Table 2. Genotype distributions of the IL-28B SNPs rs8099917 and rs12979860 in HCV genotype 1b- and 2a-infected patients compared with healthy controls

	rs8099917				P^*	OR† (95 %CI)	rs12979860				P^*	OR† (95 %CI)
	TT	TG	GG	T (%)	G (%)		CC	CT	TT	C (%)	T (%)	
HCV 1b (Set-1, -2, -3)	1180 (75.5)	352 (22.5)	30 (1.9)	86.8	13.2	2.9×10^{-3}	0.60 (0.43–0.84)	1198 (75.1)	367 (23.0)	31 (1.9)	86.6	13.4	1.7×10^{-3}	0.59 (0.42–0.82)
HCV 2a (Set-4)	441 (81.4)	93 (17.2)	8 (1.5)	89.9	10.1	0.41	0.85 (0.58–1.25)	452 (81.6)	92 (16.6)	10 (1.8)	89.9	10.1	0.45	0.86 (0.59–1.23)
Healthy control	236 (83.7)	45 (16.0)	1 (0.4)	91.7	8.3			236 (83.7)	44 (15.6)	2 (0.7)	91.5	8.5		

*P-value compared with healthy controls under a dominant model for the minor allele.
†OR of subjects infected with HCV having a favourable genotype relative to healthy control subjects.

the LD block defined by the criteria of Gabriel *et al.* (2002) spans about 9 kb (Fig. 1), we carefully resequenced the 42 kb genomic region encompassing IL-28B and IL-28A loci using specific PCR primer sets and identified 71 common SNPs, including 22 novel SNPs with a minor allele frequency greater than 0.05.

Among the 71 common SNPs identified by resequencing, 34 that showed strong linkage disequilibrium (r^2 >0.64) with the landmark SNP (rs8099917) were further genotyped. rs8099917 was found to have the strongest association with treatment outcome, and 14 SNPs exhibited comparable associations, with *P*-values varying by less than one order of magnitude (Table 3 and Fig. 2). Haplotype analysis provided little additional information compared with the single marker analysis (data not shown). These results suggest that the association signal was driven by one of the identified SNPs.

Initial response for SNP rs8099917 in the IL-28B locus

Out of the patients infected with HCV genotype 1b who were treated with PEG-IFN and ribavirin combined therapy (Set-1 and Set-2), 443 patients with sufficient viral load data were analysed for viral kinetics. As shown in Fig. 2(a, b), viral reduction in patients with rs8099917 TT is significantly greater than in those with rs8099917 TG or GG in both weeks 2 and 4. Baseline viral load in patients with rs8099917 TT were slightly but significantly higher than in those with rs8099917 TG or GG (Fig. 2c).

Multivariate logistic analysis

Using the patients infected with HCV genotype 1b who were treated with PEG-IFN, a stepwise forward logistic regression analysis was performed to assess the impact of the IL-28B polymorphism. There were 217 patients with sufficient data for the analysis. We evaluated the following seven factors: age, sex, SNP rs8099917, fibrosis and baseline HCV-RNA level. We found that SNP rs8099917 is an independent factor associated with treatment outcome [OR, 0.27; 95 % confidence interval (CI), 0.12–0.87]. Other independent factors meeting the criteria for inclusion in the model are shown in Table 4.

DISCUSSION

Various viral and host factors determine the outcome of IFN (+ribavirin) therapy (Akuta *et al.*, 2007; Bressler *et al.*, 2003; Casiraghi *et al.*, 2004; Enomoto *et al.*, 1996; Kenny-Walsh & Irish Hepatology Research Group, 1999; Manns *et al.*, 2001; Romero-Gómez *et al.*, 2005). Ethnic differences in the response to IFN therapy and in the rate of spontaneous clearance in chronic hepatitis C suggest the influence of genetic factors (Conjeevaram *et al.*, 2006; Welzel *et al.*, 2009). In the current study, we found that the influence of SNPs in the IL-28 locus was quite high.

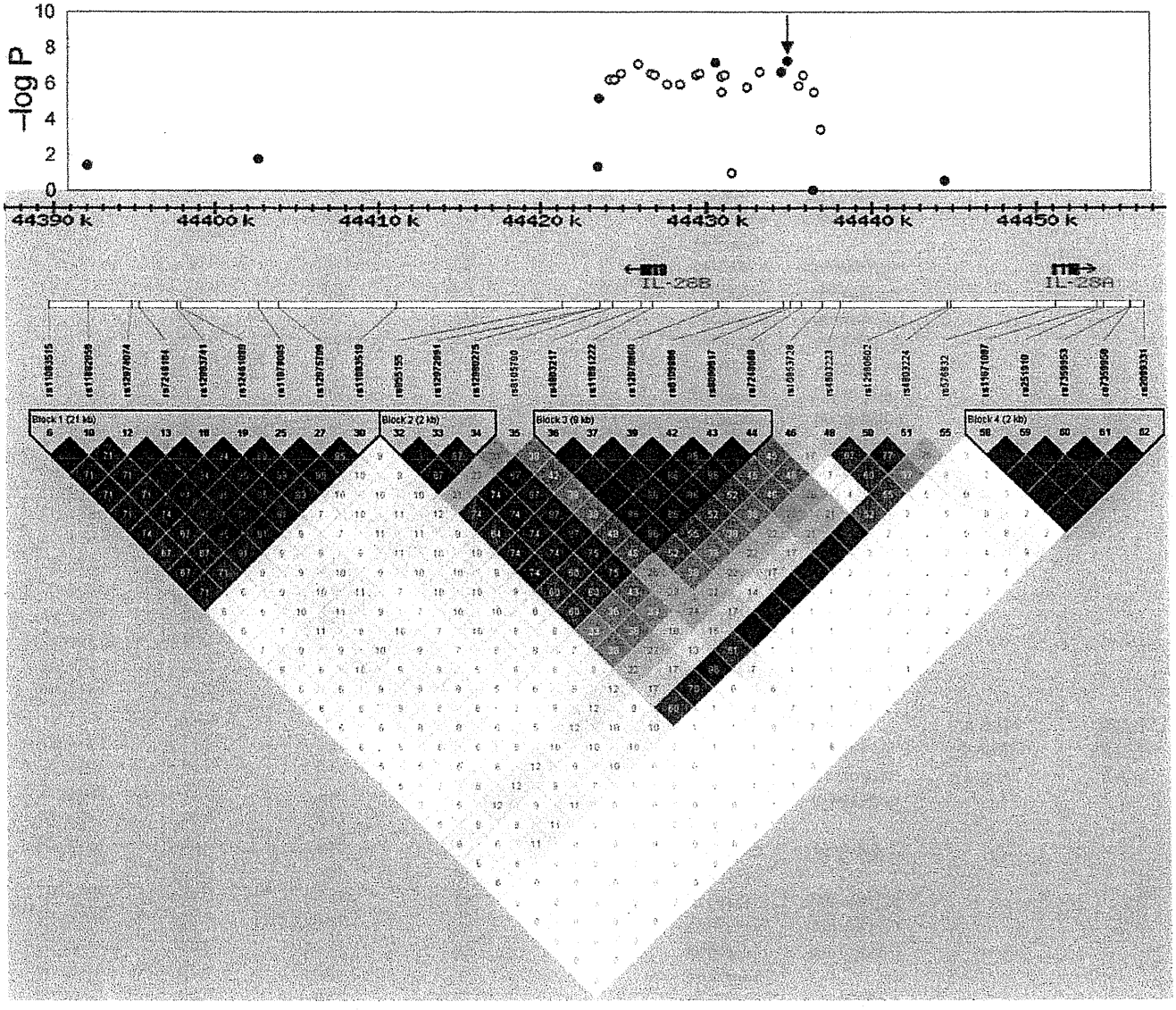

Fig. 1. LD mapping and probabilities for SNPs around the IL-28B locus. The lower panel depicts the haplotype structure around the IL-28B locus. Phase II HapMap JPT genotype data and the HAPLOVIEW program were used to define the LD block. The solid arrow represents the landmark SNP (rs8099917). The block structure is based on the criteria established by Gabriel *et al.* (2002) using pairwise estimates of the disequilibrium coefficient (r^2). Dark grey, regions with high r^2 values; light grey, regions with low r^2 values. The upper panel shows *P*-value plots of case–control association results. *P*-values by the Cochran–Armitage trend test are plotted on a $\log_{10}$ scale. Closed and open dots represent SNP *P*-value in the genome-wide association study (GWAS) and Set-1 fine-mapping stages, respectively.

Despite the fact that the total dose of PEG-IFN or IFN that was administered to each patient varied according to HCV genotype and viral titre based on the current standard regimen, the effect of the therapy in all patient cohorts showed significant association with the SNP genotypes. For replication analyses, we also found significant associations in the case of IFN monotherapy (Sets 3 and 4 in Table 1). Although combination therapy with PEG-IFN and ribavirin is currently the recommended treatment for chronic HCV infection, such analysis is still important because patients cannot always receive PEG-IFN and/or ribavirin due to severe side effects or other reasons. Consistent with earlier findings, the heterozygous genotypes show an intermediate effect level of IFN resistance.

Recent studies have demonstrated that genotype frequency of IL-28B polymorphism varied considerably by ethnicity, which may explain much of the difference in response between different population groups and support the prediction that favourable alleles would be very much more

Table 3. Results of resequencing and fine-mapping of the IL-28B locus

Gene	Location	SNP Id	Position	Alleles		Minor allele frequency (%)	r^{2}*	P† (GWAS set)
			Chr 19	Major	Minor			
		rs11083519	44411103	T	A	46.9	0.04	
		rs60635720	44411511	A	G	47.9	0.05	
		rs57593994	44411591	G	C	46.9	0.04	
			44412803	C	G	6.3	0.02	
		rs12976234	44412840	T	A	49.0	0.06	
		rs8107090	44413755	A	T	49.0	0.07	
		rs35408086	44418650	A	G	47.9	0.04	
		rs11883239	44419320	A	G	46.9	0.04	
		rs11883201	44419330	G	A	47.9	0.02	
		rs12979140	44420380	C	T	49.0	0.00	
		rs12460005	44421017	G	C	33.3	0.10	
		rs955155	44421319	G	A	13.5	0.64	4.77e–02
		rs958039	44422141	T	A	17.7	0.66	4.79e–05
		rs35790907	44422595	A	T	17.7	0.66	1.24e–04
		rs12972991	44423587	A	C	17.4	0.69	4.53e–02
		rs12980275	44423623	A	G	18.8	0.73	6.97e–06
			44424552	A	G	18.8	0.87	6.15e–07
			44424592	G	A	19.8	0.81	6.95e–07
			44423744	T	C	19.8	0.81	8.78e–05
		rs8105790	44424341	T	C	16.7	1.00	6.33e–07
			44424963	C	T	19.1	0.87	3.21e–07
		rs4803217	44426060	C	A	18.8	0.87	9.18e–08
IL-28B	Exon 5		44426165	G	A	5.2	0.01	
IL-28B	Intron 2	rs11881222	44426763	A	G	18.8	0.87	3.12e–07
IL-28B	Exon 2	rs8103142	44426946	T	C	18.8	0.87	3.91e–07
		rs28416813	44427484	C	G	18.8	0.87	8.08e–07
			44427759	C	T	18.8	0.87	1.17e–06
		rs8107030	44428559	A	G	18.8	1.00	1.15e–06
			44428927	A	C	6.3	0.01	
		rs73930703	44429353	C	T	18.8	0.87	7.16e–07
		rs11882871	44429450	A	G	18.8	0.87	3.46e–07
		rs12971396	44429706	C	G	15.6	0.93	3.20e–07
			44430157	A	C	17.7	0.79	1.19e–05
		rs12979860	44430627	C	T	17.7	0.94	7.32e–08
		rs4803221	44430969	C	G	15.6	0.93	4.67e–07
			44430995	TT	G	18.8	0.87	3.12e–06
		rs4803222	44431193	G	C	18.8	0.87	4.03e–07
		rs1549928	44431549	T	C	6.3	0.01	1.20e–01
			44432515	C	A	14.1	0.74	1.68e–06
		rs12983038	44432964	G	A	12.8	0.41	
			44433305	C	T	13.8	0.64	2.33e–07
		rs8109886	44434602	C	A	19.8	0.81	2.41e–07
		rs8109889	44434610	C	T	16.7	1.00	9.67e–07
		rs8113007	44434943	A	T	18.8	0.87	8.20e–07
		rs8099917	44435005	T	G	16.7	–	6.62e–08
		rs7248668	44435661	G	A	16.0	1.00	1.61e–06
			44435942	–	GA	16.7	0.81	4.02e–07
		rs10612351	44436647	AC	–	19.8	0.74	3.32e–06
			44436767	C	T	5.2	0.01	
			44436898	C	T	14.6	0.71	3.73e–03
		rs10853728	44436986	C	G	29.2	0.39	4.38e–04
		rs57401101	44437536	–	C	37.5	0.25	
		rs61665163	44438053	–	AAT	13.5	0.00	
		rs4803223	44438059	A	G	8.3	0.19	1.23e–01

Table 3. cont.

Gene	Location	SNP Id	Position	Alleles		Minor allele frequency (%)	r^{2}*	P† (GWAS set)
			Chr 19	Major	Minor			
			44438119	A	G	12.5	0.02	
		rs35811883	44438306	T	C	18.8	0.02	
		rs56116812	44438930	G	A	12.5	0.00	
		rs8101517	44439581	A	C	21.9	0.07	
			44440345	C	T	6.4	0.01	
			44441174	G	A	6.3	0.00	
		rs10424607	44441762	A	C	20.8	0.09	
		rs10407161	44443231	A	C	44.0	0.13	
		rs6508852	44444102	A	G	27.1	0.07	
			44445071	A	C	11.5	0.03	
		rs6508853	44445966	T	C	6.3	0.01	
			44448557	A	G	5.2	0.01	
		rs2596807	44449766	T	C	5.2	0.01	
			44450018	A	G	20.8	0.10	
IL-28A	Intron 3	rs62120533	44451758	T	G	6.3	0.00	
IL-28A	Intron 3		44451814	T	C	6.3	0.00	
IL-28A	Exon 5	rs59746524	44452275	T	C	12.5	0.32	

*Correlation coefficient (r^{2}) with the landmark SNP (rs8099917).
†Statistical comparisons were performed using the Cochran–Armitage trend test.

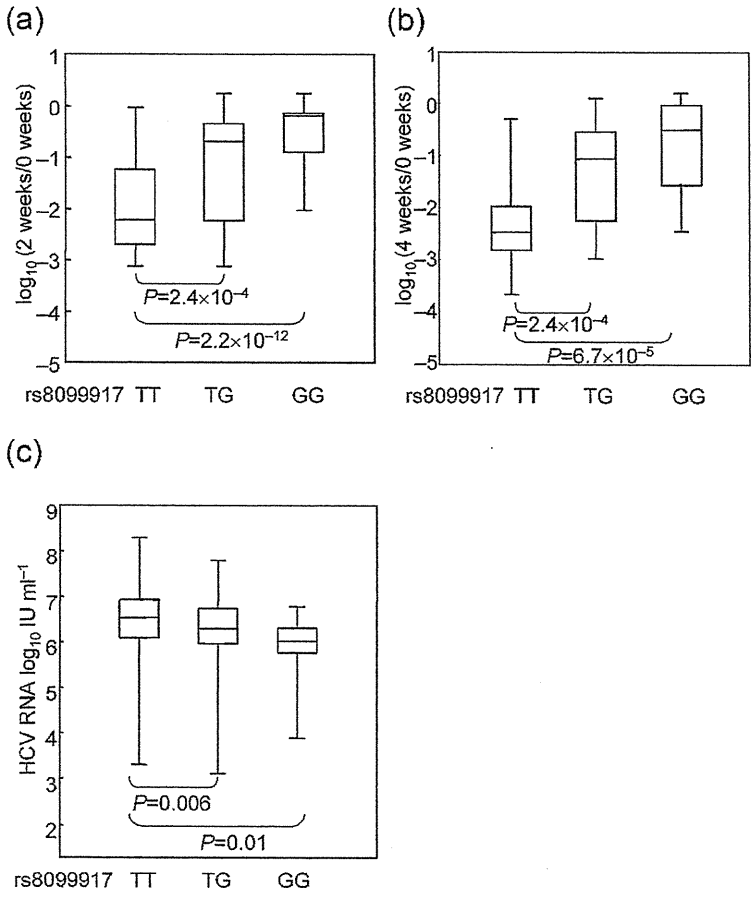

Fig. 2. Association between early response to combination therapy and the top SNP rs8099917 in the IL-28B locus. Viral reduction and basal viral load are shown according to rs8099917 genotype. (a) Viral response at 2 weeks; (b) viral response at 4 weeks; (c) baseline value of viral load. The box indicates the inter-quartile range (25 and 75 %) and the line within the box represents the median. The whiskers represent the range.

Table 4. Predictive factors for treatment outcome in patients with HCV genotype 1b determined by multivariate logistic regression analysis

Variable	*P*	OR	95 %CI
rs8099917	0.0022	0.24*	0.094–0.60
log viral load	0.0100	0.33†	0.140–0.77
Age	0.0180	0.67‡	0.480–0.93
Fibrosis	0.0200	0.40§	0.190–0.86

*Dominant model (GG+GT vs TT).
†Per 1 log increase.
‡Per 10 years increase.
§Severe versus mild.

common in Asian populations than in other ethnic groups (Ge *et al.*, 2009; Thomas *et al.*, 2009). We genotyped rs8099917 and rs12979860 in 282 healthy Japanese volunteers, and the allele frequencies of these SNPs were found to be comparatively high (91 % for both), consistent with the relatively high SVR rates of East Asian populations. Sarrazin *et al.* (2010) reported that genotype 1-infected patients had a lower frequency of the favourable genotype of rs12979860 compared with healthy controls and that genotypes 2/3 patients had an intermediate frequency. This finding was confirmed in this study (Table 2). On the other hand, they reported that the rs12979860 CC genotype, but not the rs8099917 and rs12980275 genotypes was significantly associated with SVR to (PEG-) IFN-α/ribavirin combination therapy in genotypes 2/3-infected patients. In this study, however, rs12979860 and rs8099917 were both strongly associated with SVR. This discrepancy may be due to differences among populations in the degree of linkage disequilibrium between these SNPs, and, in fact, these SNPs were in strong linkage disequilibrium in our healthy control subjects as well.

Of note in our study was that there was a significant difference in the initial viral reduction based on SNP genotype in patients treated with the PEG-IFN and ribavirin combination therapy. This finding holds important implications for the strategy of IFN-based anti-HCV therapy.

The mechanism by which polymorphisms within this locus influence the outcome of therapy has not been established. One of the polymorphisms in strong linkage disequilibrium with the two SNPs is a missense substitution within the IL-28B coding region. Recently, Urban *et al.* (2010) reported that there was no difference in antiviral potency between wild-type IL-28B and amino-acid substituted variant *in vitro* using an HCV replicon system. On the other hand, it has been reported that genetic variation in the IL-28B locus is associated with expression levels of IL-28B (plus IL-28A) IFN in peripheral blood mononuclear cells (Suppiah *et al.*, 2009; Tanaka *et al.*, 2009), although findings by Ge *et al.* (2009) are contradictory. Using a specific assay, Honda *et al.* (2010) and Urban *et al.* (2010) reported that hepatic IL-28B expression was not associated with IL-28B genotype in HCV-infected patients. Post-transcriptional regulation might be affected by the IL-28B variant, which could not be evaluated by the RT-PCR method, although further functional studies are needed to address this issue. IL-28A, IL-28B and IL-29 (IFN-λ II, III and I, respectively) are recently identified IFNs (Kotenko *et al.*, 2003; Sheppard *et al.*, 2003) similar to type I IFNs in terms of biological activity and mechanism of action, but differ structurally and genetically (Maher *et al.*, 2008). The antiviral effects of IFN-λ against hepatitis B virus and HCV have already been reported (Robek *et al.*, 2005). Furthermore, IFNs α and λ act synergistically against HCV (Marcello *et al.*, 2006; Pagliaccetti *et al.*, 2008; Zhu *et al.*, 2005). Whether such synergy actually modulates the effect of IFN therapy against HCV in the liver requires further study. In this regard, recent studies have identified higher pre-treatment hepatic levels of IFN-stimulated genes in NRs than in responders (Chen *et al.*, 2005; Feld *et al.*, 2007; Sarasin-Filipowicz *et al.*, 2008). In addition, association between intrahepatic levels of ISG expression and IL-28B genotype have also been reported (Honda *et al.*, 2010; Urban *et al.*, 2010). On the other hand, the IL-28B variant has recently been reported to be associated with treatment response following liver transplantation in patients infected with HCV (Charlton *et al.*, 2011; Fukuhara *et al.*, 2010). These findings suggest that the IL-28B polymorphism may be associated with innate as well as adaptive immunity.

The OR for the effect of the PEG-IFN and ribavirin combination therapy associated with IL-28B polymorphisms in this study is much lower than the previous report that analysed a similar effect in Japanese patients (Tanaka *et al.*, 2009) and is closer to those of Caucasian, Hispanic and African patients (Ge *et al.*, 2009; Suppiah *et al.*, 2009). Tanaka *et al.*'s non-viral responder (NVR) criteria seem to be very constrained compared with those of other groups including ours. To evaluate possible effects of such limited criteria on ORs for treatment outcome, we divided our NRs into NVRs and transient viral responders (TVRs) by the use of Tanaka and colleagues criteria based on 2 log viral decline at 12 weeks. Among the Set-1 and Set-2 samples, 344 samples were available for this analysis (NVR, 58; TVR, 122; SVR, 164). The ORs in the analyses for week 12 NVR versus SVR and for NVR versus VR (TVR plus SVR) are much greater than those based on our criteria and nearly comparable to those of Tanaka *et al.* (2009) (OR: 20.2, 17.2, respectively). Accordingly, the extreme clinical phenotype of NRs, strongly dictated by early viral response, might account for the strongly associated OR values. Our viral kinetics study also supports this interpretation.

In conclusion, we have shown that polymorphisms located near the IL-28B locus (represented by rs8099917), reported to be associated with the outcome of PEG-IFN and

ribavirin combined therapy, are also associated with that of IFN monotherapy and significantly affect early viral decline. Our resequencing and fine-mapping study identified 15 common genetic variants on the IL-28B locus that are associated with the outcome of IFN therapy for HCV infection. These findings would contribute to better targeting of PEG-IFN plus ribavirin therapy and increase overall treatment efficacy. Genotyping of IL-28B polymorphisms may be useful for predicting treatment outcome as well as estimating the optimal duration of PEG-IFN plus ribavirin combination therapy for viral eradication in HCV patients. Further study is needed to clarify the mechanism and molecular function of the IL-28B polymorphism to establish a more optimal, tailor-made treatment regimen for each patient.

METHODS

Study populations. A total of 2112 patients with chronic HCV infection were included in the study who were treated either with PEG-IFN and ribavirin therapy or with IFN monotherapy at Toranomon Hospital Department of Hepatology, Hiroshima University Hospital, Hiroshima University-Affiliated Hospitals, and the National Taiwan University Hospital. All patients had abnormal levels of serum alanine transaminase for more than 6 months and were positive for both anti-HCV antibody and serum HCV RNA. All patients were negative for hepatitis B surface antigen, had no evidence of other liver diseases, and had not received immunosuppressive therapy before enrollment in the study. Table 5 lists the demographic features of the subjects. Patients were classified into the following two groups based on treatment outcome: SRs and NRs. SRs had no evidence of viraemia at 24 weeks after completion of IFN therapy, whereas NRs were still viraemic at this stage. For IFN monotherapy, patients were treated with 6 million units of IFN intramuscularly every day for 8 weeks, followed by the same dose twice a week for 16 weeks, with a total dose of 528 million units. In the PEG-IFN plus ribavirin combination therapy, subjects received weekly injections of PEG-IFN at 1.5 μg kg^{-1} body weight and oral administration of ribavirin for 48 weeks (for 24 weeks for Taiwanese patients). The amount of ribavirin was adjusted based on the subject's body weight (600 mg for <60 kg, 800 mg for 60–80 kg and 1000 mg for >80 kg). Only patients with greater than 75 % compliance with prescribed doses of PEG-IFN and ribavirin were included in this study. For patients who underwent IFN-based therapies more than once, only parameters related to the most recent therapy were analysed. In addition, 282 healthy Japanese volunteers were genotyped for rs8099917 and rs12979860. All subjects in the present study received a detailed explanation, and all signed a written informed consent. This study was approved by the Ethical Committee of each participating medical centre and by the Ethical Committee at the SNP Research Center, the Institute of Physical and Chemical Research (RIKEN), Yokohama.

Genotyping. In the association study, we divided the Japanese patients who received PEG-IFN plus ribavirin into two independent groups based on the time of entry into the study (Set-1 and Set-2 in Table 5). For the GWAS stage, 594 Japanese patients with HCV genotype 1b treated with PEG-IFN plus ribavirin (315 SRs and 279 NRs) were initially genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip (Set-1 in Table 5). The other samples were genotyped using the Invader assay, the TaqMan assay, or by direct sequencing as described previously (Ohnishi *et al.*, 2001; Suzuki *et al.*, 2003). The other patients were organized into the following sets: Set-2, 185 genotype 1b Japanese patients treated with PEG-IFN plus ribavirin (67 SRs vs 118 NRs); Set-3, 750 genotype 1b Japanese patients treated with IFN monotherapy (217 SRs vs 533 NRs); Set-4, 513 genotype 2a Japanese patients treated with IFN monotherapy (342 SRs vs 171 NRs); Set-5, 44 genotype 1b Taiwanese patients treated with PEG-IFN plus ribavirin (25 SRs vs 19 NRs); and Set-6, 29 genotype 2a Taiwanese patients treated with PEG-IFN plus ribavirin (25 SRs vs 4 NRs) (Table 5).

Quality control criteria for the genome-wide study. For the genome-wide survey, we applied the following quality control criteria. Individual samples with genotype call rates less than 98 % and SNPs with call rates less than 99 % were removed, as were non-autosomal SNPs, minor alleles with a frequency less than 0.01, or SNPs deviating from the Hardy–Weinberg equilibrium ($P<1\times10^{-6}$). Related individuals were detected by identity-by-state analysis performed with the PLINK software. One subject from each of the cryptically related pairs of individuals (PI_HAT >0.4) was excluded. We assessed

Table 5. Clinical characteristics of cohorts

SR, Sustained responder; NR, non-responder. PEG+riba, pegylated IFN-α-2b plus ribavirin combination therapy; IFN, IFN-α monotherapy.

Group	Treatment outcome	No. samples	Male (%)	Mean age in years (±SD)	HCV genotype	Therapy	Ethnicity
Set-1	SR	304	197 (64.8)	54.4 (±13.2)	1b	PEG+riba	Japanese
	NR	279	139 (49.8)	59.9 (±10.7)			
Set-2	SR	67	33 (49.3)	57.1 (±13.3)	1b	PEG+riba	Japanese
	NR	118	49 (41.5)	62.7 (±8.5)			
Set-3	SR	217	147 (67.7)	57.2 (±13.8)	1b	IFN	Japanese
	NR	533	311 (58.3)	56.4 (±10.7)			
Set-4	SR	341	203 (59.5)	56.4 (±14.7)	2a	IFN	Japanese
	NR	169	86 (50.9)	54.9 (±12.3)			
Set-5	SR	25	12 (50.0)	50.5 (±9.8)	1b	PEG+riba	Taiwanese
	NR	19	8 (42.1)	51.7 (±7.0)			
Set-6	SR	25	14 (56.0)	49.4 (±12.4)	2a	PEG+riba	Taiwanese
	NR	4	4 (100.0)	48.2 (±13.0)			
Healthy control		282	151 (53.5)	44.1 (±15.0)			Japanese

population stratification using the smartpca program from the EIGENSOFT package (http://genepath.med.harvard.edu/~reich/Software.htm) using genotypes for about 70 000 SNPs informative for the Japanese population according to the method described previously (Yamaguchi-Kabata *et al.*, 2008). Analysis was performed using the Set-1 samples and all four of the HapMap population datasets (CEU/YRI/JPT/CHB). Outliers from JPT/CHB clusters were excluded from the association analysis. Genotype-based associations were tested with the Cochran–Armitage trend test. The genomic control method was applied to evaluate whether the inflation of false-positive rates, indicated by inflation factor λ, was within an acceptable level.

Resequencing. Resequencing around the IL-28B locus was performed by direct sequencing of DNA from 48 unrelated Japanese HCV patients from the enrolled subjects. We used Phase II HapMap JPT genotype data and the HAPLOVIEW program to define an LD block.

HCV RNA level. The HCV RNA level was analysed at three time points: before IFN therapy, at the end of the second week and at the end of the fourth week, using an RT-PCR-based method (the original method, the high range method or the TaqMan RT-PCR test). The measurement ranges of these assays were 0.5–850 kilo (K) IU ml^{-1}, 5–5000 KIU ml^{-1} and 1.2–7.8 log IU, respectively. Set-1 and Set-2 patients with sufficient viral load data falling within the measurable range were included in the viral kinetics analysis.

Liver biopsy. Liver biopsy specimens were evaluated by a pathologist at each institution and were scored for the stage of liver fibrosis according to the classification of Desmet *et al.* (1994). The patients were divided into two categories: mild fibrosis (F0–1) and severe fibrosis (F2–4).

Statistical analysis. Genotype-based associations were tested using the allele model χ^2-test. SNP effects were also evaluated using the dominant, recessive and co-dominant models (using the Cochran–Armitage trend test). Combined analysis was performed following the Mantel–Haenszel method. Viral kinetics were assessed using the non-parametric Kruskal–Wallis test followed by the Steel–Dwass test. Multivariate logistic regression analysis with stepwise forward selection was performed with criteria of $P<0.05$ for inclusion and removal of variables using the StatFlex 5.0 software package (Artec Inc.).

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Efficacy and safety of telaprevir, a new protease inhibitor, for difficult-to-treat patients with genotype 1 chronic hepatitis C

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SUMMARY. The aims of this phase III study were to assess the efficacy and safety of telaprevir in combination with peginterferon alfa-2b (PEG-IFN) and ribavirin (RBV) for difficult-to-treat patients who had not achieved sustained virological response (SVR) to prior regimens in Japan. The subjects were 109 relapsers (median age of 57.0 years) and 32 nonresponders (median age of 57.5 years) with hepatitis C virus genotype 1. Patients received telaprevir (750 mg every 8 h) for 12 weeks and PEG-IFN/RBV for 24 weeks. The SVR rates for relapsers and nonresponders were 88.1% (96/109) and 34.4% (11/32), respectively. Specified dose modifications of RBV that differed from that for the standard of care were introduced to alleviate anaemia. RBV dose reductions were used for 139 of the 141 patients. The SVR rates for relapsers did not depend on RBV dose reduction for 20–100% of the planned dose (SVR rates 87.5–100%, $P < 0.05$). Skin disorders were observed in 82.3% (116/141). Most of the skin disorders were controllable by anti-histamine and/or steroid ointments. The ratios of discontinuation of telaprevir only or of all the study drugs because of adverse events were 21.3% (30/141) and 16.3% (23/141), respectively. A frequent adverse event leading to discontinuation was anaemia. Telaprevir in combination with PEG-IFN/RBV led to a high SVR rate for relapsers and may offer a potential new therapy for nonresponders even with a shorter treatment period.

Keywords: direct-acting antiviral, peginterferon, ribavirin, sustained virological response, treatment failure.

INTRODUCTION

Hepatitis C virus (HCV) affects approximately 170 million people worldwide [1]; patients with chronic hepatitis C (CHC) eventually develop cirrhosis and hepatocellular carcinoma (HCC) [2,3]. The standard of care (SOC) with peginterferon plus ribavirin (RBV) for 48 weeks is most effective for eradicating HCV genotype 1 [4], which is a dominant genotype for CHC [1]. However, the sustained virological response (SVR) rate of SOC for the treatment of naïve patients with genotype 1 is approximately <50% [5,6]. The retreatment regimen for patients who do not achieve SVR is limited to exposure to peginterferon plus RBV with modification of dose and treatment duration. Some studies have been conducted to estimate the effectiveness of peginterferon plus RBV for 48 weeks for nonresponders to prior interferon-based combination therapy, and the SVR rates in most studies did not exceed 20% [7–9]. A large randomized study of patients who had not responded to previous treatment with peginterferon alfa-2b (PEG-IFN) plus RBV gave SVR rates for peginterferon alfa-2a 180 μg/kg plus RBV for 72 weeks that were not as high as those for 48 weeks (14%, 9%) [10]. HCV patients who had failed to achieve SVR with the combination therapy displayed high risk rates of decompensated cirrhosis, HCC and liver-related mortality [11]. Therefore, it is very important to establish new regimens to increase the SVR rate and shorten the treatment period for patients who do not achieve SVR with prior treatments.

Telaprevir, classified as a direct-acting antiviral agent, is a reversible, selective, orally bioavailable inhibitor of the nonstructural NS3/4A HCV serine protease [12]. Two phase II studies (PROVE 1 and PROVE 2) on the treatment of naïve patients with genotype 1 were conducted to assess the

Abbreviations: CHC, chronic hepatitis C; ETR, end of treatment response; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; PEG-IFN, peginterferon alfa-2b; RBV, ribavirin; RVR, rapid viral response; SOC, standard of care; SVR, sustained virological response.

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

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

PATIENTS AND METHODS

Study patients

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

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

All patients provided written informed consent before participating in the study. These studies were approved by each site's institutional review board and conducted in accordance with good clinical practice and the Declaration of Helsinki.

Study design

All patients received PEG-IFN (PegIntron®; MSD, Tokyo, Japan) at a dose of 1.5 μg/kg per week subcutaneously, RBV (Rebetol®; MSD) at a dose of 600 mg per day (for body weight ≤60 kg), 800 mg per day (for body weight >60 to ≤80 kg) or 1000 mg per day (for body weight >80 kg) and telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) at a dose of 750 mg every 8 h after food. The patients were treated with telaprevir, PEG-IFN and RBV for 12 weeks, followed by PEG-IFN and RBV (PEG-IFN/RBV) for 12 weeks. All patients had a 24-week follow-up period after the last dosing of study drugs to assess SVR.

Dose modification of study drugs

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

Stopping rules

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

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

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

Efficacy assessments

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

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

Safety assessments

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

Statistical analysis

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

Table 1 Baseline characteristics of study patients

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

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

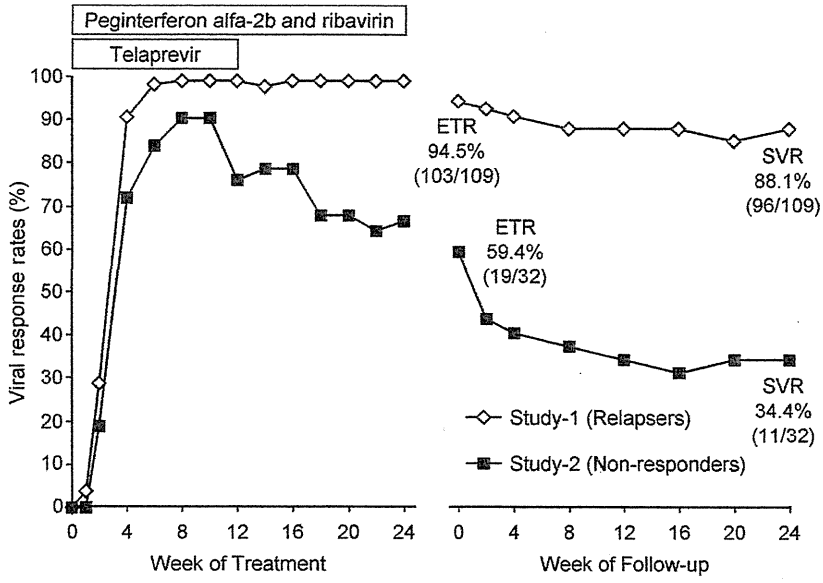

Fig. 1 Undetectable hepatitis C virus RNA rates at each measurement point. SVR, sustained virological response; ETR, end-of-treatment response.

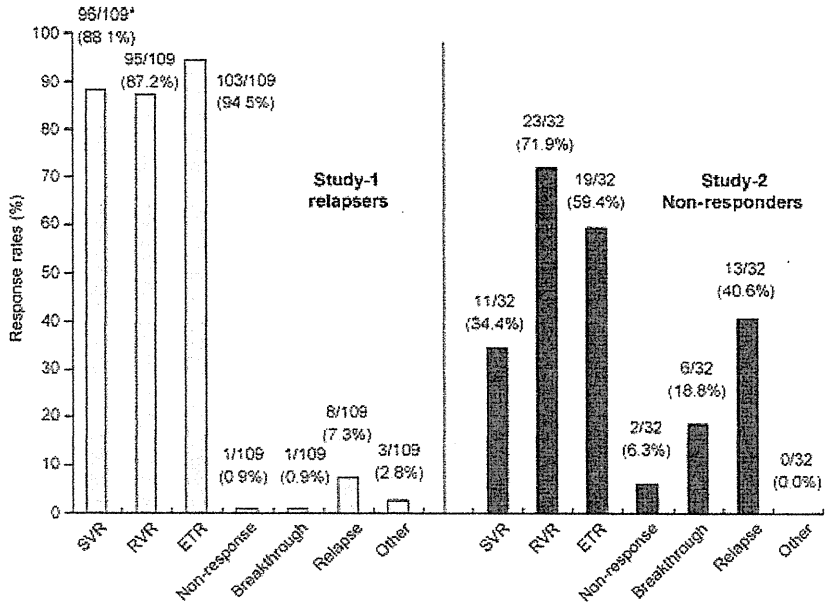

Fig. 2 Response rates of patients with virological response. *Number of patients who achieved SVR in each subgroup/ *N* (%). SVR, sustained virological response; RVR, rapid viral response; ETR, end-of-treatment response.

RESULTS

Study patients

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

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

Efficacy in study 1 (relapsers)

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

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

Efficacy in study 2 (nonresponders)

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

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

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

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

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

Safety

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

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

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

DISCUSSION

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

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

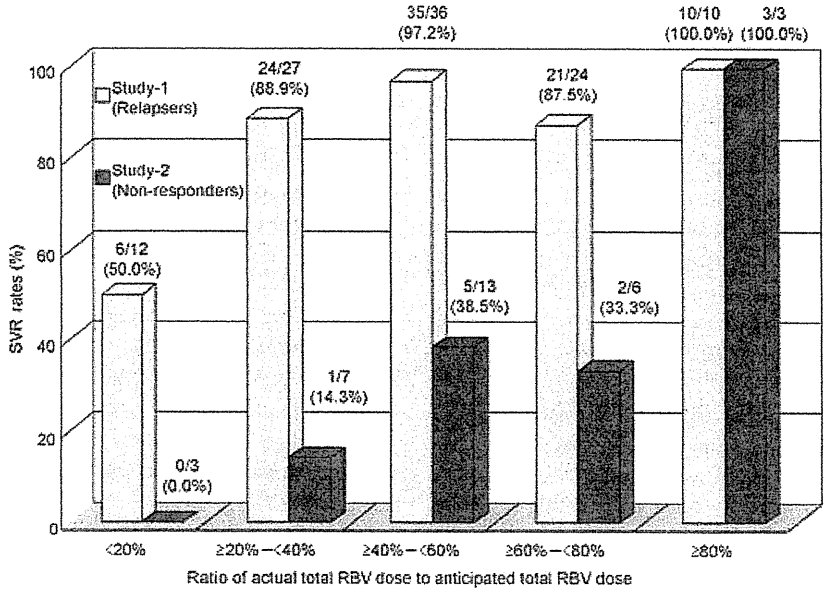

Fig. 3 Sustained virological response rates according to adherence to the ribavirin dose.

Table 3 Most common adverse events

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

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

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

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

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

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

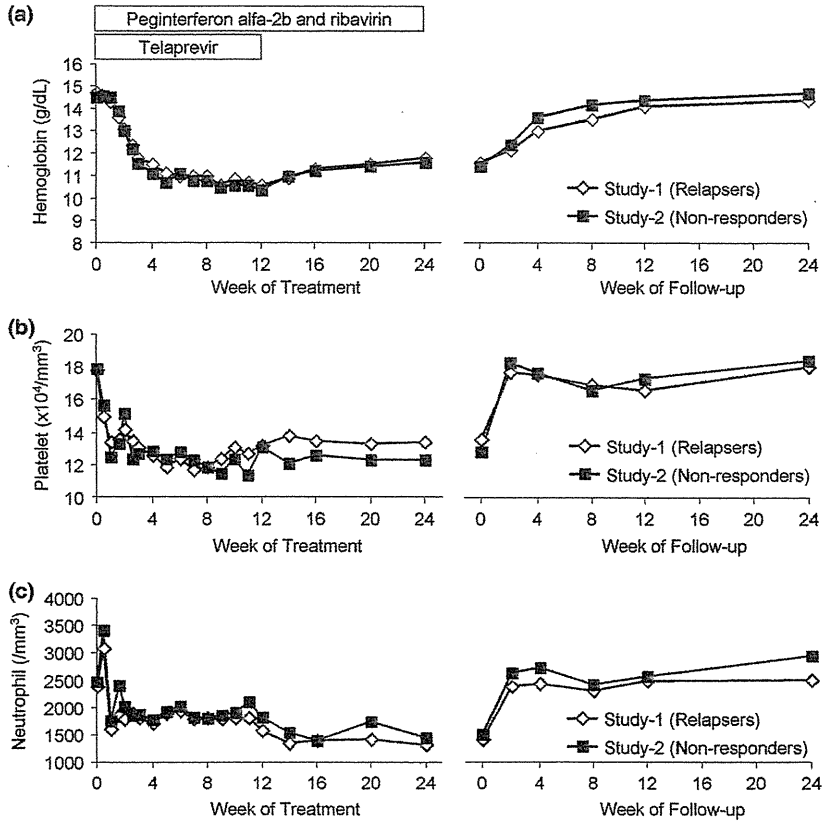

Fig. 4 Changes in hematology parameters. Median haemoglobin levels (a), median platelet counts (b) and median neutrophil counts (c) were plotted during treatment and follow-up periods.

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

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

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

DISCLOSURES

None to declare.

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APPENDIX

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

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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)