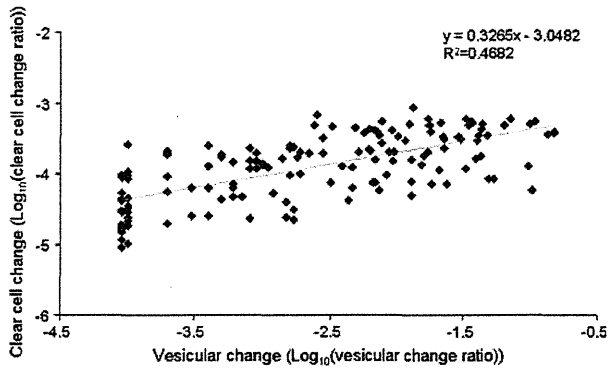

Fig. 4 Association between vesicular and clear cell change. The vesicular and clear cell change ratios were performed with logarithmic transformation, and the distributions were compared. The association between the distribution of vesicular and clear cell change are shown by a scatter diagram

accounts for more than 70% of chronic hepatitis C cases in Japan and is the best studied with respect to IL28B polymorphisms. Because ethanol intake of more than 20 g/day is associated with progressive liver damage [29, 30], patients whose daily alcohol intake exceeded this threshold were defined as alcohol positive. To evaluate fatty degeneration objectively, the distribution of brightness in liver tissue images was used. The brightness was calculated with Dynamic cell count BZ-H1C software and divided into 256 gradation sequences, and the fatty degeneration area was determined with the fixed brightness range. To evaluate the association between fatty degeneration and other factors, statistical analysis using continuous values may be more precise than using thresholds, assuming a large sample size with homoskedastic, normally distributed data. When the data were analyzed using continuous values in univariate

Table 2 Association between vesicular change and clinical background

	Vesicular change ratio		Univariate analysis	Multiple logistic regression analysis		
	<0.002 ($N = 76$)	≥0.002 ($N = 77$)	*P* value	*P* value	Adjusted odds ratio	95% CI
Age (years)[a]	60 (22–76)	61 (15–76)	0.774			
Gender (M:F)	44:32	39:38	0.368**			
BMI (kg m^{-2})[a]	22.0 (16.2–30.6)	23.5 (17.7–39.4)	0.009	0.033	1.147	1.011–1.301
Alcohol (+:−)	26:49	21:54	0.379**			
Platelet count ($\times 10^3$ μl^{-1})[a]	144 (23–759)	143 (49–327)	0.436			
Prothrombin activity (%)[a]	99 (56–124)	100 (12–166)	0.727			
Total bilirubin (mg dl^{-1})[a]	0.7 (0.4–14.7)	0.7 (0.4–2.1)	0.922			
Aspartate aminotransferase (IU l^{-1})[a]	42 (20–2617)	53 (14–250)	0.008	0.317		
Alanine aminotransferase (IU l^{-1})[a]	48 (11–2707)	61 (2–327)	0.093	0.318		
Lactate dehydrogenase (IU l^{-1})[a]	197 (129–899)	204 (43–473)	0.286			
Gamma glutamyl transpeptidase (IU l^{-1})[a]	34 (12–187)	55 (14–295)	<0.001	0.110		
Albumin (g dl^{-1})[a]	4.4 (2.9–5.3)	4.2 (3.1–5.1)	0.069			
Total cholesterol (mg dl^{-1})[a]	174 (100–312)	174 (122–263)	0.975			
Triglycerides (mg dl^{-1})[a]	97 (33–339)	113 (35–2466)	0.141			
Glucose (mg dl^{-1})[a]	98 (68–334)	103 (70–237)	0.533			
HCV RNA (log IU ml^{-1})[a]	6.4 (4.2–7.9)	6.2 (4.2–7.5)	0.031	0.068		
Core 70 (wild:mutant)	37:12	40:27	0.075**	0.230		
Core 91 (wild:mutant)	21:28	33:34	0.495**			
ISDR (0:≥1)	15:33	23:43	0.687**			
IL28B (TT:TG or GG)	65:11	51:26	0.005**	0.001	8.158[b]	2.412–27.589
Fibrosis (F0–1:F2–3)	36:40	23:54	0.026**	0.041	2.541[c]	1.040–6.207
Inflammatory activity (A0–1:A2–3)	24:51	17:60	0.168**			

Univariate analysis was performed with Mann–Whitney *U* test and **chi-square test

Multiple logistic regression analysis was performed using variables that were significant ($P < 0.05$) or marginally significant ($P < 0.10$) in univariate analysis

[a] Median (range)

[b] IL28B genotypes were coded as 0 or 1 depending on whether the subject carried the minor allele

[c] Fibrosis was coded as 0 for patients with mild fibrosis (F0–1) and 1 for patients with severe fibrosis (F2–3)

Table 3 Association between clear cell change and clinical background

	Clear cell change ratio		Univariate analysis	Multiple logistic regression analysis		
	<0.03 ($N = 77$)	≥0.03 ($N = 76$)	P value	P value	Adjusted odds ratio	95% CI
Age (years)[a]	60 (22–76)	61 (15–76)	0.408			
Gender (M:F)	47:29	36:41	0.061**	0.057		
BMI (kg m^{-2})[a]	23.0 (16.2–32.3)	22.7 (17.6–39.4)	0.477			
Alcohol (+:−)	27:47	20:56	0.179**			
Platelet count ($\times 10^3$ μl^{-1})[a]	152 (23–759)	131 (49–327)	0.175			
Prothrombin activity (%)[a]	99 (56–166)	100 (12–136)	0.829			
Total bilirubin (mg dl^{-1})[a]	0.7 (0.4–14.7)	0.7 (0.4–2.1)	0.815			
Aspartate aminotransferase (IU l^{-1})[a]	42 (14–2617)	56 (15–250)	<0.001	0.824		
Alanine aminotransferase (IU l^{-1})[a]	48 (11–2707)	65 (2–327)	0.018	0.809		
Lactate dehydrogenase (IU l^{-1})[a]	197 (123–899)	209 (43–473)	0.193			
Gamma glutamyl transpeptidase (IU l^{-1})[a]	37 (12–187)	47 (13–295)	0.049	0.928		
Albumin (g dl^{-1})[a]	4.3 (2.9–5.1)	4.2 (3.0–5.3)	0.376	0.200		
Total cholesterol (mg dl^{-1})[a]	174 (122–270)	173 (100–312)	0.161			
Triglycerides (mg dl^{-1})[a]	102 (33–2466)	97 (35–517)	0.861			
Glucose (mg dl^{-1})[a]	97 (68–334)	104 (70–284)	0.092	0.456		
HCV RNA (log IU ml^{-1})[a]	6.3 (4.2–7.9)	6.2 (4.2–7.5)	0.060	0.101		
Core 70 (wild:mutant)	35:14	42:25	0.325**			
Core 91 (wild:mutant)	20:29	34:33	0.290**			
ISDR (0:≥1)	14:34	24:42	0.421**			
IL28B (TT:TG or GG)	64:12	52:25	0.016**	0.011	3.000[b]	1.282–7.019
Fibrosis (F0–1:F2–3)	33:43	26:51	0.220**			
Inflammatory activity (A0–1:A2–3)	25:50	16:61	0.081**	0.066		

Univariate analysis was performed with Mann–Whitney U test and **chi-square test

Multiple logistic regression analysis was performed using variables that were significant ($P < 0.05$) or marginally significant ($P < 0.10$) in univariate analysis

[a] Median (range)

[b] IL28B genotypes were coded as 0 or 1 depending on whether the subject carried the minor allele

Table 4 Association between fatty degeneration and IL28B genotype

	IL28B		Univariate analysis
	TT	TG or GG	P value
Obesity group (BMI ≥25 kg m^{-2}, $N = 36$)			
Vesicular change ratio (<0.002:≥0.002)	12:19	2:3	1.000*
Clear cell change ratio (<0.033:≥0.033)	20:11	2:3	0.357*
Non-obesity group (BMI <25 kg m^{-2}, $N = 114$)			
Vesicular change ratio (<0.002:≥0.002)	52:30	9:23	0.001
Clear cell change ratio (<0.033:≥0.033)	48:34	10:22	0.009

Univariate analysis was performed with chi-square test and *Fisher's exact test

analysis using the Mann–Whitney U test, the IL28B genotype was found to be significantly associated with clear cell change and vesicular change ($P = 0.001$, $P < 0.001$, respectively). When continuous values were used in multiple regression, the association between IL28B genotype and fatty degeneration was also observed. *IL28B* genotype, HCV RNA titer and liver fibrosis stage were identified as independent factors for vesicular change ($P = 0.003$, $P = 0.022$, $P = 0.047$, respectively), and the *IL28B* genotype was identified as an independent factor for clear cell change ($P < 0.001$). Genotypes of rs738409 within the Patatin-like phospholipase domain-containing 3 (PNPLA3) locus were also recently reported to be associated with hepatic steatosis in chronic hepatitis C patients

(odds ratio 1.90–2.55) [38–40]. It would be interesting to analyze the effect of the PNPLA3 genotype on fatty degeneration in the present study; however, given the small number of study subjects and the small odds ratios reported in these studies, this study lacks the statistical power to verify the association between hepatic steatosis and PNPLA3.

Chronic HCV infection is known to be associated with fatty change of the liver, and the incidence of fatty change in chronic hepatitis C patients is higher than in those with other chronic liver dysfunctions [36, 41]. The mechanisms of fatty change in chronic hepatitis C patients are still unclear, but the induction of liver steatosis was observed in the presence of the HCV core protein in vitro and in vivo. In a transgenic mouse study, all the male and approximately 50% of the female mice developed liver steatosis by the age of 6 months [42]. Furthermore, the HCV core transfected cells were shown to activate the deposition of lipid [36, 37, 41, 43]. Thus it was hypothesized that chronic HCV infection might be associated with fatty change and the effect of clinical background. As shown in Tables 2 and 3, hepatic histological changes, including both vesicular and clear cell change, were significantly associated with BMI, aspartate aminotransferase, γ-GTP and HCV RNA levels in univariate analysis, whereas clear cell change was not associated with BMI. As shown in Fig. 2, clear cell change was strongly related to HCV infection, whereas it was rarely observed in the liver tissues of patients with non-alcoholic fatty liver disease. These findings suggest that vesicular change is associated with obesity or other lipid depositions, and that clear cell change is associated with chronic HCV infection.

Recently, several groups have reported significant associations between several linked SNPs in the *IL28B* locus and HCV eradication with IFN therapy based on genome-wide association analysis. The sustained virological response rate in chronic hepatitis C patients homozygous for the major allele (genotypes rs8099917 TT or rs12980275 CC or rs12979860 CC) was significantly higher than in patients heterozygous or homozygous for the minor allele [24–27]. Serum γ-GTP levels and liver histological fibrosis and inflammation levels in chronic hepatitis C patients with the favorable TT or CC SNP genotypes were also significantly lower than in those with the minor genotypes [28]. Although other host factors, such as liver steatosis or insulin resistance, have been demonstrated to be associated with virological response [23, 44], this study demonstrates that SNPs in the *IL28B* locus might affect steatosis in chronic hepatitis C patients. As the influence of the *IL28B* genotype was observed with both clear cell change and vesicular change (Tables 2, 3), and these associations became more remarkable in the non-obese group (Table 4), it is tempting to speculate that differences in IL28B expression might cause an aberration of lipid metabolism in chronic hepatitis C patients. An association between IL28B genotype at rs12979860 and hepatic steatosis in chronic hepatitis C patients was demonstrated in a previous report [45]. The results of this study were very similar given the strong association between the rs8099917 and rs12979860 genotypes [35], but the methods used to evaluate hepatic steatosis were quite different. In the previous report, hepatic steatosis was subjectively evaluated by pathologists using the Brunt classification, whereas in the present study, hepatic steatosis was evaluated objectively using a quantitative method, and two different classes of steatotic change, clear cell change and vesicular change were analyzed separately.

In conclusion, the relationship between clinical background and fatty change in liver tissue was analyzed using an operator-independent method, and significant associations between fatty change level and *IL28B* genotypes or HCV RNA level were identified. These findings suggest that these factors are connected to an aberration of lipid metabolism in chronic hepatitis C patients.

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Conflict of interest None.

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MAJOR ARTICLE

Toward the Establishment of a Prediction System for the Personalized Treatment of Chronic Hepatitis C

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Background. Although several direct-acting antivirals (DAAs) are now available, the therapy regimen for chronic hepatitis C will continue to include pegylated interferon and ribavirin for the foreseeable future. Despite their improved rate of sustained virological response (SVR), DAAs pose increased risks of side effects and selection for antiviral resistance. Not all patients require DAA to achieve SVR, whereas others are unlikely to respond even to triple therapy. Therefore, a personalized approach to candidate selection is necessary.

Methods. In this retrospective study, data from 640 Japanese patients who were treated for chronic hepatitis C genotype 1, 2, or 3 with pegylated interferon plus ribavirin combination therapy was compiled to identify robust pretreatment predictive factors for SVR.

Results. A logistic regression model for personalized therapy was developed based on age, viral genotype, initial viral load, aspartate aminotransferase/alanine aminotransferase ratio, α-fetoprotein levels, and *IL28B* single-nucleotide polymorphism genotype. The area under the receiver-operating characteristic curve (AUC) was 0.85. The mean AUC following 10 rounds of 10-fold cross validation was 0.82, with a true positive rate of 78.2%.

Conclusions. A personalized approach to therapy may better inform treatment decisions and reduce incidence of side effects and antiviral resistance.

The hepatitis C virus (HCV) affects >100 million people worldwide and is a major global cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma [1–5]. The current standard of care, pegylated interferon (PEG-IFN) plus ribavirin combination therapy, is both expensive and poorly tolerated, and treatment efficacy is <50% for genotype 1b [6].Telaprevir and boceprevir, 2 direct-acting antiviral (DAA) protease inhibitors, have recently been approved for clinical use in the United States [7] and are expected to improve the rate of sustained virological response (SVR) to 65%–75% [8]. However, the addition of a DAA to the current standard of care increases the risk of side effects, including anemia and rash, and failure to achieve SVR may pose an increased risk of accumulating protease inhibitor–resistant viral strains that may be recalcitrant to future treatment [8]. Consequently, it may be advantageous to identify patients who are unlikely to respond to therapy, as well patients who are likely to achieve SVR under the current standard of care without requiring a DAA. Patients who are able to achieve at least a transient response (relapsers) under combination therapy are more likely to achieve a SVR under triple therapy, whereas patients who fail to respond to combination therapy are also less likely to respond to triple therapy [9]. Therefore, it may be possible for patients who are highly likely to respond to combination therapy to be spared the additional risks

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and costs of triple therapy, but to determine the optimal treatment for each patient, reliable and inexpensive pretreatment predictors are needed for SVR.

A number of pretreatment predictors associated with SVR or nonresponse have been reported. Older female patients have been shown to respond poorly to therapy in Japan [10–12], and metabolic factors such as obesity [13], insulin resistance [13], hepatic steatosis [14], low-density lipoprotein (LDL) cholesterol levels [15, 16], and γ-glutamyl transpeptidase (γ-GTP) levels [17] have been shown to influence treatment outcome. Baseline virus titer is also an important predictor of treatment outcome [14, 18]. HCV genotypes differ in the response to interferon therapy, with genotypes 1 and 4 considered more difficult to treat than genotypes 2 and 3 [18, 19]. The importance of these and other factors in triple therapy remains unclear, although they may influence the effectiveness of interferon and ribavirin in suppressing emergence of resistant strains.

Genetic differences among patients also influence response to treatment and incidence of side effects. Genomewide association studies have reported common single-nucleotide polymorphisms (SNPs) predictive of response to interferon therapy. A set of linked SNPs within the *IL28B* locus on chromosome 19 has recently been shown to be the strongest predictor of sustained virological response as well as spontaneous viral clearance [20–26]. So far, the SNP also appears to be the strongest predictor for triple therapy [9, 27]. Other SNPs are associated with the occurrence of side effects. In particular, SNPs in the *ITPA* locus have been found to be associated with anemia in patients treated with PEG-IFN plus ribavirin combination therapy [28–30] and appear to be predictive of anemia in triple therapy as well [31]. Although there are currently few options for treating HCV, SNP genotyping may nonetheless help gauge expectations and help identify patients at risk for severe side effects that may disrupt the course of therapy.

Even though telaprevir and boceprevir are now available for use in clinical practice, DAAs must be coadministered with PEG-IFN and ribavirin, to prevent rapid selection for resistance mutations [32]. As a result, patients who respond poorly to PEG-IFN and ribavirin may not only fail to achieve SVR under triple therapy but may be more likely to encounter viral breakthrough, with confounding effects on future treatment efforts. Consequently, there remains a need to identify robust predictors for response to PEG-IFN and ribavirin to establish a personalized approach for treatment of chronic hepatitis C.

METHODS

Patients

Data from 640 patients who were treated with PEG-IFN plus ribavirin combination therapy for chronic HCV infection were compiled from hospitals belonging to the Hiroshima Liver Study Group (http://home.hiroshima-u.ac.jp/naika1/e/) in Hiroshima, Japan. All patients were interferon treatment–naive and were infected with HCV genotype 1, 2, or 3. Study participants tested positive for HCV RNA over a span of >6 months, tested negative for hepatitis B and human immunodeficiency virus (HIV), and showed no evidence for other liver diseases. All patients gave written informed consent to participate in the study in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and according to the process approved by the ethical committees of Hiroshima University and the SNP Research Center at the Institute of Physical and Chemical Research in Yokohama. Patient profiles are shown in Table 1 and Figure 1.

PEG-IFN Plus Ribavirin Combination Therapy

Patients received weekly injections of PEG-IFN-α-2b at 1.5 μg/kg body weight for 48 weeks. Ribavirin was administered orally, and the dosage was determined based on the patient's body weight (600 mg for <60 kg, 800 mg for 60–80 kg, 1000 mg for >80 kg), based on guidelines by the Ministry of Health, Labor, and Welfare of Japan [33]. The ribavirin dose was reduced when hemoglobin levels fell below 10 g/dL, and both PEG-IFN and ribavirin were discontinued when hemoglobin levels dropped to <8.5 g/dL.

Outcome of Therapy

We evaluated treatment success based on SVR, defined as undetectable HCV RNA levels 24 weeks after cessation of treatment. Some patients showed a transient response (relapsers), in which HCV RNA dropped to undetectable levels during treatment but then rebounded during the follow-up period. In nonresponders, HCV-RNA levels failed to decline by 2 $\log_{10}$ IU/mL by week 12 of treatment and never dropped below detectable levels. Histopathological diagnosis was made as described previously [34].

HCV RNA Levels

We monitored HCV RNA levels throughout the course of therapy using polymerase chain reaction (PCR)–based methods (the original Amplicor method, the high-range method, or the TaqMan real-time PCR test). The measurement ranges of these assays were 0.5–850 KIU/mL, 5–5000 KIU/mL, and 1.2–7.8 $\log_{10}$ IU, respectively. Samples exceeding the measurement range were diluted with phosphate-buffered saline and reanalyzed. All values were reported as $\log_{10}$ international units per milliliter.

SNP Genotyping

We genotyped each patient for 2 SNPs: rs8099917 in the *IL28B* locus, which is associated with therapy outcome, and rs1127354 in the *ITPA* locus, which is associated with ribavirin-induced anemia. Although there are 2 SNPs associated with *ITPA* enzyme activity in white patients [28], 1 of these SNPs appears to

Table 1. Patient Profiles[a]

	Total (N = 640)	SVR (n = 388)	TR (n = 119)	NR (n = 119)
Age, years	59 (10–82)	56 (10–77)	63 (24–78)	63 (27–82)
Sex, M/F	327/313	221/167	47/72	52/67
BMI	22.09 (14–32)	21.95 (15–32)	22.22 (14–32)	22.62 (16–31)
Viral genotype, 1b/others	441/186	223/153	98/21	108/11
Virus titer, $\log_{10}$ IU/mL	6.1 (3.6–7.5)	6 (3.6–7.3)	6.25 (4–7.4)	6.4 (5.1–7.3)
Fibrosis, 0/1/2/3/4	7/213/156/74/17	4/140/99/41/4	1/44/28/15/3	2/26/26/17/9
Activity, 0/1/2/3/4	108/147/207/32/0	64/89/130/24/0	18/31/37/3/0	24/25/35/5/0
rs8099917, TT/GT, GG	476/161	330/57	90/29	46/72
rs1127354, CC/CA, AA	468/171	273/114	88/31	100/19
γ-GTP level, IU/L	40 (8–535)	38.5 (8–535)	34 (8–341)	52 (12–213)
Hemoglobin level, g/dL	14 (7.8–18)	14.15 (7.8–18)	13.6 (9.7–18)	13.8 (9.2–16)
ALT level, IU/L	50 (10–512)	53.5 (11–512)	48 (11–408)	46 (10–224)
AST level, IU/L	43 (0–312)	41 (0–312)	45 (16–197)	47 (0–142)
α-Fetoprotein level, μg/L	5 (0.8–262)	5 (0.8–262)	6.8 (1.9–152)	7.5 (1–244)
Ferritin level, μg/L	121.2 (0.56–1057)	127 (3–1057)	110.1 (0.56–1023)	135 (7.7–769)
LDL cholesterol level, mg/dL	173 (93–271)	171 (99–264)	178.5 (102–271)	171 (93–260)
Triglyceride level, mg/dL	93 (20–541)	90.5 (35–541)	99 (44–303)	104.5 (20–305)
HDL cholesterol level, mg/dL	57.5 (21–167)	57 (27–101)	57 (27–114)	63 (21–106)
Iron count, μ/dL	128.5 (11–339)	123 (11–339)	138 (61–305)	156.5 (31–286)
Fasting blood sugar level, mg/dL	98 (15–248)	96 (15–248)	99 (76–243)	101 (80–211)
White blood cell count, cells/mm^3	4800 (4.4–10 760)	4970 (4.4–10 760)	4600 (5.4–8780)	4460 (2160–8400)
Platelet count × 10^4 cells/mm^3	16.4 (4.8–246)	17.5 (5–229)	15.3 (5.6–246)	15.05 (5–116)
IFN reduction, Y/N	93/547	50/338	20/99	21/98
Ribavirin reduction, Y/N	208/432	114/274	50/69	43/76
Anemia reported, Y/N	158/482	83/305	44/75	30/89
Stopped, Y/N	15/610	0/382	0/115	1/113

Abbreviations: ALT, aspartate aminotransferase; AST, alanine aminotransferase; BMI, body mass index; γ-GTP, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; IFN, interferon; NR, nonresponse; SVR, sustained virological response; TR, transient response (relapse).

[a] All patients were interferon treatment–naive and were treated with pegylated interferon plus ribavirin combination therapy. Counts are listed for categorical values and the median and range are reported for continuous variables.

be fixed in the Japanese population, and so only rs1127354 was genotyped [29]. Samples were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip or the Invader or TaqMan assay, as described previously [35].

Statistical Analysis

All analysis was performed using the R statistical package (http://www.r-project.org). Nonparametric tests (χ^2 and Mann–Whitney *U* tests) were used to detect significant associations. All statistical analyses were 2 sided, and $P < .05$ was considered significant. Multiple logistic regression analysis with forward/backward stepwise selection of variables was used to identify independent factors associated with SVR. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for each factor. Receiver-operating characteristic (ROC) curves and areas under the curve (AUC) were calculated for each model using the ROCR software package. CIs for predicted SVR probabilities were calculated over a range of ages and viral loads, and results were stratified by *IL28B* SNP genotype and viral genotype using the rms software package. Models were validated based on 10 rounds of 10-fold cross-validation using the WEKA data-mining package [36].

RESULTS

Patient Characteristics

Patient profiles are shown in Table 1. In total, 388 (61%) patients achieved SVR, 119 (19%) were transient responders, and 119 (19%) were nonresponders. The frequency of the deleterious allele for the *IL28B* SNP rs8099917 (G) was 0.14. A total of 476 patients had the favorable TT genotype, and 148 and 13 patients had the unfavorable GT and GG genotypes, respectively. Genotype data for rs12979860, another commonly reported *IL28B* SNP, were not available for all patients, but the 2 SNPs are in high linkage disequilibrium and genotypes are highly correlated (0.99). The frequency of the favorable allele for the *ITPA* SNP rs1127354 (A) was 0.15. A total of 468 patients had the anemia-susceptible CC genotype, and 152 and 19 patients had the protective AC and AA genotypes, respectively.

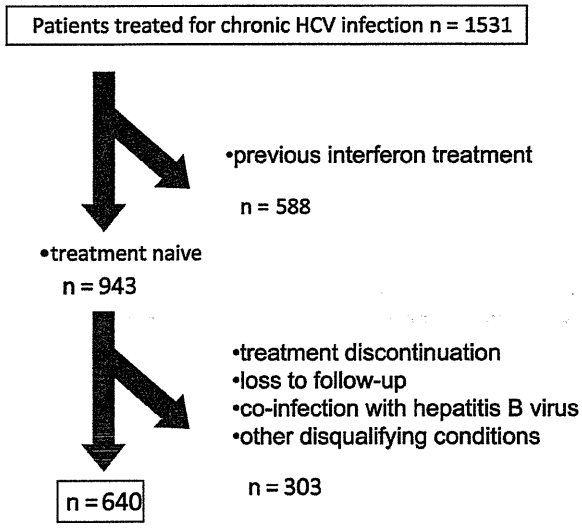

Figure 1. Patient-selection flowchart. Beginning with a database of 1531 patients treated for chronic hepatitis C virus (HCV) infection with pegylated interferon and ribavirin combination therapy, 588 patients were excluded due to prior history of interferon treatment and another 303 patients were excluded because of treatment discontinuation, loss to follow-up, or other disqualifications as described in the Methods, leaving 640 patients who were included in the analysis.

Predictive Factors for SVR

The following predictors were significantly associated with SVR using univariate analysis after Bonferroni correction for 26 tests: male sex, age, genotype 1b, initial viral load, aspartate aminotransferase/alanine aminotransferase (AST/ALT) ratio, rs8099917 TT genotype, diabetes mellitus, α-fetoprotein level, white blood cell count, platelet count, and hemoglobin level (Table 2). When all factors were included in multivariate analysis, age, genotype 1b, initial viral load, AST/ALT ratio, rs8099917 TT genotype, and α-fetoprotein were identified as independent predictors for SVR (Table 3) with an AUC of 0.85 (Figure 2). The mean AUC based on 10 rounds of 10-fold cross-validation was 0.82 with a true-positive rate of 78.2%, a false-positive rate of 21.8%, and a κ statistic of 0.53. Figure 2 shows predicted probabilities of achieving SVR for patients with genotype 1b stratified by the rs8099917 genotype and the initial viral load. The probability of SVR can be calculated using the following prediction formula:

log odds (SVR) = 11.7007–0.0479*age – 1.5417*genotype1b – 1.2804*log(viral load) – 0.7638*log(AST/ALT) + 1.6610* rs8099917TT – 0.3449*log(α-fetoprotein)

DISCUSSION

In this study, we present a simple predictive model for outcome of PEG-IFN plus ribavirin combination therapy for patients infected with HCV. Although the *IL28B* SNP is the best single predictor of SVR, not all patients with the favorable genotype achieved SVR, whereas some patients with an unfavorable genotype were able to achieve SVR. Inclusion of other viral and host factors is therefore expected to improve the accuracy of treatment-outcome predictions. Although a number of predictors have been reported, this study achieves relatively high accuracy using only a simple subset of pretreatment predictors, the most important of which are *IL28B* SNP genotype, age, viral genotype, and initial viral load.

A prediction equation based on the coefficients in Table 3 was used to generate the predicted response over a range of ages and viral loads (Figure 3). For example, a 60-year-old patient with the favorable *IL28B* SNP genotype and HCV genotype 1b has a probability of SVR of 0.61, whereas the probability is only 0.23 for a patient with an unfavorable *IL28B* genotype. On the other hand, the probability increases to 0.80 for a 40-year-old patient or 0.88 for a patient with genotype 1a, 2, or 3. Based on this model, it appears that older patients who have high viral load for genotype

Table 2. Univariate Predictors for Sustained Virological Response

Variable	OR	(95% CI)	*P* Value	
Sex	1.93	(1.39–2.7)	.000101	***
Age	0.948	(.933–0.963)	1.66×10^{-12}	***
BMI	0.961	(.91–1.01)	.1035	
Genotype 1b vs others	0.202	(.127–.311)	1.09×10^{-13}	***
Virus titer, log IU/mL	0.39	(.285–.524)	7.12×10^{-9}	***
ALT	1.28	(1.01–1.63)	.07166	
AST/ALT ratio	0.311	(.177–.528)	5.97×10^{-6}	***
rs8099917 TT genotype	4.24	(2.9–6.26)	2.75×10^{-6}	***
rs1127354 CC genotype	0.64	(.432–.939)	.0237	(*)
γ-GTP	0.999	(.996–1)	.04023	(*)
Fibrosis	0.744	(.595–.929)	.02826	(*)
Activity	1.12	(.909–1.39)	.2868	
α-Fetoprotein	0.69	(.559–.844)	1.30×10^{-5}	***
LDL cholesterol	0.997	(.992–1)	.3184	
Triglycerides	0.998	(.995–1)	.04564	(*)
HDL cholesterol	0.993	(.976–1.01)	.558	
Iron	0.995	(.99–1)	.01145	(*)
Fasting blood sugar	0.989	(.981–.997)	.01578	(*)
White blood cells	1	(1–1)	.000859	***
Platelets	2.32	(1.46–3.82)	.000342	***
Hemoglobin	1.25	(1.11–1.41)	.000224	***
Core aa 70 substitution	1.35	(.517–3.69)	.548	
Core aa 91 substitution	1.24	(.491–3.19)	.6604	
ISDR substitutions (0–1 vs >1)	3.87	(1.4–12)	.01034	(*)
Hypertension	0.497	(.278–.878)	.01599	(*)
Diabetes mellitus	0.206	(.0841–.454)	6.90×10^{-5}	***

Abbreviations: ALT, aspartate aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; γ-GTP, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; OR, odds ratio.

***$P < .001$; **$P < .01$; *$P < .05$; (*): $P < .05$ but not significant following Bonferroni correction for multiple testing (α: 0.05/26 = .0019)

Table 3. Multivariate Predictors for Sustained Virological Response

Variable	Coeff	OR	(95% CI)	*P* Value	
Age	−0.0479	0.953	(.93–.976)	9.71×10^{-5}	***
Genotype 1b vs others	−1.542	0.214	(.107–.405)	4.97×10^{-6}	***
Log viral load	−1.28	0.278	(.166–.445)	3.57×10^{-7}	***
AST/ALT ratio	−0.7638	0.466	(.226–.931)	.03396	*
rs8099917 TT genotype	1.661	5.26	(2.98–9.57)	2.16×10^{-8}	***
α-Fetoprotein	−0.3449	0.708	(.549–.91)	.007249	**

Abbreviations: ALT/AST, aspartate aminotransferase/alanine aminotransferase ratio; CI, confidence interval; Coeff, coefficient; OR, odds ratio.

***$P < .001$; **$P < .01$; *$P < .05$; (*): $P < .05$.

1b and lack the favorable *IL28B* genotype are less likely to benefit from combination therapy and possibly triple therapy, whereas young patients with the favorable *IL28B* genotype and low viral load have a high probability of achieving SVR with combination therapy alone and may not benefit from the addition of a DAA. The model presented here includes data from 640 patients and achieves an AUC score greater than 0.82 following 10-fold cross-validation using some pretreatment predictors. However, in future studies, this model should also be validated against external data sets based on patients from different populations and ethnic groups.

Several predictive models for outcome of combination therapy for HCV have been reported and have used a variety of different approaches. Several studies have used artificial neural networks [37–39] and support vector machines [40] to predict SVR, although these types of models are more difficult to interpret than regression-based methods and are less amenable to adoption in clinical use. Other studies have used decision trees [41] and classification and regression tree analysis [42], both of which provide an intuitive, flowchart-based approach to prediction. However, small changes can dramatically alter the topology of the tree, and individual paths through the tree make use of only a fraction of the available data. Medrano et al proposed a logistic-regression SVR prediction model for patients in Spain coinfected with HCV and HIV [43]. The model achieved high accuracy (AUC, 0.85–0.89) using 4 predictors: *IL28B* SNP genotype, liver stiffness, viral genotype, and viral load. For HCV-monoinfected patients, O'Brien et al [44] proposed a prediction model for SVR in European–American patients with genotype 1 based on *IL28B* SNP genotype, viral load, AST/ALT ratio, fibrosis score, and prior ribavirin treatment. The model proposed here is similar to the model proposed by O'Brien et al, differing mainly in patient ethnicity (Japanese vs European–American) and treatment history (prior ribavirin treatment vs treatment-naive), although patients in the model of O'Brien et al had more severe fibrosis (Ishak fibrosis score ≥3 vs 0–4), and were younger (median age, 49 vs 59 years) and more likely to be male (73% vs 51%). Both models had similar factors and AUC scores (0.79 vs 0.82), and the inclusion of various host and viral factors in both models underscores the variability in response to therapy and the limitations of *IL28B* SNP genotype alone in predicting the outcome of therapy. Presumably, future studies will introduce models geared specifically for response to triple therapy, but until additional data become available, predictions based on response to combination therapy may help guide patient selection.

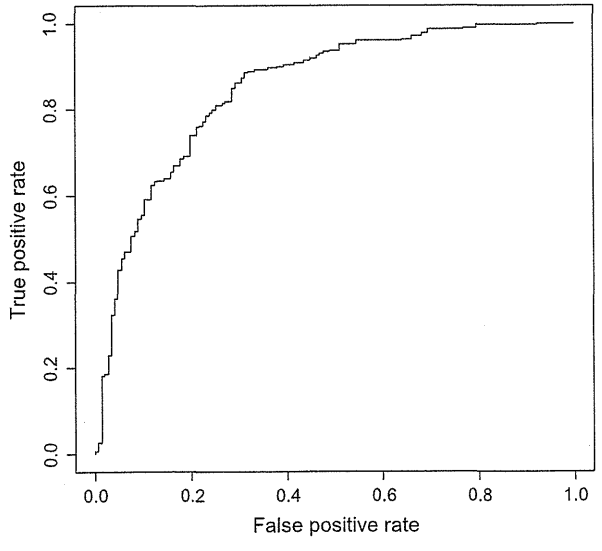

Figure 2. Receiver-operating characteristic curves for prediction of sustained virological response. The area under the curve was 0.85 for the full data set, and the mean of 10 rounds of 10-fold cross-validation was 0.82.

CONCLUSIONS

Pretreatment predictors based on clinical and viral factors may be used to predict the outcome of therapy. Regardless of the approach or the specific predictors analyzed, most prediction studies report a consistent set of important predictive factors, including viral genotype, *IL28B* SNP genotype, age, viral load, and ≥1 clinical factors reflecting liver function (eg, γ-GTP, LDL cholesterol, blood sugar, α-fetoprotein, and platelet count). By adopting a personalized approach to treatment, clinicians may be better able to determine the most appropriate course of therapy for individual patients while minimizing the risk of side effects.

Notes

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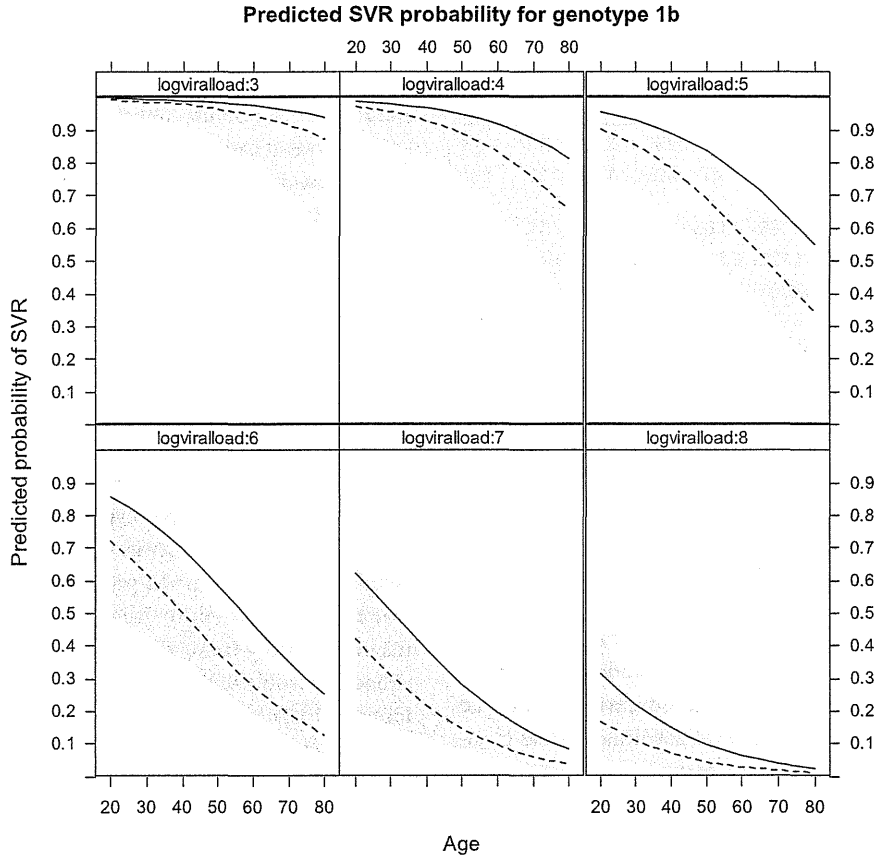

Figure 3. Predicted probabilities of sustained virological response (SVR) for patients with genotype 1b. Confidence intervals for predicted probability of SVR based on logistic regression by age, grouped by rs8099917 genotype and initial viral load for patients with genotype 1b, are shown. Solid lines represent the favorable rs8099917 TT single-nucleotide polymorphism genotype, and dashed lines represent the unfavorable GT or GG genotype.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Research Article

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

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

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

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

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

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

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

Introduction

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

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

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

Patients and methods

Patients

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

Table 1. Baseline characteristics of patients.

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

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

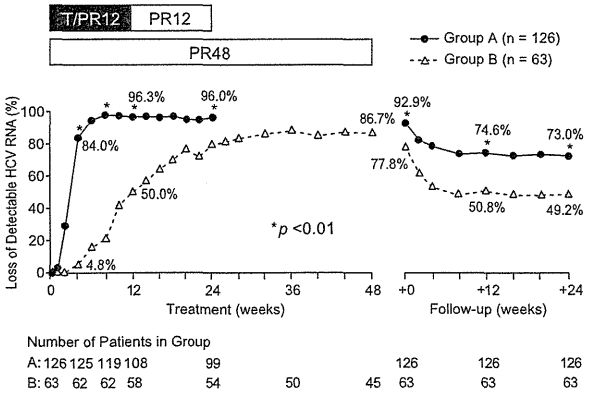

Fig. 1. Loss of detectable HCV RNA in patients in Groups A and B. Statistical tests were performed at weeks 4, 8, 12, and 24 in the treatment period, end of treatment, and weeks 12 and 24 in the follow-up period. An asterisk (*) indicates p <0.01 differences. The number of patients at each time point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

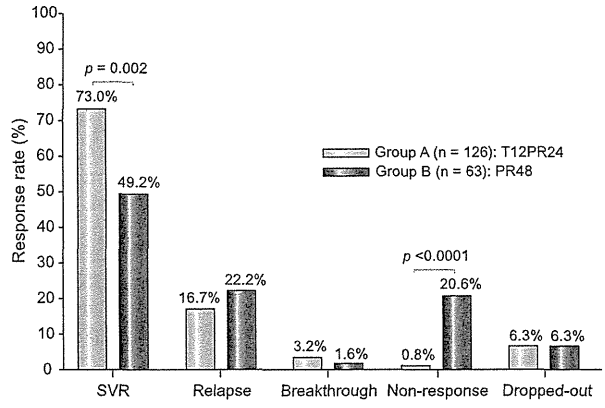

Fig. 2. Comparison of treatment responses between patients in Groups A and B. SVR, sustained virological response (HCV RNA negative 24 weeks after the completion of treatment); relapse, reappearance of HCV RNA in serum during follow-up period; breakthrough, reappearance of HCV RNA in serum during treatment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

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

Study design

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

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

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

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

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

HCV RNA and genotypes

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

Antiviral treatments

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

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

Statistical analysis

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

Results

Patient cohorts

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

Loss of HCV RNA during treatment

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

Responses to treatments

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

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

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

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

Adverse events

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

Hematological disorders

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

Skin disorders

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

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

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

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

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

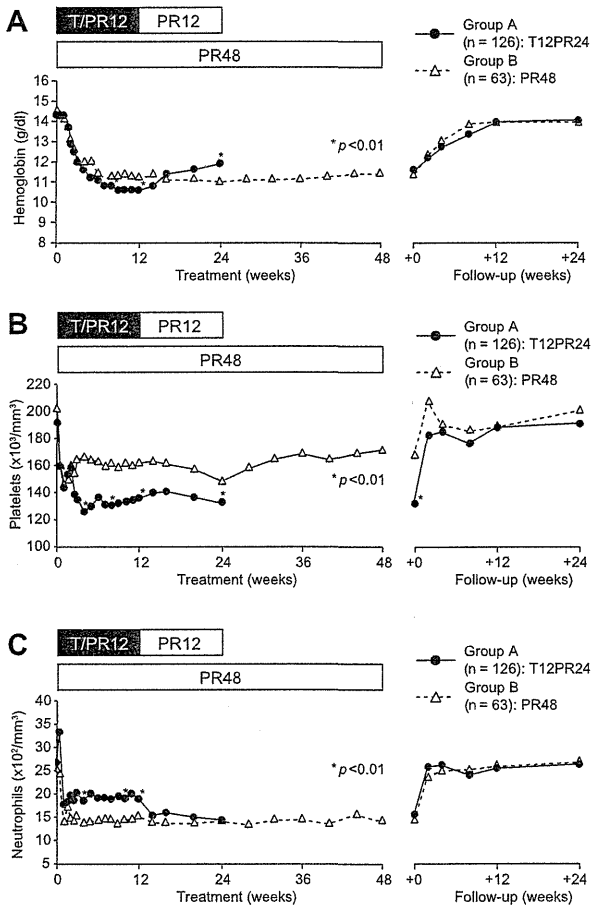

Fig. 3. Comparison of hematopoietic disorders between patients in Groups A and B. (A) Median hemoglobin levels, (B) platelet counts, and (C) neutrophil counts are plotted during treatment and follow-up. Ranges from 25% to 75% are omitted for visual clarity. Statistical tests were performed at weeks 4,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

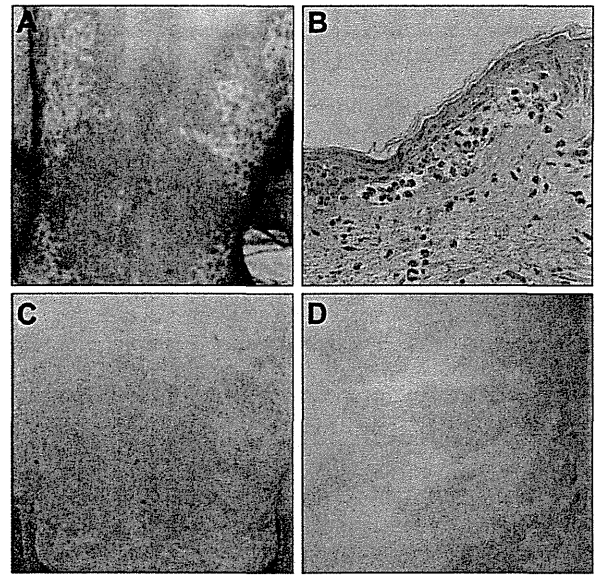

Fig. 4. Grade 4 skin regions in patients who received the triple therapy. (A) Erythema and (B) histopathology of the skin in the patient with Stevens–Johnson syndrome, as well as (C and D) generalized erythema in the patient developing drug rashes with eosinophilia and systemic symptoms (DRESS), are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

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

Discussion

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

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

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

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

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

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

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

Conflict of interest

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HEPATOLOGY

Interleukin-28B single nucleotide polymorphism of donors and recipients can predict viral response to pegylated interferon/ribavirin therapy in patients with recurrent hepatitis C after living donor liver transplantation

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Key words
core, hepatitis C virus, interferon sensitivity-determining region, interleukin-28B, liver transplantation.

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Abstract

Background and Aim: Interleukin-28B (*IL28B*) single nucleotide polymorphism (SNP) influences viral response (VR) to interferon (IFN) therapy in patients with hepatitis C. We studied the relationship between VR and the *IL28B* polymorphism (rs8099917) in patients on long-term pegylated IFN plus ribavirin (PEGIFN/RBV) therapy for recurrent hepatitis C after living-donor liver transplantation (LDLT).

Methods: Thirty-five patients with recurrent hepatitis C after LDLT were treated with PEGIFN/RBV. We evaluated the effect of *IL28B* SNP on the outcome in 20 patients infected with hepatitis C virus genotype 1 who completed IFN therapy.

Results: The sustained VR (SVR) rate was 54% (19/35) for all patients; 46% (13/28) for genotype 1. The SVR rate of donors' TT group (major genotype) was higher than that of donors' TG + GG group (minor genotype) (73% *vs* 20%), while that of recipients' TT group was similar to that of recipients' TG + GG group (64% *vs* 50%). With regard to the combined effect of donors' and recipients' *IL28B* SNP, the SVR rates of TT : TT (donors' : recipients'), TT : TG + GG, TG + GG : any group were 81%, 50%, and 20%, respectively. The VR rate of TT : TT, TT : TG + GG and TG + GG : any group at 12 weeks were 28%, 0%, and 0%; those at 48 weeks were 70%, 50%, 20%, and those at the end of treatment were 100%, 50%, 20%, respectively. The multivariate analysis identified *IL28B* of donors : recipients (TT : TT) as the only independent determinant of SVR (odds ratio 15.0, $P = 0.035$).

Conclusion: Measurement of donors' and recipients' *IL28B* SNP can predict the response to PEGIFN/RBV therapy, and the donors' *IL28B* SNP might be a more significant predictor than that of the recipients.

Introduction

Hepatitis C virus (HCV) has infected 170 million people worldwide, and such infection sometimes progresses to liver cirrhosis and/or hepatocellular carcinoma.[1] The current treatment for patients infected with HCV genotype 1 (HCV-1) is the combination of pegylated interferon-α and ribavirin (PEGIFN/RBV) for 48 weeks.[2] However, this treatment results in sustained viral response (SVR) in only approximately 50% of patients with HCV-1 infection.

In a recent genome-wide association study, a single nucleotide polymorphism (SNP) upstream of the interleukin (IL)-28B (*IL28B*) gene on chromosome 19, coding for IFN-λ-3, was found to be strongly associated with SVR rate in treatment-adherent HCV-1 patients.[3–8] The G nucleotide of rs8099917 was associated with a poor response to treatment (minor allele), whereas a T nucleotide was found to be associated with a fair response to treatment (major allele) in Japanese patients.

HCV-related end-stage liver disease is currently the leading indication for liver transplantation (LT). However, the outcome of LT for patients with HCV-related liver disease has been less satisfactory than those with HCV-negative liver disease.[9–15] HCV recurrence is universal after LT with accelerated progression of liver fibrosis. Approximately 20–25% of HCV-positive