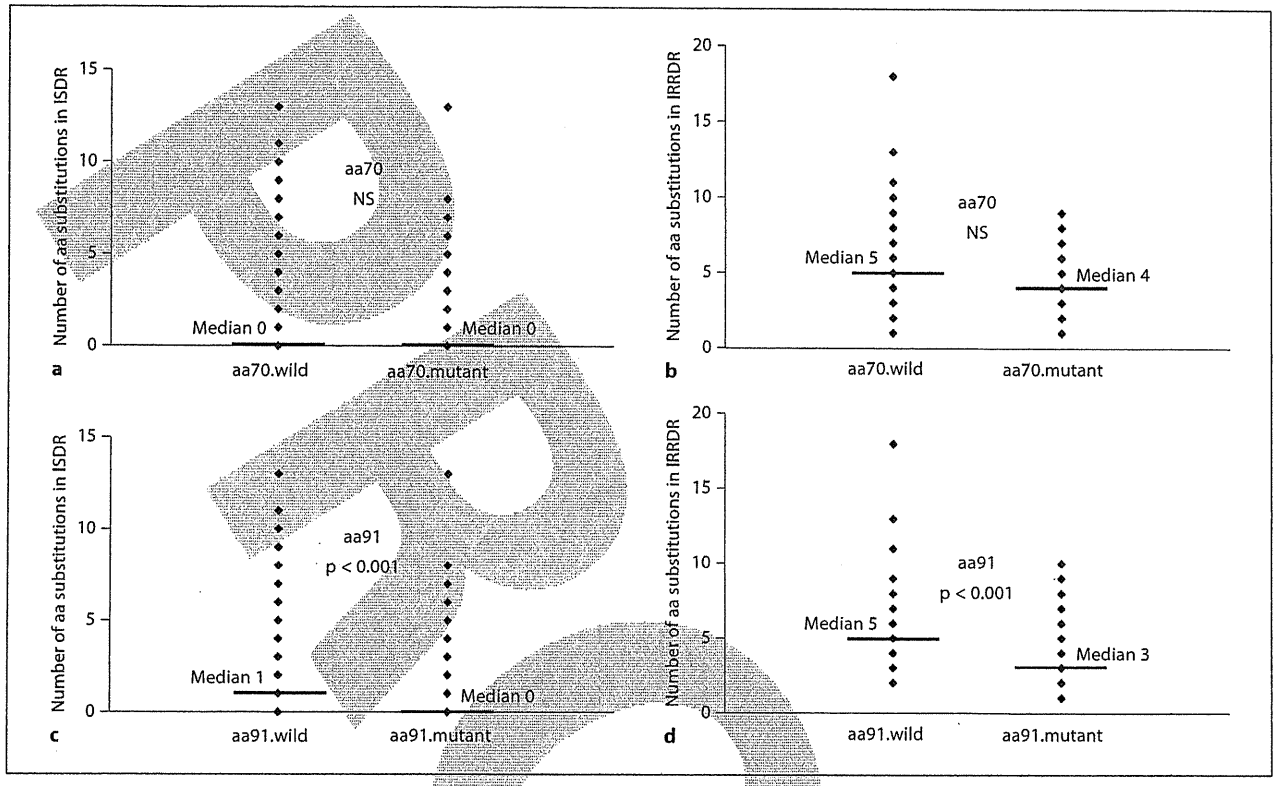

Fig. 3. aa substitutions in the core region and NS5A-ISDR/IRRDR. **a**, **b** Concerning the substitution of core aa 70, the number of aa substitutions in ISDR/IRRDR of patients with Arg70 was not significantly different from that of patients with Gln70 (His70). **c**, **d** Concerning the substitution of core aa 91, the number of aa substitutions in ISDR/IRRDR of patients with Leu91 was significantly higher than that of patients with Met91 ($p < 0.001$).

Predictors of SVR as Determined by Uni- and Multivariate Analyses

Univariate analysis identified 15 parameters that correlate with SVR: gender (male sex; $p < 0.001$), age (<55 years; $p < 0.001$), ribavirin dose (≥11.0 mg/kg; $p = 0.006$), AST (<58 IU/l; $p = 0.039$), leukocyte count (≥4,500/mm^3; $p = 0.043$), hemoglobin (≥14.0 g/dl; $p = 0.001$), platelet count (≥15.0 × 10^4/mm^3; $p < 0.001$), GGT (<50 IU/l; $p = 0.028$), uric acid (≥5.5 mg/dl; $p = 0.005$), level of viremia (<6.0 log IU/ml; $p < 0.001$), α-fetoprotein (<10 μg/l; $p < 0.001$), genetic variation in rs8099917 (genotype TT; $p < 0.001$), substitution of aa 70 (Arg70; $p < 0.001$), the number of aa substitutions in ISDR (non-WT; $p < 0.001$) and IRRDR (≥4; $p = 0.039$). Figure 6 shows the SVR rate according to aa substitution in the core/NS5A region and genetic variation near *IL28B* by univariate analysis.

Multivariate analysis that included the above variables identified 3 parameters that independently influenced SVR: genetic variation in rs8099917 (genotype TT; $p < 0.001$), gender (male sex; $p < 0.001$), and the number of aa substitutions in ISDR (non-WT; $p = 0.027$) (table 2).

Predictors of ETR as Determined by Uni- and Multivariate Analyses

Univariate analysis identified 14 parameters that correlated with ETR: gender (male sex; $p = 0.001$), age (<55 years; $p = 0.004$), AST (<39 IU/l; $p = 0.027$), hemoglobin (≥14.0 g/dl; $p = 0.035$), platelet count (≥15.0 × 10^4/mm^3; $p < 0.001$), albumin (≥3.9 g/dl; $p = 0.014$), GGT (<50 IU/l; $p < 0.001$), uric acid (≥5.5 mg/dl; $p = 0.003$), level of viremia (<6.0 log IU/ml; $p = 0.001$), low-density lipoprotein cholesterol (≥85 mg/dl; $p = 0.004$), α-fetoprotein (<10 μg/l; $p < 0.001$), genetic variation in rs8099917 (genotype TT; $p < 0.001$), substitution of aa 70 (Arg70; $p < 0.001$), and the number of aa substitutions in ISDR (non-WT; $p = 0.021$). Figure 7 shows the ETR rate according to aa

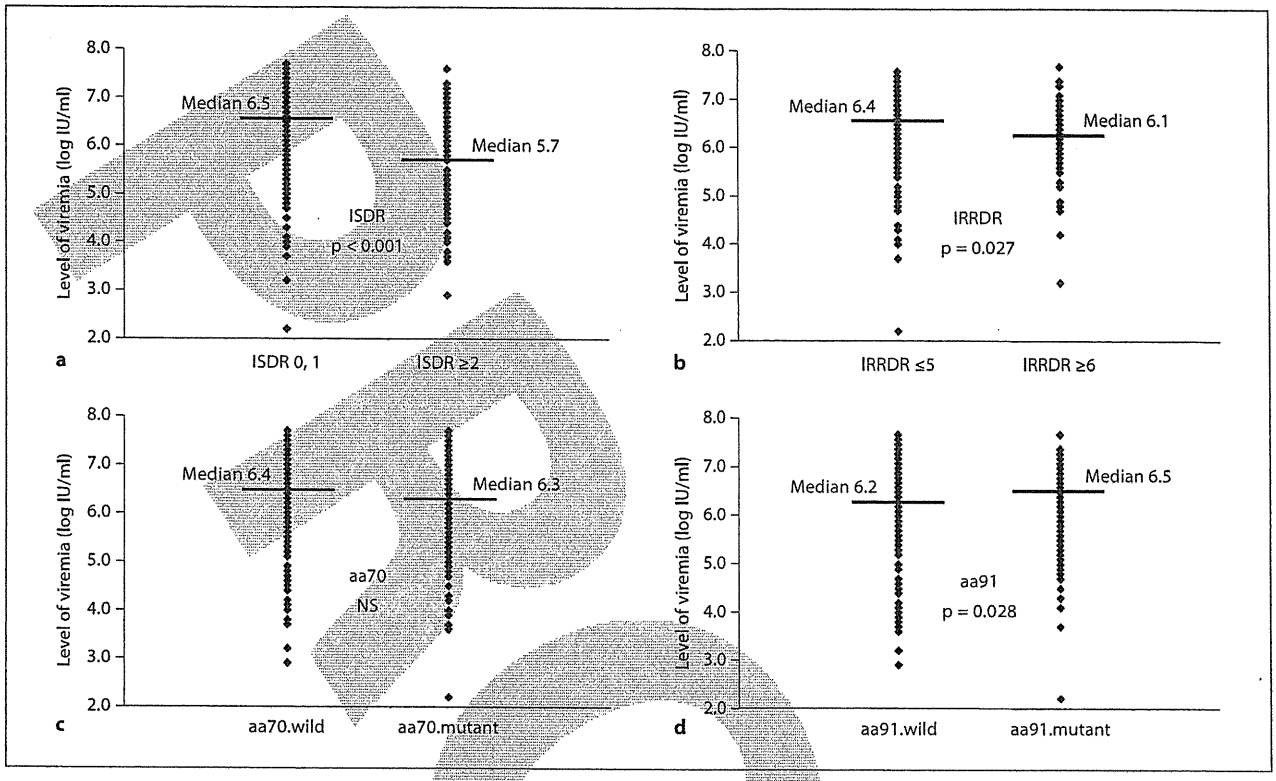

Fig. 4. Viremia level and aa substitutions in core region/ISDR/IRRDR. **a** Concerning the number of substitutions in ISDR, viremia levels of patients with WT were significantly higher than those of patients with non-WT ($p < 0.001$). **b** Concerning the number of substitutions in IRRDR, viremia levels of patients with ≤5 aa substitutions were significantly higher levels than those of patients with ≥6 ($p = 0.027$). **c** Concerning the substitution of core aa 70, viremia levels of patients with Arg70 were not significantly different from those of patients with Gln70 (His70). **d** Concerning the substitution of core aa 91, viremia levels of patients with Met91 were significantly higher than those of patients with Leu91 ($p = 0.028$). Thus, levels of viremia might be influenced by aa substitutions in core aa 91 and ISDR/IRRDR.

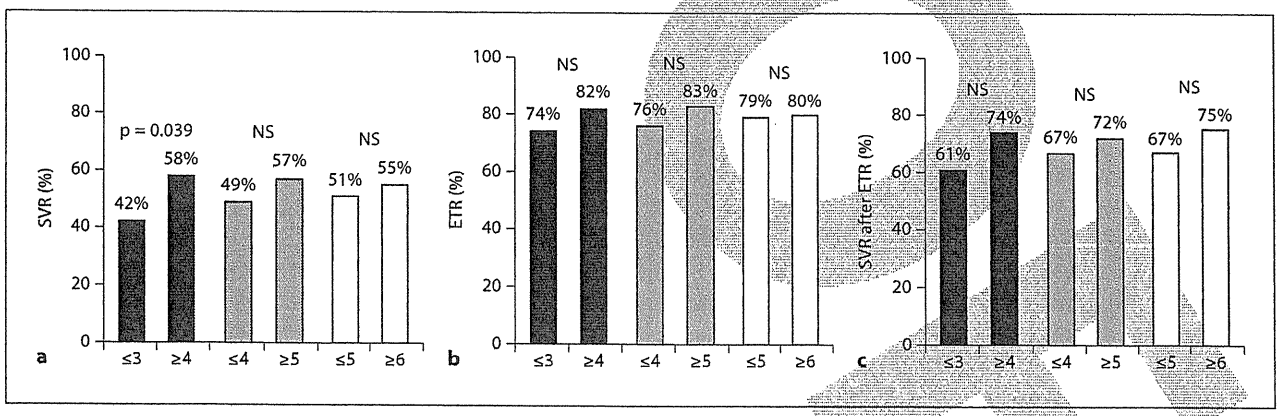

Fig. 5. Treatment response according to the number of aa substitutions in NS5A-IRRDR. **a** A significantly higher proportion of patients with ≥4 (58%) aa substitutions showed SVR compared to patients with ≤3 (42%) ($p = 0.039$), and it was useful as predictor of SVR to categorize into two groups of ≤4 and ≥5 aa substitutions by univariate analysis. **b**, **c** ETR and SVR after ETR rates were not significantly different according to the number of aa substitutions in IRRDR.

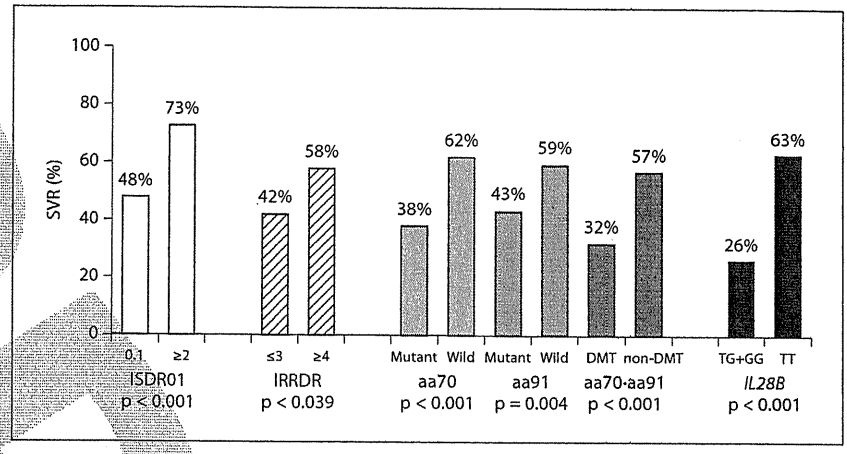

Fig. 6. SVR rate according to aa substitution in core/NS5A region and genetic variation near *IL28B* by univariate analysis.

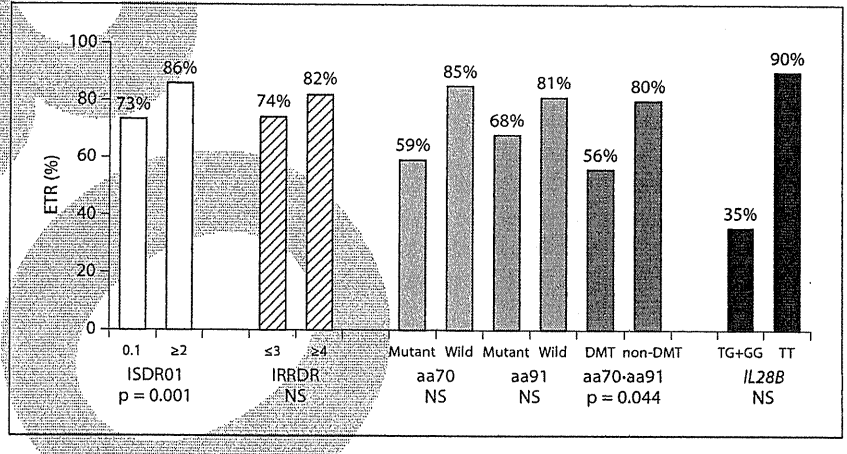

Fig. 7. ETR rate according to aa substitution in core/NS5A region and genetic variation near *IL28B* by univariate analysis.

Table 2. Factors associated with SVR to 48-week IFN + ribavirin combination therapy in patients infected with HCV-1b, identified by multivariate analysis

Factor	Category	OR (95% CI)	p
rs8099917 genotype	1: TG+GG 2: TT	1 16.7 (4.54–61.3)	<0.001
Gender	1: Female 2: Male	1 10.5 (3.47–32.3)	<0.001
ISDR of NS5A	1: WT 2: Non-WT	1 5.68 (1.22–26.3)	0.027

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.

Table 3. Factors associated with ETR response to 48-week IFN + ribavirin combination therapy in patients infected with HCV-1b, identified by multivariate analysis

Factor	Category	OR (95% CI)	p
rs8099917 genotype	1: TG+GG 2: TT	1 18.2 (6.29–52.6)	<0.001
Level of viremia log IU/ml	1: ≥6.0 2: <6.0	1 9.20 (2.59–32.6)	0.001
Core aa 70	1: Gln70 (His70) 2: Arg70	1 4.68 (1.65–13.3)	0.004
Serum albumin g/dl	1: <3.9 2: ≥3.9	1 3.08 (1.11–8.47)	0.030

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.

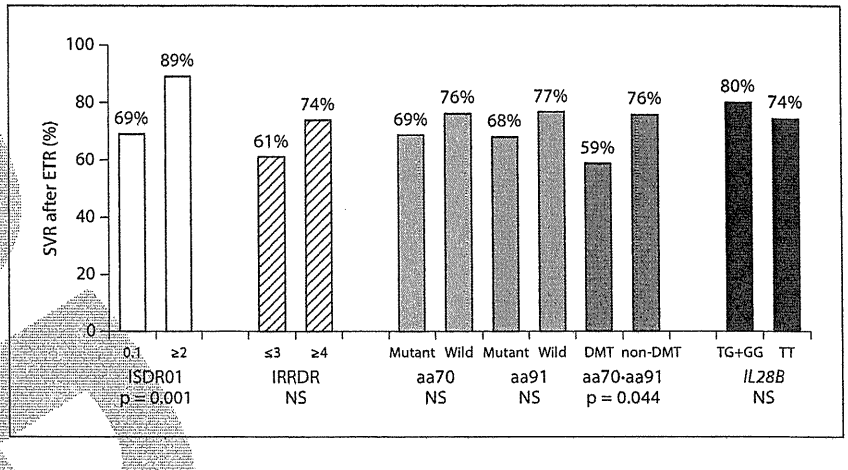

Fig. 8. SVR after ETR rate according to aa substitution in core/NS5A region and genetic variation near *IL28B* by univariate analysis.

substitution in the core/NS5A region and genetic variation near *IL28B* by univariate analysis.

Multivariate analysis that included the above variables identified 4 parameters that independently influenced ETR: genetic variation in rs8099917 (genotype TT; $p < 0.001$), level of viremia (<6.0 log IU/ml; $p = 0.001$), substitution of aa 70 (Arg70; $p = 0.004$), and albumin (≥3.9 g/dl; $p = 0.030$) (table 3).

Predictors of SVR after ETR as Determined by Uni- and Multivariate Analyses

Univariate analysis identified 11 parameters that influenced SVR after ETR: gender (male sex; $p < 0.001$), age (<55 years; $p < 0.001$), ribavirin dose (≥11.0 mg/kg; $p = 0.025$), leukocyte count (≥4,500/mm^3; $p = 0.033$), hemoglobin (≥14.0 g/dl; $p = 0.025$), platelet count (≥15.0 × 10^4/mm^3; $p = 0.001$), level of viremia (<6.0 log IU/ml; $p = 0.020$), total cholesterol (<170 mg/dl; $p = 0.017$), α-fetoprotein (<10 μg/l; $p = 0.004$), substitution of aa 70 and 91 (Arg70 and/or Leu91; $p = 0.044$), and the number of aa substitutions in ISDR (non-WT; $p = 0.001$). Figure 8 shows the SVR after ETR rate according to aa substitution in the core/NS5A region and genetic variation near *IL28B* by univariate analysis.

Multivariate analysis that included the above variables identified 6 parameters that independently influenced the SVR after ETR: gender (male sex; $p < 0.001$), ribavirin dose (≥11.0 mg/kg; $p = 0.002$), the number of aa substitutions in ISDR (non-WT; $p = 0.012$), substitution of aa 70 and 91 (Arg70 and/or Leu91; $p = 0.023$), platelet count (≥15.0 × 10^4/mm^3; $p = 0.033$), and α-fetoprotein (<10 μg/l; $p = 0.042$) (table 4).

Comparison of Factors Associated with Treatment Efficacy Identified by Multivariate Analysis

Table 5 shows the variables that achieved statistical significance on multivariate logistic regression for each evaluation of treatment efficacy. Rs8099917 genotype was an important predictor of ETR and SVR. With regard to viral factors, core region was an important predictor of ETR, and SVR after ETR. ISDR was an important predictor of SVR, and SVR after ETR. Level of viremia was an important predictor of ETR. Thus, genetic variation near *IL28B* and viral factors (core region, ISDR, and level of viremia) were important predictors of treatment efficacy. Furthermore, gender, α-fetoprotein, albumin, and platelet count were also identified as other important predictors of treatment efficacy, in addition to genetic variation near *IL28B* and viral factors.

Discussion

Using multivariate analysis, the present study identified viral- (aa substitutions in core aa 70/91 and NS5A-ISDR/IRRDR) and host-related factors (genetic variation near *IL28B* gene) that influenced treatment efficacy to 48-week IFN/ribavirin combination therapy, which is in agreement with recent findings [22, 23]. Identification of these viral and host factors before the start of IFN/ribavirin combination therapy should help to select better therapeutic regimens, including triple therapy of telaprevir/PEG-IFN/ribavirin [24–26], for those patients who are less likely to achieve SVR.

According to the number of substitutions in ISDR, a previous report showed that levels of viremia were sig-

Table 4. Factors associated with SVR in patients who achieved ETR response to 48-week IFN + ribavirin combination therapy in patients infected with HCV-1b, identified by multivariate analysis

Factor	Category	OR (95% CI)	p
Gender	1: Female	1	
	2: Male	4.27 (2.15–8.55)	<0.001
Ribavirin dose, mg/kg	1: <11.0	1	
	2: ≥11.0	2.95 (1.48–5.86)	0.002
ISDR of NS5A	1: WT	1	
	2: Non-WT	4.00 (1.35–11.8)	0.012
Core aa 70 and 91	1: Gln70 (His70) and Met91	1	
	2: Arg70 and/or Leu91	2.96 (1.16–7.52)	0.023
Platelet count $\times 10^4/mm^3$	1: <15.0	1	
	2: ≥15.0	2.19 (1.07–4.50)	0.033
α-Fetoprotein μg/l	1: ≥10	1	
	2: <10	2.66 (1.04–6.80)	0.042

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.

Table 5. Comparison of factors associated with efficacy of 48-week IFN + ribavirin combination therapy in patients infected with HCV-1b, identified by multivariate analysis

Factor	ETR response (at 48 weeks)	SVR after ETR response	SVR
IL28B	rs8099917 p < 0.001, 18.2 (6.29–52.6)[a]		rs8099917 p < 0.001, 16.7 (4.54–61.3)[a]
Virus	Core aa 70 p = 0.004, 4.68 (1.65–13.3)[a]	Core aa 70 and 91 p = 0.023, 2.96 (1.16–7.52)[a]	
	Level of viremia p = 0.001, 9.20 (2.59–32.6)[a]	ISDR p = 0.012, 4.00 (1.35–11.8)[a]	ISDR p = 0.027, 5.68 (1.22–26.3)[a]
Others	Albumin p = 0.030, 3.08 (1.11–8.47)[a]	α-Fetoprotein p = 0.042, 2.66 (1.04–6.80)[a]	
		Platelet count p = 0.033, 2.19 (1.07–4.50)[a]	
		Gender p < 0.001, 4.27 (2.15–8.55)[a]	Gender p < 0.001, 10.5 (3.47–32.3)[a]
		Ribavirin dose p = 0.002, 2.95 (1.48–5.86)[a]	

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.
[a] OR (95% CI).

nificantly lower in patients with non-WT of ISDR than in those with WT [8]. The present study indicated that substitution of IRRDR and core aa 91, in addition to substitution of ISDR, also significantly influenced levels of viremia. Furthermore, there was a significant positive correlation between the number of aa substitutions in ISDR and those in IRRDR, and the number of aa substitutions in ISDR/IRRDR of patients with Leu91 was significantly higher than that of patients with Met91. To our knowledge, this is the first report of the relationship between viremia levels and aa substitutions in core region/ISDR/IRRDR. This result might be interpreted to mean

that core aa 91/ISDR/IRRDR might be associated with viremia levels involved in resistance to combination therapy. Further studies that examine the functional impact of aa substitutions to combination therapy should be conducted to confirm the above finding.

The present results showed that α-fetoprotein, albumin, platelet count, and gender were predictors of virological response to IFN/ribavirin combination therapy. Previous data indicated that absence of advanced liver fibrosis was a positive predictor of SVR to IFN monotherapy and IFN/ribavirin combination therapy [2, 3, 13, 27–29], and that advanced liver fibrosis was usually associated with higher levels of α-fetoprotein, and lower levels of albumin and platelet count [1, 3, 30–32]. Furthermore, gender is also a predictor of treatment response to IFN/ribavirin combination therapy [2, 3, 14]. In the present study based on a large number of patients, histopathological changes in the liver and gender were identified as independent predictors of virological response, in addition to genetic variation near *IL28B* and viral factors (core region, ISDR, and level of viremia).

In a previous study, multivariate analysis identified core region, gender, and stage of liver fibrosis as parameters that independently influenced the SVR of patients who achieved early virological response, but ISDR was not entered into uni- and multivariate analysis [3]. To our knowledge, the present study based on multivariate analysis is the first report to identify ISDR as pretreatment predictor of SVR after ETR to combination therapy. Interestingly, ISDR was not a predictor of ETR, but was a significant predictor of SVR to combination therapy. Thus, the underlying mechanisms of failure to develop SVR in those patients who achieve HCV-RNA negativity remain unclear. Further studies that examine the impact of aa substitutions of ISDR to combination therapy should be conducted to confirm the above finding.

One limitation of the present study was that aa substitutions in areas other than the core region and NS5A-ISDR/IRRDR of the HCV genome were not examined. Other limitations were differences in host factors including race [24, 33, 34] and differences in viral factors, such as the distribution of HCV-1a or -1b, and geographic diversities of HCV-1b [35]. Further large-scale prospective studies are necessary to investigate whether the present results relate to the efficacy of 48-week IFN/ribavirin combination therapy, and further understanding of the complex interaction between virus- and host- related factors should facilitate the development of more effective therapeutic regimens.

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

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

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

Introduction

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

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