Table 2 Factors associated with sustained virological response

Variable	Simple			Multiple		
	OR	95 % CI	*P* value	OR	95 % CI	*P* value
Host-related factor						
Age (year)	1.00	0.96–1.04	0.9488			
Sex male vs. female	1.23	0.56–2.72	0.6019			
Body weight (kg)	0.99	0.97–1.02	0.7195			
Body mass index (kg/m^2)	0.97	0.87–1.08	0.5494			
Cirrhosis absence vs. presence	2.20	0.93–5.18	0.0711			
Treatment-naïve or relapsers vs. non-responders	12.58	4.92–32.21	< 0.0001	5.58	1.28–24.38	0.0224
rs8099917 TT vs. non-TT	29.93	9.54–93.92	< 0.0001	73.65	11.28–480.93	< 0.0001
White blood cells (/μL)	1.00	1.00–1.00	0.0098			
Hemoglobin (g/dL)	1.16	0.87–1.55	0.3196			
Platelets (×10^4/μL)	1.09	1.01–1.18	0.0299			
Aspartate aminotransferase I(U/L)	0.99	0.98–1.00	0.1034			
Alanine aminotransferase I(U/L)	1.00	0.99–1.00	0.3574			
Gamma-glutamyl-transpeptidase I(U/L)	0.99	0.99–1.00	0.0014			
Albumin (g/dL)	3.14	0.65–15.22	0.1548			
Total cholesterol (mg/dL)	1.01	1.00–1.03	0.0467			
Low-density lipoprotein-cholesterol (mg/dL)	1.03	1.01–1.05	0.0080			
Alpha-fetoprotein (ng/mL)	0.97	0.95–1.00	0.0175			
Virus-related factor						
HCV RNA ($\log_{10}$IU/mL)	1.01	0.64–1.60	0.9695			
Core amino acid substitution 70 wild-type vs. mutant-type	4.01	1.75–9.17	0.0010			
ISDR of NS5A non-wild-type vs. wild type	2.13	0.46–9.79	0.3319			
Treatment-response factor						
Rapid virological response + vs. −	9.43	3.89–22.87	< 0.0001	12.59	2.33–69.97	0.0032
Reduction in HCV RNA level at week 1 ≥4.7 $\log_{10}$/mL vs. <4.7 $\log_{10}$IU/mL	10.11	2.92–34.99	0.0003	18.99	2.74–131.63	0.0029
Treatment-related factor						
Administration intervals of telaprevir q8 vs. q12 h	1.20	0.54–2.67	0.6572			
Initial daily dose of telaprevir 2250 vs. 1500 mg	1.46	0.65–3.26	0.3545			
Duration of therapy (weeks)	0.66	0.92–1.13	1.0226			
Adherence of PEG-IFN (%)	1.00	0.98–1.01	0.5762			
Adherence of ribavirin (%)	1.00	1.00–1.00	0.8539			
Adherence of telaprevir (%)	1.01	0.99–1.03	0.4877			

HCV hepatitis C virus, *ISDR* interferon sensitivity-determining region, *Peg-IFN* PEG-interferon

genotype ($P < 0.0001$, OR = 73.65, 95 % CI = 11.28–480.93), reduction of ≥4.7 $\log_{10}$IU/mL in HCV RNA at week 1 ($P = 0.0029$, OR = 18.99, 95 % CI = 2.74–131.63), achievement of RVR ($P = 0.0032$, OR = 12.59, 95 % CI = 2.33–69.97), and treatment-naïve patients or relapsers ($P = 0.0224$, OR = 5.58, 95 % CI = 1.28–24.38) (Table 2).

When analyses focused on patients with the *IL28B* non-TT genotype alone, previous relapsers ($P = 0.0020$), higher white blood cell count ($P = 0.0255$) and platelet count ($P = 0.0161$), lower body mass index ($P = 0.0400$), aspartate aminotransferase level ($P = 0.0303$), alpha-fetoprotein level ($P = 0.0304$), achievement of RVR ($P = 0.0011$), and reduction of ≥4.7 $\log_{10}$IU/mL in HCV RNA levels at week 1 ($P = 0.0003$) were identified as factors associated with SVR by univariate analysis. The multiple logistic regression analysis identified the following three independent factors: a reduction of ≥4.7 $\log_{10}$IU/mL in HCV RNA at week 1 ($P = 0.0043$, OR = 29.35, 95 % CI = 2.88–299.22), achievement of RVR

Table 3 Factors associated with sustained virological response in patients with the *IL28B* non-TT genotype

Variable	Simple			Multiple		
	OR	95 % CI	*P* value	OR	95 % CI	*P* value
Host-related factor						
Age (year)	0.99	0.94–1.05	0.7963			
Sex male vs. female	1.56	0.50–4.83	0.4421			
Body weight (kg)	0.97	0.93–1.02	0.2324			
Body mass index (kg/m^2)	0.82	0.69–0.99	0.0400			
Cirrhosis absence vs. presence	1.80	0.50–6.43	0.3657			
Relapsers vs. treatment-naïve or non-responders	13.64	2.60–71.46	0.0020	9.18	1.04–81.16	0.0461
White blood cells (/μL)	1.00	1.00–1.00	0.0255			
Hemoglobin (g/dL)	1.26	0.87–1.82	0.2145			
Platelets ($\times 10^4$/μL)	1.14	1.02–1.26	0.0161			
Aspartate aminotransferase I(U/L)	0.97	0.95–1.00	0.0303			
Alanine aminotransferase I(U/L)	0.98	0.96–1.00	0.0564			
Gamma-glutamyl-transpeptidase I(U/L)	0.99	0.99–1.00	0.1852			
Albumin (g/dL)	2.30	0.42–12.72	0.3380			
Total cholesterol (mg/dL)	1.00	0.98–1.02	0.9274			
Low-density lipoprotein cholesterol (mg/dL)	1.01	0.99–1.03	0.3557			
Alpha-fetoprotein (ng/mL)	0.90	0.82–0.99	0.0304			
Virus-related factor						
HCV RNA ($\log_{10}$IU/mL)	0.67	0.28–1.59	0.3590			
Core amino acid substitution 70 wild-type vs. mutant-type	1.56	0.50–4.83	0.4421			
ISDR of NS5A non-wild-type vs. wild type	1.87	0.29–12.33	0.5130			
Treatment-response factor						
Rapid virological response + vs. −	15.27	2.96–78.81	0.0011	17.96	1.73–186.57	0.0156
Reduction in HCV RNA level at week 1 ≥4.7 $\log_{10}$/mL vs. <4.7 $\log_{10}$IU/mL	15.00	3.43–65.59	0.0003	29.35	2.88–299.22	0.0043
Treatment-related factor						
Administration intervals of telaprevir q8 vs. q12 h	0.60	0.19–1.84	0.3698			
Initial daily dose of telaprevir 2250 vs. 1500 mg	0.71	0.21–2.41	0.5781			
Duration of therapy (weeks)	1.16	0.93–1.44	0.1973			
Adherence of PEG-IFN (%)	1.04	0.99–1.08	0.1084			
Adherence of ribavirin (%)	1.01	0.98–1.04	0.4767			
Adherence of telaprevir (%)	0.99	0.96–1.03	0.6851			

HCV hepatitis C virus, *ISDR* interferon sensitivity-determining region, *Peg-IFN* PEG-interferon

($P = 0.0156$, OR = 17.96, 95 % CI = 1.73–186.57), and previous relapsers ($P = 0.0461$, OR = 9.18, 95 % CI = 1.04–81.16) (Table 3).

Combination of the *IL28B* genotype and reduction in HCV RNA levels at week 1 after the start of therapy to identify patients with a high likelihood of SVR

Figure 4 shows the schematic representation of the process used to identify patients with a high likelihood to achieve SVR by combining the two factors most strongly associated with SVR. Patients with the *IL28B* TT genotype presented a high SVR rate [102 of 106 patients (96.2 %)], regardless of the reduction in HCV RNA levels at week 1 after the start of therapy. In contrast, patients with the non-TT genotype showed a high SVR rate [15 of 18 patients (83.3 %)] if they presented a reduction of ≥4.7 $\log_{10}$IU/mL in the HCV RNA levels at week 1 after the start of therapy. In contrast, the SVR rate was significantly lower [8 of 32 patients [25.0 %)] when patients did not present a reduction of ≥4.7 $\log_{10}$IU/mL at week 1 ($P = 0.0001$). In patients with the *IL28B* non-TT genotype and a reduction

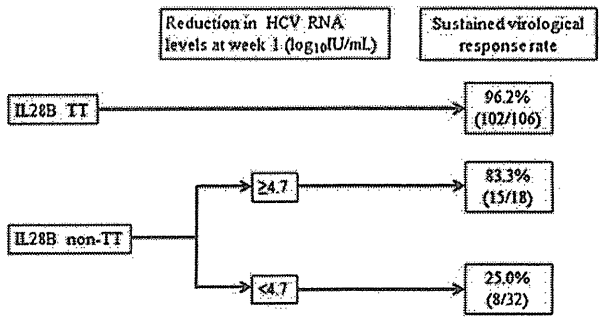

Fig. 4 Prediction of a sustained virological response (SVR) by the *IL28B* (rs8099917) genotype and reduction in HCV RNA level at week 1 after the start of therapy. In patients with the TT genotype, the SVR rate was high (96.2 %), regardless of the reduction in HCV RNA at week 1. In contrast, in patients with the non-TT genotype, the SVR rate was significantly higher in patients with a reduction of ≥4.7 log_{10}IU/mL in the HCV RNA level at week 1 after the start of therapy than in those with a reduction of <4.7 log_{10}IU/mL in the HCV RNA levels at week 1 [15 of 18 patients (83.3 %) vs. 8 of 32 patients (25.0 %), $P = 0.0001$]

of ≥4.7 log_{10}IU/mL in the HCV RNA levels at week 1, the sensitivity, specificity, PPV, NPV, and accuracy for SVR were 62.5, 88.9, 83.3, 75.0, and 78.0 %, respectively. Furthermore, in patients with the non-TT genotype, when both a reduction of ≥4.7 log_{10}IU/mL in the HCV RNA levels at week 1 and RVR were used, the sensitivity, specificity, PPV, NPV, and accuracy for SVR were 60.9, 100, 100, 75.0, and 82.0 %, respectively.

Discussion

Multiple logistic regression analysis revealed that the *IL28B* genotype was the most significant factor predicting SVR to a 24-week regimen of TVR-based triple combination therapy. The impact of the *IL28B* genotype on SVR found for this treatment regimen was in agreement with the findings of previous studies in Japan [7, 11, 14–16, 24]. In addition, a reduction of ≥4.7 log_{10}IU/mL in the HCV RNA levels at week 1 after the start of therapy was identified as a strong independent on-treatment predictor for SVR in a multiple logistic regression analysis.

The reduction in HCV RNA levels at week 1 was particularly relevant in patients with the *IL28B* non-TT genotype. Whereas patients with the *IL28B* TT genotype showed a high SVR rate regardless of the on-treatment response of HCV RNA, a significant difference in SVR rate was observed based on the reduction in HCV RNA levels at week 1 in patients with the unfavorable non-TT genotype. In this patient subpopulation, the reduction in HCV RNA level at week 1 was the factor most strongly associated with SVR, and this finding is of clinical value to identify patients with a low likelihood of achieving SVR as early as possible. Furusyo et al. [11] previously reported that the serum HCV RNA levels at day 3 presented a significant difference between SVR and non-SVR patients. The ability of the very early viral response to predict SVR shown by both Furusyo et al. and our study may be explained by the strong antiviral effect of TVR. However, Furusyo et al. did not enter serum HCV RNA levels at day 3 into a multiple logistic regression analysis to identify significant independent predictors of SVR. Therefore, in that study, it was not clear whether the serum HCV RNA level at day 3 was an independent factor of SVR when including host-related, virus-related, and on-treatment factors. In the present study, the median serum HCV levels at week 1 was significantly lower for SVR patients (1.9 log_{10}IU/mL) than for non-SVR patients (2.2 log_{10}IU/mL) ($P = 0.0136$, data not shown). In the present study, the reduction in HCV RNA levels at week 1 after the start of therapy was an independent predictive factor for SVR. This reduction in HCV RNA level at week 1 may represent early viral kinetics closely correlated with the antiviral effect. The predictive ability of the reduction in HCV RNA level at day 3 and week 1 after the start of therapy should therefore be compared based on the *IL28B* genotype.

This study is the first report to demonstrate that a reduction of ≥4.7 log_{10}IU/mL in the HCV RNA level at week 1 is a useful on-treatment predictive factor associated with SVR to a 24-week TVR-based triple combination therapy in clinical practice, especially in patients with the *IL28B* non-TT genotype. In 'real-world' clinical practice in some cases, it may be impossible to differentiate between previous null and partial responders because of the absence of relevant historical data from medical records. Therefore, for these treatment-experienced patients, *IL28B* genotyping may have clinical utility, as it may serve as a pretreatment marker for interferon responsiveness to guide patients and physicians. In patients with the *IL28B* non-TT genotype, both a reduction of <4.7 log_{10}IU/mL in the HCV RNA levels at week 1 and positivity for HCV RNA at week 4 (non-RVR) indicated a high likelihood of treatment failure. Hence, these patients should not undergo TVR-based triple combination therapy to avoid unnecessary treatment. This study identified that measurement of the HCV RNA level not only at week 4, but also at week 1, provides important information for predicting SVR, particularly in patients with the *IL28B* non-TT genotype.

There were some limitations to this study. First, the number of patients was too low to conclusively identify factors contributing to SVR. In particular, the number of non-responders was very small. Second, TVR-resistant variants were not analyzed. Resistant variants have been reported to occur in 56 % of HCV genotype 1b patients who did not achieve SVR [35]. Therefore, resistance variants should be identified in patients with treatment

failure. Third, this study regimen was limited to T12PR24. Only a 24-week TVR-based triple combination therapy (triple therapy for 12 weeks followed by an additional 12 weeks of PEG-IFN and RBV) is allowed by the Japanese National Insurance System. In the US, Canada, and EU, triple combination therapy is administered for either 12 or 36 additional weeks after PEG-IFN and RBV, according to the response-guided regimen based on the early viral response in each category, i.e., treatment-naïve patients and previous relapsers or partial responders and null responders.

Recently, the second-generation direct-acting antiviral agent simeprevir (SMV), which is once-daily oral NS3/4A protease inhibitor, was approved in September 2013 in Japan. Hayashi et al. [36] reported a Japanese phase II study. In treatment-naïve patients, the SVR rate was 77–92 % by triple combination therapy with SMV, PEG-IFN-α-2a and RBV. During the first 3–7 days of SMV-based therapy, an initial rapid reduction in HCV RNA was evident. Mean reduction in HCV RNA at week 1 in our study in TVR-based therapy was 4.5 $\log_{10}$IU/mL (data not shown). Mean reduction in HCV RNA at week 1 was not shown with the numerical value in this SMV-based therapy, but that seems to be similar to our TVR-based therapy. However, in this study, the *IL28B* genotypes were not investigated. Therefore, in clinical practice, from now on, prospective studies should be necessary to confirm whether the reduction in HCV RNA at week 1 is predictive for SVR in SMV-based therapy based on the *IL28B* genotype as well as in TVR-based therapy.

In conclusion, this prospective, multicenter study of a 24-week TVR-based triple combination therapy for Japanese genotype 1b CHC patients showed that the *IL28B* SNP genotype is the most important baseline factor for predicting SVR, and a reduction of $\geq$4.7 $\log_{10}$IU/mL in the HCV RNA levels at week 1, i.e., viral kinetics earlier than week 4, could be a useful on-treatment predictor of SVR, especially in patients with the *IL28B* non-TT genotype. Further large-scale prospective studies including SMV-based triple combination therapy are necessary to confirm these findings and develop the individual tailoring and optimization of therapeutics.

Conflict of interest The authors declare that they have no conflict of interest.

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Journal of Medical Virology 86:461–472 (2014)

α-Fetoprotein Is a Surrogate Marker for Predicting Treatment Failure in Telaprevir-Based Triple Combination Therapy for Genotype 1b Chronic Hepatitis C Japanese Patients With the *IL28B* Minor Genotype

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Even when treated with telaprevir-based triple therapy, some patients fail to achieve a sustained virological response. This study identified factors related closely to treatment failure. A total of 146 Japanese genotype 1b chronic hepatitis C patients were enrolled in this prospective, multicenter study and received a 24-week regimen of triple therapy. The end-of-treatment response rate was significantly lower in patients with the interleukin 28B (*IL28B*) (rs8099917) non-TT genotype (85.2%) than in those with the TT genotype (100%, $P=0.0002$). Multiple logistic regression analysis identified high α-fetoprotein levels as an independent factor related to non-end-of-treatment response in patients with the non-TT genotype. A cut-off value of 20 ng/ml was determined for a non-end-of-treatment response; sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were 75.0%, 95.7%, 75.0%, 75.0%, and 92.6%, respectively. Multiple logistic regression analysis for a sustained virological response identified the *IL28B* TT genotype, low α-fetoprotein levels, non-responders, and a rapid virological response. The sustained virological response rate was significantly lower in patients with the non-TT genotype (59.3%) than in those with the TT genotype (96.7%, $P<0.0001$). In patients with the non-TT genotype, α-fetoprotein was the most significant predictor for non-sustained virological response by univariate analysis. A cut-off value of 7.4 ng/ml α-fetoprotein was determined for non-sustained virological response; sensitivity, specificity, PPV, NPV, and accuracy were 63.6%, 87.5%, 77.8%, 77.8%, and 77.8%, respectively. For the non-TT patients, serum α-fetoprotein levels may be a surrogate marker for predicting treatment failure in telaprevir-based therapy for genotype 1b chronic hepatitis C. ***J. Med. Virol. 86:461–472, 2014.***

KEY WORDS: HCV; telaprevir; α-fetoprotein; *IL28B*

INTRODUCTION

Even when treated with standard care consisting of peginterferon (Peg-IFN) and ribavirin including an extended 72-week treatment course, a sustained virological response was achieved only in approximately 40–53% of genotype 1 chronic hepatitis C

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Accepted 27 September 2013
DOI 10.1002/jmv.23824
Published online 28 October 2013 in Wiley Online Library (wileyonlinelibrary.com).

patients [Manns et al., 2001; Fried et al., 2002; McHutchison et al., 2009a; Toyoda et al., 2012; Tsubota et al., 2012; Yoshizawa et al., 2013]. In aging patients in Japan, improving the sustained virological response rate through more effective treatment methods is required urgently to reduce mortality from liver failure and hepatocellular carcinoma.

Of the many drugs under investigation, the most promising are direct-acting antiviral agents, such as nonstructural (NS) 3/4A protease inhibitors [Pawlotsky, 2013]. Telaprevir, a NS3/4A serine protease inhibitor, was approved in the United States, Canada, the European Union (EU), and Japan in 2011. In treatment-naïve genotype 1 chronic hepatitis C patients, telaprevir-based triple combination therapy for a shortened period was reported to remarkably improve the sustained virological response rate compared with Peg-IFN and ribavirin alone [Hézode et al., 2009; McHutchison et al., 2009b; Kumada et al., 2012]. In treatment-experienced patients, the effect of telaprevir-based therapy was prescribed according to the patient's response to previous Peg-IFN and ribavirin combination therapy [McHutchison et al., 2010; Chayama et al., 2011; Muir et al., 2011; Zeuzem et al., 2011; Akuta et al., 2012, 2013; Hayashi et al., 2012].

Pivotal genome-wide association studies demonstrated that genetic variations near the interleukin 28B (*IL28B*) gene (rs8099917 and rs12979860) are associated strongly with treatment outcome of Peg-IFN and ribavirin combination therapy [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009]. These genetic variations appear to be strong predictors of the sustained virological response to telaprevir-based triple therapy as well as Peg-IFN and ribavirin combination therapy [Akuta et al., 2010, 2013; Chayama et al., 2011; Bota et al., 2013; Furusyo et al., 2013; Muir, 2013]. Additionally, fibrosis of liver likely influences treatment outcome. Particularly, presence of cirrhosis decreased the sustained virological response rate, even in telaprevir-based therapy [McHutchison et al., 2010; Jacobson et al., 2011]. Furthermore, the importance of a rapid virological response, defined as undetectable serum hepatitis C virus (HCV) RNA at treatment week 4, and extended rapid virological response, defined as undetectable serum HCV RNA at both treatment weeks 4 and 12, were also reported as significant predictors of telaprevir-based treatment outcome [Chayama et al., 2011; Jacobson et al., 2011; Sherman et al., 2011; Furusyo et al., 2013].

Indeed, telaprevir-based triple combination therapy remarkably improves the sustained virological response rate in chronic hepatitis C patients with the difficult-to-treat HCV genotype 1. However, some patients still fail to achieve a sustained virological response. Adverse events occurred more frequently and were more severe in patients treated with telaprevir-based therapy than in those treated with Peg-IFN and ribavirin alone [Hézode et al., 2009; McHutchison et al., 2009b, 2010; Zeuzem et al., 2011; Kumada et al., 2012]. Additionally, telaprevir-based therapy is very costly. In clinical practice, predictive factors of treatment failure are necessary for preventing unnecessary treatment as well as physical and economic burdens. This prospective, multicenter study was conducted to identify factors related closely to treatment failure in telaprevir-based triple combination therapy for genotype 1b chronic hepatitis C patients.

PATIENTS AND METHODS

Patients

Between December 2011 and May 2012, 146 Japanese genotype 1b-monoinfected chronic hepatitis C patients were enrolled in this study at Shinmatsudo Central General Hospital, Nippon Medical School Chiba Hokusoh Hospital, Jikei University School of Medicine Katsushika Medical Center, Jikei University School of Medicine Kashiwa Hospital, and Nippon Medical School Hospital. Inclusion criteria for the study included persistently positive sera for HCV RNA for >6 months as determined using quantitative real-time polymerase chain reaction (PCR) (COBAS AmpliPrep/COBAS TaqMan HCV test, Roche Diagnostics, Tokyo, Japan), age of 18–75 years, and body weight >35 kg at the time of entry into the study. Exclusion criteria included: (1) decompensated cirrhosis, (2) positive for hepatitis B surface antigen or antibody against human immunodeficiency virus, (3) previous or current development of hepatocellular carcinoma, (4) co-existence of other liver diseases such as autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, Wilson disease, and alcoholic liver disease, (5) renal disease or creatinine clearance ≤50 ml/min at baseline, (6) hemoglobin level <12 g/dl, white blood cell count <2,000/μl, neutrophil count <1,500/μl, and platelet count $<8.0 \times 10^4$/μl at baseline, (7) depression, schizophrenia or its history, and history of suicide attempt, (8) pregnancy in progress or planned during the study period for either partner. For all patients, liver biopsy was conducted within 12 months of enrollment and the presence or absence of cirrhosis was established according to the Metavir score [The French METAVIR Cooperative Study Group, 1994].

Patient profiles are shown in Table I. In this study, all treatment-experienced patients were treated with Peg-IFN and ribavirin combination therapy. Patients in this study were categorized as relapsers (HCV RNA undetectable at the end of treatment and then positive in follow up), partial responders ($\geq 2 \log_{10}$ IU/ml reduction in HCV RNA at week 12 but never undetectable), null responders ($<2 \log_{10}$ U/ml reduction in HCV RNA at week 12). In this study, partial responders and null responders were analyzed as non-responders.

All patients were treated with Peg-IFN-α-2b, ribavirin, and telaprevir triple therapy. Telaprevir (Telavic;

TABLE I. Characteristics of Patients

Variables	All	Naïve	Relapsers	Partial responders	Null responders
Number of patients	146	62	58	16	10
Gender, male/female (male%)	65/81 (44.5%)	28/34 (45.2%)	21/37 (36.2%)	12/4 (75.0%)	4/6 (40.0%)
Age (years)	57.4 (10.2)	55.1 (11.6)	59.4 (8.9)	56.6 (9.6)	60.3 (5.6)
Body weight (kg)	60.0 (11.9)	60.3 (11.7)	58.5 (11.6)	62.8 (12.9)	62.1 (12.0)
Body mass index (kg/m^2)	23.2 (3.4)	23.5 (3.5)	22.7 (3.1)	23.3 (3.3)	24.2 (3.7)
Absence or presence of cirrhosis (non-cirrhosis/cirrhosis) (cirrhosis %)	107/39 (26.7%)	47/15 (24.2%)	44/14 (24.1%)	11/5 (31.3%)	5/5 (50.0%)
rs8099917 (TT/TG/GG) (TT %)	92/52/2 (63.0%)	46/16/0 (74.2%)	40/17/1 (69.0%)	6/10/0 (37.5%)	0/9/1 (0.0%)
rs1127354 (CC/CA/AA)	123/22/1	50/11/1	50/0	14/2/0	9/1/0
Core amino acid substitution 70 (wild-type/mutant-type)	97/49	44/18	40/18	11/5	2/8
Core amino acid substitution 91 (wild-type/mutant-type)	100/46	44/18	40/18	10/6	6/4
ISDR of NS5A (wild-type/non-wild-type)	123/23	48/14	52/6	13/3	10/0
White blood cells (/μl)	5,032 (1,466)	5,241 (1,305)	4,803 (1,613)	5,105 (1,457)	4,941 (1,318)
Hemoglobin (g/dl)	14.0 (1.5)	14.2 (1.5)	13.6 (1.4)	14.7 (1.4)	14.0 (1.4)
Platelets (×10^4%L)	17.6 (5.2)	17.9 (5.1)	17.5 (5.5)	17.8 (5.0)	15.9 (3.6)
Aspartate aminotransferase (IU/L)	53.7 (38.5)	54.8 (36.5)	51.8 (44.7)	47.1 (14.3)	68.1 (34.8)
Alanine aminotransferase (IU/L)	61.7 (54.0)	66.2 (52.7)	56.8 (60.1)	54.9 (27.5)	73.2 (52.3)
γ-Glutamyl-transpeptidase (IU/L)	51.4 (62.3)	59.9 (59.6)	58.2 (50.8)	67.2 (76.0)	114.4 (87.7)
Albumin (g/dl)	4.2 (0.4)	4.1 (0.3)	4.2 (0.4)	4.2 (0.4)	4.2 (0.4)
Low density lipoprotein-cholesterol (mg/dl)	101.0 (29.4)	103.8 (31.7)	101.5 (27.1)	92.6 (22.9)	93.1 (31.7)
Fasting plasma glucose (mg/dl)	100.5 (22.8)	105.2 (23.7)	105.4 (23.8)	104.9 (19.4)	109.1 (14.1)
Homeostasis model assessment-insulin resistance	4.0 (5.9)	4.0 (6.7)	3.7 (4.9)	3.7 (4.6)	6.0 (6.9)
α-Fetoprotein (ng/ml)	11.3 (22.9)	13.8 (26.0)	6.5 (7.3)	4.4 (2.2)	34.6 (48.2)
HCV RNA ($\log_{10}$ IU/ml)	6.4 (0.8)	6.5 (0.8)	6.3 (0.9)	6.5 (1.0)	6.6 (0.3)
Initial dose of Peg-IFN (μg/kg)	1.5 (0.2)	1.5 (0.1)	1.5 (0.2)	1.5 (0.2)	1.6 (0.2)
Initial dose of ribavirin (mg/kg)	11.1 (1.9)	11.1 (1.8)	10.6 (1.9)	11.8 (1.6)	12.3 (0.9)
Initial daily dose of telaprevir (1,500/2,250 mg)	67/79	24/38	33/25	5/11	5/5
The administration intervals of telaprevir (q8h/q12q)	98/48	38/24	41/17	11/5	8/2

Data are expressed as numbers or mean (standard deviation).
ISDR, interferon sensitivity-determining region; HCV, hepatitis C virus; Peg-IFN, peginterferon.

Mitsubishi Tanabe Pharma, Osaka, Japan) was administered every 8 hr after meals (q8h) at 500 or 750 mg or every 12 hr after meals (q12h) at 750 mg or 1,125 mg. The initial daily dose of telaprevir (1,500 mg per day or 2,250 mg per day) and administration intervals (q8h or q12h) were determined by each attending physician according to age, gender, body weight, and hemoglobin level. Peg-IFN-α-2b (Peg-Intron. MSD, Tokyo, Japan) was injected subcutaneously at a median dose of 1.5 μg/kg/week. Ribavirin (Rebetol, MSD) dose was adjusted by body weight (600 mg for <60 kg; 800 mg for ≥60 to <80 kg; and 1,000 mg for ≥80 kg, and in the case of hemoglobin <13 g/dl at start of therapy, ribavirin was reduced by 200 mg), based on the guidelines of the Ministry of Health, Labor and Welfare of Japan. The drug was administered orally after breakfast and dinner. Triple therapy was given for 12 weeks, followed by an additional 12 weeks of Peg-IFN-α-2b and ribavirin combination therapy (T12PR24). Each drug was reduced appropriately or withdrawn when a serious adverse event was suspected of developing or if a serious adverse event occurred during the treatment course.

In addition, treatment was stopped for patients who had HCV RNA >3 $\log_{10}$ IU/ml at week 4 or detectable at week 12 or those showing a 2 $\log_{10}$ IU/ml increase from the lowest level during therapy because these patients had a low likelihood of achieving a sustained virological response and a high risk of developing antiviral resistance.

Adherence to Peg-IFN was calculated based on an initial weekly dose and that to ribavirin was calculated based on an initial daily dose. Adherence to telaprevir was defined as 100% when 2,250 mg was given per day for 12 weeks, which is the recommended daily dose.

Virological response was analyzed on an intent-to-treat basis. Treatment efficacy was evaluated based on HCV RNA negativity at the end of treatment (end-of-treatment response) and a successful endpoint

of treatment was a sustained virological response for patients showing undetectable HCV RNA for 24 weeks after cessation of treatment. In patients with relapse, HCV RNA levels became undetectable by the end-of-treatment but became positive during the follow-up period. In patients with viral breakthrough, HCV RNA became undetectable during the treatment period but then became positive before the end of the treatment period. In patients with non-response, HCV RNA was always detected during therapy. Furthermore, a rapid virological response was defined as undetectable HCV RNA at week 4 of starting treatment, while an extended rapid virological response was defined as undetectable HCV RNA at both weeks 4 and 12 of starting treatment.

All patients provided written informed consent. This study protocol was prepared following ethics guidelines established in conformity with the 2008 Declaration of Helsinki and was approved by the Ethics Committee of Shinmatsudo Central General Hospital, Nippon Medical School, and Jikei University (Approval numbers: 2012001, 523029, and 23-246, respectively).

Measurement of HCV RNA

HCV genotype was determined by direct sequencing followed by phylogenic analysis of the NS5B region [Simmonds et al., 1996]. The antiviral effects of the therapy on HCV were assessed by measuring serum HCV RNA levels. In this study, HCV RNA levels during treatment were evaluated at least once every 4 weeks before, during, and after therapy. HCV RNA concentrations were determined using the COBAS AmpliPrep/CABAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2–7.8 $\log_{10}$ IU/ml, and undetectable samples were defined as negative.

Detection of Amino Acid Substitution in Core and NS5A Regions of HCV Genotype 1b

Core amino acid substitutions at positions 70 and 91 were determined according to a previously described method [Akuta et al., 2007a]. Core amino acid substitutions at position 70 were defined as wild-type (arginine) or mutant-type (glutamine or histidine), and core amino acid substitutions at position 91 were defined as wild-type (leucine) or mutant-type (methionine). Additionally, substitutions at amino acids 2290–2248 of the NS5A region (interferon-sensitivity determining region, ISDR) were determined using a previously described method [Enomoto et al., 1996]. Amino acid substitutions in ISDR were defined as wild-type (0 or 1) or non-wild-type ($\geq$2).

Single Nucleotide Polymorphism Genotyping

Genomic DNA was extracted from whole blood using the MagNA Pure LC and a DNA Isolation Kit (Roche Diagnostics). Genetic polymorphisms, rs8099917 near the *IL28B* gene [Suppiah et al., 2009; Tanaka et al., 2009] and rs1127354 at the inosine triphosphatase (*ITPA*) gene [Fellay et al., 2010], were genotyped by real-time detection PCR using the TaqMan SNP Genotyping Assays and the 7500Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). The rs8099917 genotypes were classified into two categories, including TT (major genotype) and non-TT genotype (minor genotype: TG or GG), while the rs1127354 genotypes were classified into two categories, including CC (major genotype) and non-CC genotype (minor genotype: CA or AA).

Statistical Analysis

Continuous variables are expressed as the mean and standard deviations. Categorical data were analyzed using the chi-squared test and Fisher's exact test, while continuous data were analyzed using the nonparametric Mann–Whitney *U* test and Mann–Whitney *U* test with Bonferroni's correction. Univariate and multiple logistic regression analysis were used to identify factors that significantly contributed to rapid virological response, end-of-treatment response (or non-end-of-treatment response), and sustained virological response (or non-sustained virological response). Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated. All *P* values for statistical tests were two tailed, and values of <0.05 were considered statistically significant. Variables that achieved statistical significance ($P < 0.05$) according to univariate analysis were entered into multiple logistic regression analysis to identify significant independent predictive factors of rapid virological response, end-of-treatment response or non-end-of-treatment response, and sustained virological response or non-sustained virological response.

Receiver-operating characteristics (ROC) analyses were performed to determine cut-off values for sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy for predicting treatment outcomes. Statistical analysis was performed using SPSS version 17.0 (IBM-SPSS, Chicago, IL).

RESULTS

Characteristics According to Previous Treatment Response

Characteristics of patients involved in the study are shown in Table I. The distribution of the *IL28B* SNP genotype was the most significantly different across the four categories of previous treatment response. The proportion of the TT genotype was 74.2% (46 of 62) in treatment-naïve patients, 69.0% (40 of 58) in relapsers, 37.5% (6 of 16) in partial responders, and 0% (0 of 10) in null responders ($P = 0.0001$). The α-fetoprotein levels were also significantly different as follows: null (34.6 ± 48.2 ng/ml) versus naïve (13.8 ± 26.0 ng/ml; $P = 0.0019$); null

versus relapsers (6.5 ± 7.3 ng/ml; $P = 0.0368$); and null versus partial (4.4 ± 2.2 ng/ml; $P = 0.0029$). The presence of cirrhosis was higher in partial responders and null responders than in treatment-naïve and relapsers, though not statistically significant.

According to previous treatment response, among the 62 treatment-naïve patients, 55 (88.7%) achieved a sustained virological response, 3 (4.8%) relapsed, 3 (4.8%) had viral breakthrough, and 1 (1.6%) showed a non-response. Among the 58 relapsers, 54 (93.1%) achieved a sustained virological response, 3 (5.2%) relapsed, and 1 (1.7%) had viral breakthrough. Among the 16 partial responders, 12 (75.0%) achieved a sustained virological response and 4 (25.0%) relapsed. Among the 10 null responders, none (0%) achieved a sustained virological response, 7 (70.0%) relapsed, 1 (10.0%) had viral breakthrough, and 2 (20%) showed non-response (Fig. 1). For the *IL28B* SNP genotype, among the 92 patients with the TT genotype, 89 (96.7%) achieved a sustained virological response and 3 (3.3%) relapsed. Among the 54 patients with the non-TT genotype, 32 (59.3%) achieved a sustained virological response, 14 (25.9%) relapsed, 5 (9.3%) had viral breakthrough, 3 (5.6%) showed non-response (Fig. 2).

Factors Associated With Rapid Virological Response

The rate of rapid virological response was 82.2% (120 of 146 patients). All rapid virological response patients also achieved an extended rapid virological response. According to univariate analysis, previous relapse ($P = 0.0087$), high platelet counts ($P = 0.0481$), and low aspartate aminotransferase ($P = 0.0156$), alanine aminotransferase ($P = 0.0486$), α-fetoprotein ($P = 0.0300$), and HCV RNA ($P = 0.0001$) were significant positive predictors for rapid virological response. Being previous non-responders was a significant negative predictor for rapid virological response ($P = 0.0118$). The rate of rapid virological response was higher in patients with the *IL28B* TT genotype (84.8%, 78 of 92) than in those with the non-TT genotype (77.8%, 42 of 54, respectively), although the values were not significantly different ($P = 0.2855$). Multiple logistic regression analysis revealed that low HCV RNA levels ($P = 0.0002$, OR = 0.16, 95% CI = 0.06–0.42) and previous non-responders ($P = 0.0121$, OR = 0.25, 95% CI = 0.09–0.74) were independent predictors of rapid virological response.

Factors Associated With End-of-Treatment Response

A total of 138 of 146 (94.5%) patients achieved end-of-treatment response. For the *IL28B* genotype, end-of-treatment response was achieved in all 92 (100%) patients with the TT genotype and 46 of 54 (85.2%) patients with the non-TT genotype ($P = 0.0002$). Surprisingly, all patients with the TT genotype showed a favorable end-of-treatment response. Only patients with the non-TT genotype failed in end-of-treatment response. Therefore, factors associated with failed end-of-treatment response (non- end-of-treatment response) were explored in patients with the non-TT genotype alone. High aspartate aminotransferase ($P = 0.0139$), alanine aminotransferase ($P = 0.0085$), γ-glutamyl-transpeptidase ($P = 0.0499$), and α-fetoprotein ($P = 0.0011$) were significantly associated with non-end-of-treatment response. For the non-end-of-treatment response rate, the presence of cirrhosis (5 of 16 patients, 31.2%) was significantly higher than the absence of cirrhosis (3 of 38, 7.9%; $P = 0.0413$). Multiple logistic regression analysis only identified high α-fetoprotein levels as an independent predictor for non-end-of-treatment response

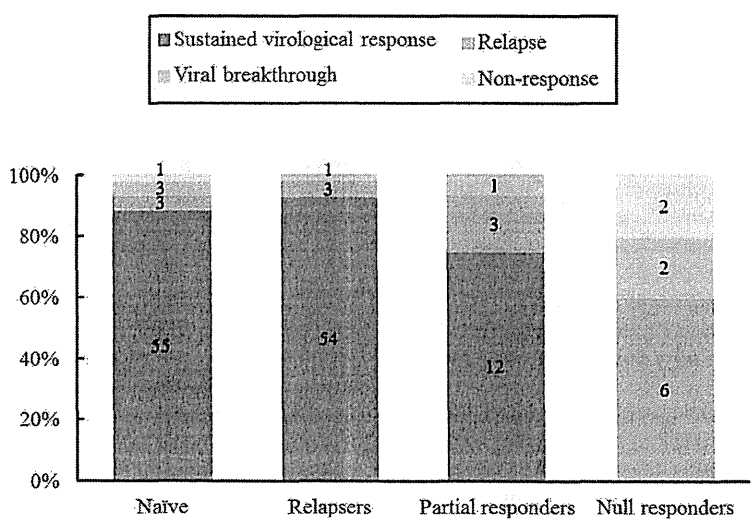

Fig. 1. Virological outcome according to previous treatment response.

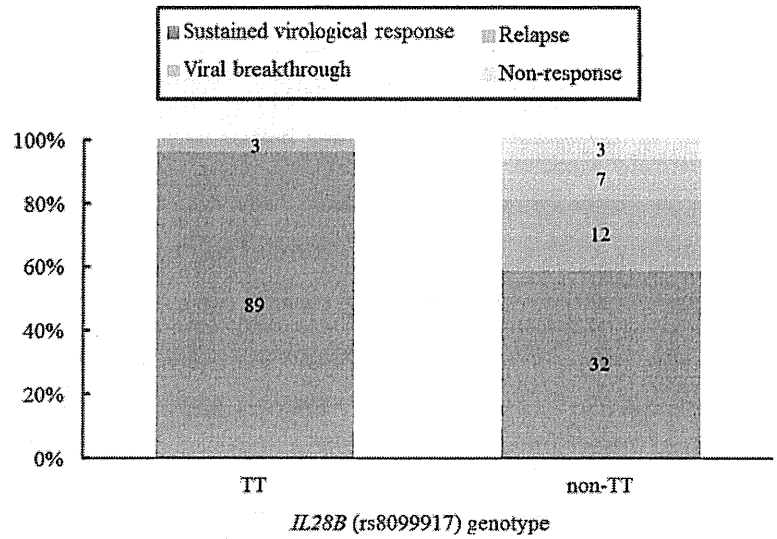

Fig. 2. Virological outcome according to the *IL28B* (rs8099917) genotype.

($P = 0.0146$, OR = 1.05, 95% CI = 1.01–1.09; Table II). The association between α-fetoprotein levels and non-end-of-treatment response was determined based on ROC curve analysis (Fig. 3). The area under the ROC curve was 0.864; the cut-off value of 20 ng/ml revealed the maximum likelihood of discriminating between end-of-treatment responses and non-end-of-treatment responses. The rate of non-end-of-treatment response was significantly higher in patients with high α-fetoprotein levels (≥20 ng/ml; 6 of 8, 75.0%) than in those with low α-fetoprotein levels (<20 ng/ml, 2 of 46, 4.3%; $P < 0.0001$, OR = 66.0, 95% CI = 7.78–559.58; Suppl. Fig. S1). The sensitivity, specificity, PPV, NPV, and accuracy of the cut-off value for non-end-of-treatment response were 75.0%, 95.7%, 75.0%, 95.7%, and 92.6%, respectively.

Factors Associated With Sustained Virological Response

A sustained virological response was achieved in 121 of 146 (82.9%) patients. For the *IL28B* genotype, a sustained virological response was achieved in 89 of 92 patients (96.7%) with genotype TT and 32 of 54 patients (59.3%) with genotype non-TT ($P < 0.0001$). All three patients with the TT genotype who failed to show a sustained virological response discontinued treatment (renal dysfunction at 4 weeks in a previous relapser, acute pancreatitis at 7 weeks in a treatment-naïve patient, and renal dysfunction at 10 weeks in a partial responder) and thereafter relapsed. All had cirrhosis.

According to univariate analysis, the following factors were associated with a sustained virological response: previous relapsers ($P = 0.0012$), absence of cirrhosis ($P = 0.0006$), *IL28B* genotype TT ($P < 0.0001$), high white blood cell counts ($P = 0.0404$), platelet counts ($P = 0.0192$), and low density lipoprotein-cholesterol ($P = 0.0178$), low γ-glutamyl-transpeptidase ($P = 0.0201$) and α-fetoprotein ($P = 0.0030$), core amino acid substitutions at position 70 wild-type ($P = 0.0032$), achievement of rapid virological response ($P < 0.0001$), longer treatment duration ($P = 0.0364$), and high dosage adherence of ribavirin ($P = 0.0413$). Being previous non-responders was a significant negative predictor for sustained virological response ($P < 0.0001$). Multiple logistic regression analysis identified the following 4 independent factors: *IL28B* TT genotype ($P = 0.0001$, OR = 36.51, 95% CI = 5.86–227.51), low α-fetoprotein levels ($P = 0.0045$, OR = 0.96, 95% CI = 0.93–0.99), previous non-responders ($P = 0.0147$, OR = 0.15, 95% CI = 0.03–0.69), and achievement of a rapid virological response ($P = 0.0261$, OR = 1.25, 95% CI = 1.25–33.59) (Table III).

Factors Associated With Non-Sustained Virological Response

Patients with the *IL28B* TT genotype showed extremely high sustained virological response rate (96.7%). Therefore, factors associated with a failed sustained virological response (non-sustained virological response) were explored in patients with the non-TT genotype alone. According to univariate analysis, previous non-responders ($P = 0.0093$), presence of cirrhosis ($P = 0.0137$), low white blood cell counts ($P = 0.0324$) and platelet counts ($P = 0.0046$), and high aspartate aminotransferase ($P = 0.0022$), alanine aminotransferase ($P = 0.0055$), and α-fetoprotein ($P = 0.0002$) were associated positively with a non-sustained virological response. Being a previous relapsers was a significant negative predictor of non-sustained virological response ($P = 0.0178$). However,

TABLE II. Factors Associated With Non-End-of-Treatment Response in Patients With the *IL28B* SNP Non-TT Genotype

	Simple	Multiple		
Variable	*P*-Value	OR	95% CI	*P*-Value
Host-related factor				
Age (year)	0.6173			
Gender, male vs. female	0.7058			
Body weight (kg)	0.9418			
Body mass index (kg/m^2)	0.4954			
Cirrhosis absence vs. presence	0.0413			
Treatment-naïve	0.2174			
Relapsers	0.2447			
Partial responders	0.3264			
Null responders	0.1561			
rs1127354 CC vs. CA+AA	0.5769			
White blood cells (/μl)	0.8646			
Hemoglobin (g/dl)	0.8359			
Platelets (×10^4/μl)	0.0860			
Aspartate aminotransferase (IU/L)	0.0139			
Alanine aminotransferase (IU/L)	0.0085			
γ-Glutamyl-transpeptidase (IU/L)	0.0499			
Albumin (g/dl)	0.1064			
Low density lipoprotein-cholesterol (mg/dl)	0.1765			
Fasting plasma glucose (mg/dl)	0.2892			
Homeostasis model assessment-insulin resistance	0.9798			
α-Fetoprotein (ng/ml)	0.0011	1.05	1.01–1.09	0.0146
Virus-related factor				
HCV RNA ($\log_{10}$ IU/ml)	0.0621			
Core amino acid substitution 70 wild-type vs. mutant-type	1.0000			
Core amino acid substitution 91 wild-type vs. mutant-type	0.7019			
ISDR of NS5A non-wild-type vs. wild type	1.0000			
Treatment-related factor				
The administration intervals of telaprevir q8h vs. q12h	0.6966			
Initial daily dose of telaprevir 2,250 mg vs. 1,500 mg	1.0000			
Duration of therapy (weeks)	0.6264			
Adherence of Peg-IFN (%)	0.9895			
Adherence of ribavirin (%)	0.2053			
Adherence of telaprevir (%)	0.7575			

HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region.

multiple logistic regression analysis did not identify any significant independent factors associated with non-sustained virological response. Since the high α-fetoprotein levels was the most significant predictor by univariate analysis, the association between α-fetoprotein levels and non-sustained virological response was determined using ROC curve analysis (Fig. 4). The area under the ROC curve was 0.798, and a cut-off value of 7.4 ng/ml yielded maximum likelihood of discrimination between non-sustained virological response and sustained virological response ($P = 0.0001$, OR = 12.25, 95% CI = 3.14–47.77). The rate of non-sustained virological response was significantly higher in patients with high α-fetoprotein levels (>7.4 ng/ml; 14 of 18, 77.8%) than in those with low α-fetoprotein levels (≤7.4 ng/ml; 8 of 36, 22.2%; Suppl. Fig. S2). The sensitivity, specificity, PPV, NPV, and accuracy of the cut-off value for non-sustained virological response were 63.6%, 87.5%, 77.8%, 77.8%, and 77.8%, respectively.

Regarding the presence or absence of cirrhosis, the rate of non-sustained virological response was significantly higher in patients with cirrhosis (11 of 16, 68.8%) than in those without cirrhosis (11 of 38, 28.9%; $P = 0.0137$). The sensitivity, specificity, PPV, NPV, and accuracy of presence of cirrhosis for non-sustained virological response were 50.0%, 84.4%, 68.8%, 71.1%, and 70.4%, respectively. The α-fetoprotein levels was significantly higher in patients with cirrhosis than in those without cirrhosis (37.4 ± 45.7 ng/ml vs. 5.7 ± 4.5 ng/ml, $P = 0.0001$).

DISCUSSION

Multiple logistic regression analysis revealed the *IL28B* genotype as the most significant factor predicting a sustained virological response to telaprevir-based triple combination therapy. The contribution of the *IL28B* genotype to a sustained virological response agreed with the results of previous studies conducted in Japan [Akuta et al., 2010, 2013; Chayama et al., 2011; Furusyo et al., 2013]. In this study, the sustained virological response rate was very high (96.7%) in patients with the *IL28B* TT genotype and numerically higher than values reported previously [Akuta et al., 2010, 2013; Chayama

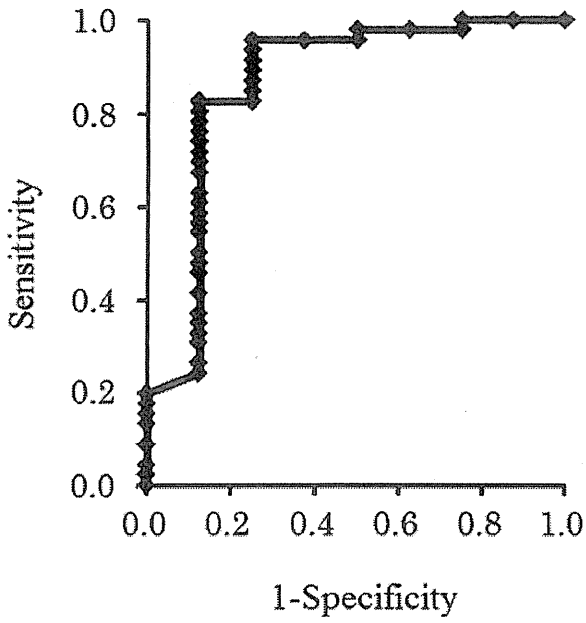

Fig. 3. The receiver operating characteristics (ROC) analysis for predicting non-end-of-treatment response to telaprevir-based triple combination therapy in patients with the *IL28B* non-TT according to serum α-fetoprotein levels. The area under the ROC curve was 0.864.

et al., 2011; Furusyo et al., 2013]. Notably, all patients with the favorable *IL28B* TT genotype achieved end-of-treatment response. A few TT genotype patients who discontinued prematurely treatment and experienced treatment failure may have achieved a sustained virological response if they had completed treatment as scheduled. Interestingly, rapid virological response did not significantly differ between patients with the TT and non-TT genotypes, despite distinct differences in the rates of end-of-treatment response and sustained virological response. Even if patients with the TT genotype failed a rapid virological response, they may have achieved a sustained virological response with a shortened 24-week treatment. Taken together, Japanese patients with the favorable TT genotype showed the strongest likelihood of achieving a sustained virological response to telaprevir-based therapy.

In contrast, the sustained virological response rate decreased to approximately 60% in patients with the unfavorable *IL28B* non-TT genotype. However, telaprevir-based therapy substantially increased the rate from approximately 14–24% [Hayes et al., 2011; Kurosaki et al., 2011; Toyoda et al., 2012; Yoshizawa et al., 2013] in Peg-IFN and ribavirin therapy to approximately 60%. Therefore, this study focused on patients with the non-TT genotype alone and investigated which factors contributed to treatment failure in the relatively unfavorable patient group. Interestingly, α-fetoprotein was associated closely with the failure of both end-of-treatment and sustained virological response. Furthermore, the cut-off value was determined using ROC analysis to discriminate between treatment success and failure.

α-Fetoprotein, known as a single-stranded glycoprotein, belongs to the albuminoid gene family, which includes albumin and the vitamin D-binding protein. α-Fetoprotein is produced in the embryonic yolk sac and fetal liver; therefore, the serum α-fetoprotein level in the fetus remains high until birth. Serum α-fetoprotein level decrease by 10 ng/ml within several weeks after birth and remain at low concentrations throughout life [Takikawa and Suzuki, 2002]. However, reactivation of α-fetoprotein production in adults occurs during liver regeneration and hepatocarcinogenesis [Liaw et al., 1986]. Therefore, serum α-fetoprotein is measured routinely as a tumor marker of hepatocellular carcinoma [Tyson et al., 2011]. However, α-fetoprotein levels are sometimes elevated in patients with chronic HCV infection who do not have hepatocellular carcinoma [Bayati et al., 1998; Goldstein et al., 1999; Chu et al., 2001]. Patients with increased α-fetoprotein levels have a very high risk of developing hepatocellular carcinoma in chronic HCV infection [Kumada et al., 2010]. Although advanced liver fibrosis is generally associated with high α-fetoprotein levels [Bayati et al., 1998; Hu et al., 2004; Chen et al., 2007], elevated α-fetoprotein levels are a risk factor for hepatocellular carcinoma, irrespective of fibrosis stage [Tateyama et al., 2011]. In Peg-IFN and ribavirin therapy for chronic hepatitis C, high pretreatment α-fetoprotein levels independently predict a lower sustained virological response [Males et al., 2007; Akuta et al., 2007b]. The increased serum α-fetoprotein levels are ascribed to hepatic damage with selective transcriptional activation of the α-fetoprotein gene [Taketa, 1990].

This study involved multiple regression and ROC analyses and is the first to demonstrate that the high levels of α-fetoprotein is a useful predictive factor for treatment failure in patients with the *IL28B* non-TT genotype by telaprevir-based triple combination therapy in clinical practice. Only one study reported that α-fetoprotein levels were related to treatment outcome in telaprevir-based therapy [Akuta et al., 2012]. The difference between the present study and the previous study is the patient group. The previous study investigated previous non-responders to Peg-IFN and ribavirin in clinical trials, while the present study investigated patients with the *IL28B* non-TT genotype in clinical practice. In the previous study, the rate of end-of-treatment response failure was significantly higher in patients with high levels of α-fetoprotein (≥10 ng/dl) than in those with low levels of α-fetoprotein (<10 ng/dl). The previous study investigated 15 non-responders, and 14 of those had the *IL28B* non-TT genotype. Therefore, these two studies appear to be similar in that patients with the *IL28B* non-TT genotype were examined. The discrepancy in cut-off value can be explained by including

TABLE III. Factors Associated With Sustained Virological Response

Variable	Simple		Multiple		
	OR	*P*-Value	OR	95% CI	*P*-Value
Host-related factor					
Age (year)	0.98	0.4171			
Gender, male vs. female	0.84	0.7008			
Body weight (kg)	1.00	0.8929			
Body mass index (kg/m^2)	0.95	0.3920			
Cirrhosis absence vs. presence	4.89	0.0006			
Treatment-naïve	2.14	0.1134			
Relapsers	4.23	0.0122			
Non-responders	0.09	<0.0001	0.15	0.03–0.69	0.0147
rs8099917 TT vs. TG+GG	20.40	<0.0001	36.51	5.86–227.51	0.0001
rs1127354 CC vs. CA+AA	0.41	0.2554			
White blood cells (/μl)	1.00	0.0404			
Hemoglobin (g/dl)	1.30	0.0902			
Platelets (×10^{4}μl)	1.13	0.0192			
Aspartate aminotransferase (IU/L)	0.99	0.0783			
Alanine aminotransferase (IU/L)	1.00	0.2192			
γ-Glutamyl-transpeptidase (IU/L)	0.99	0.0201			
Albumin (g/dl)	2.95	0.0651			
Low density lipoprotein-cholesterol (mg/dl)	1.02	0.0178			
Fasting plasma glucose (mg/dl)	1.02	0.1291			
Homeostasis model assessment-insulin resistance	0.97	0.4013			
α-Fetoprotein (ng/ml)	0.97	0.0030	0.96	0.93–0.99	0.0045
Virus-related factor					
HCV RNA ($\log_{10}$ IU/ml)	0.81	0.4525			
Core amino acid substitution 70 wild-type vs. mutant-type	3.84	0.0032			
Core amino acid substitution 91 wild-type vs. mutant-type	1.93	0.1438			
ISDR of NS5A non-wild-type vs. wild type	2.41	0.2554			
Treatment response factor					
Rapid virological response + vs. −	7.05	<0.0001	6.48	1.25–33.59	0.0261
Treatment-related factor					
The administration intervals of telaprevir q8h vs. q12h	0.76	0.5694			
Initial daily dose of telaprevir 2,250 mg vs. 1,500 mg	1.11	0.8161			
Duration of therapy (weeks)	1.10	0.0364			
Adherence of Peg-IFN (%)	1.02	0.0899			
Adherence of ribavirin (%)	1.02	0.0413			
Adherence of telaprevir (%)	1.00	0.7031			

HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; Peg-IFN, peginterferon.

treatment-naïve patients and relapsers and the difference in baseline α-fetoprotein levels. Baseline α-fetoprotein levels in the present study were higher compared to those in the previous study (15.1 ± 29.0 ng/dl vs. 10.5 ± 11.2 ng/dl).

In this study, the sustained virological response rate was significantly lower in patients with the *IL28B* non-TT genotype. Therefore, this study focused on patients with the non-TT genotype alone and attempted to identify factors contributing to treatment failure in patients with the unfavorable *IL28B* genotype. As a result, high levels of α-fetoprotein may be a surrogate marker for predicting non-sustained virological response as well as non-end-of-treatment response in patients with the *IL28B* non-TT genotype. Although the populations and aims of the studies differed, the cut-off value of α-fetoprotein levels (7.4 ng/ml) for non-sustained virological response in this study were surprisingly similar (7.3 ng/ml) for high risk of hepatocellular carcinoma in chronic hepatitis C patients before interferon therapy [Asahina et al., 2013]. The results suggest that patients with baseline α-fetoprotein levels with a high risk of hepatocarcinogenesis experience difficulty in achieving a sustained virological response. Even when treated with telaprevir-based triple combination therapy, high α-fetoprotein concentrations appeared to attenuate successful treatment outcome.

This study also found that α-fetoprotein was more useful as a predictor of non-sustained virological response than the presence of cirrhosis. Although liver biopsy remains the gold standard for evaluating fibrosis, there are several limitations, such as invasiveness, variability in sampling and pathological interpretation, and high cost. However, measuring serum α-fetoprotein is easy, inexpensive, non-invasive, and superior in quantitative capability. Thus, measuring serum α-fetoprotein is simpler and more useful than histological evaluation by liver biopsy for identifying patients with treatment failure in telaprevir-based therapy, particularly for the *IL28B* non-TT genotype. However, in the patients who had high

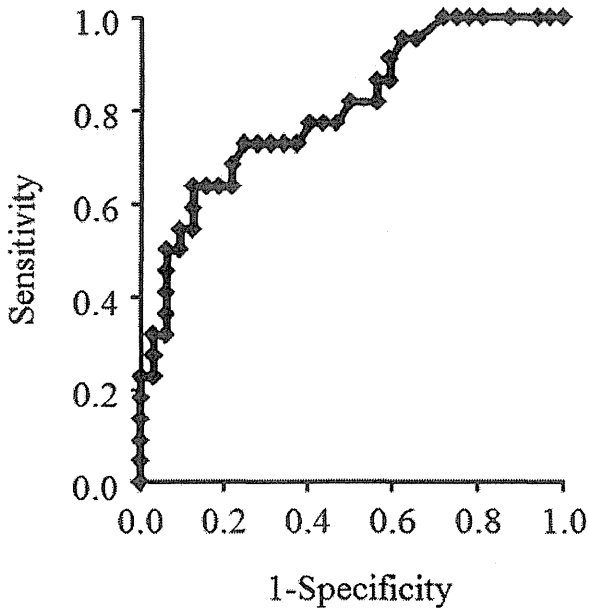

Fig. 4. The receiver operating characteristics (ROC) analysis for predicting non-sustained virological response to telaprevir-based triple combination therapy in patients with the *IL28B* genotype non-TT according to serum α-fetoprotein levels. The area under the ROC curve was 0.798.

levels of α-fetoprotein, the population ratio of cirrhosis would be high. Therefore, that would be a main reason that α-fetoprotein could be a surrogate marker for treatment failure.

In this study, the proportions of the *IL28B* genotype were different mostly based on previous treatment response. In treatment-experienced patients, the proportions of the unfavorable *IL28B* non-TT genotype increased with refractoriness to treatment. In the REALIZE study, the proportions of the unfavorable *IL28B* (rs12979860) non-CC genotype (CT/TT) were increased similarly, at 72.9% in relapsers, 86.9% in partial responders, and 94.0% in null responders [Pol et al., 2013]. Treatment outcome is associated clearly with previous treatment response. The close relationship between these factors may arise largely from the proportions of the unfavorable *IL28B* genotype. Furthermore, α-fetoprotein levels and the frequency of cirrhosis in null responders were higher compared to those in others. There may be close relationship among these unfavorable factors. Thus, previous null responders showed an extremely poor response, even to telaprevir-based therapy. These patients also had a high risk of developing hepatocellular carcinoma. Hence, a new combination therapy with potent direct-acting antiviral agents with or without interferon is needed urgently.

There were some limitations to this study. First, the number of patients was too small to identify factors contributing to treatment failure conclusively. In particular, the number of non-responders was only 26. Second, telaprevir-resistant variants were not analyzed. Resistant variants were reported to occur in 56% of HCV genotype 1b patients who did not achieve a sustained virological response [Sullivan et al., 2013]. Therefore, resistance variants should be identified in patients with treatment failure. Third, this study regimen was limited in T12PR24. Only 24-week telaprevir-based triple combination therapy is allowed by the Japanese National Insurance System. In the US, Canada, and EU, triple combination therapy is administered for either 12 or 36 additional weeks after Peg-IFN and ribavirin, according to the response-guided regimen based on early viral response in each category: treatment-naïve patients and previous relapsers or partial responders and null responders.

In conclusion, this prospective, multicenter study of telaprevir-based triple combination therapy for Japanese genotype 1b chronic hepatitis C patients showed that the *IL28B* SNP genotype is the most important pretreatment factor for predicting a sustained virological response and indicated that high levels of α-fetoprotein could be a surrogate marker for predicting treatment failure in patients with the *IL28B* non-TT genotype. Further large-scale prospective studies are necessary to confirm these findings and to facilitate development of more rational and effective therapeutic regimens.

ACKNOWLEDGEMENTS

We are grateful to Hiroyuki Tachibana MD, Ken Yonezawa MD, Kai Yoshizawa MD, Ai Nakagawa MD, and Norio Itokawa MD for excellent clinical assistance. We also appreciate Gotaro Toda MD, PhD for providing advice on this manuscript.

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Journal of Gastroenterology and Hepatology

JGHF

doi:10.1111/jgh.12402

HEPATOLOGY

Impact of *IL28B* polymorphisms on 24-week telaprevir-based combination therapy for Asian chronic hepatitis C patients with hepatitis C virus genotype 1b

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Key words
chronic hepatitis C virus infection, *IL28B* single nucleotide polymorphism, pegylated interferon, ribavirin, sustained virological response, telaprevir.

Accepted for publication 5 September 2013.

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Funding: This work was supported in part by Clinical Research Funds from Department of Gastroenterology and Hepatology, Katsushika Hospital, Jikei University School of Medicine and Nippon Medical School Chiba Hokusoh Hospital.

Competing interests: None declared with respect to this manuscript.

Abstract

Background and Aim: The aim of this study was to clarify which or how factors could influence the probability of sustained virological response (SVR) in 24-week telaprevir-based triple combination therapy for East Asian chronic hepatitis C patients infected with hepatitis C virus genotype 1b.

Methods: Of 140 patients who were enrolled in this study, 137 received 12-week telaprevir combined with 24-week pegylated interferon alpha-2b plus ribavirin and were subjected to the analysis. Factors associated with SVR were analyzed by multiple logistic regression analysis.

Results: Of the 137 patients, 112 (82%) achieved SVR. Of 87 patients with *IL28B* single nucleotide polymorphism rs8099917 genotype TT, 84 (97%) achieved SVR. By contrast, 28 of 50 (56%) patients with the genotype TG/GG had SVR ($P = 3.29 \times 10^{-9}$). Fifty-three of 60 (88%) naïve patients and 50 of 54 (93%) prior relapsers achieved SVR. Nine of 13 (69%) prior partial responders and none of 10 (0%) prior null responders achieved SVR. Multivariable analysis identified four independent factors that were significantly associated with SVR: *IL28B* SNP rs8099917 genotype ($P = 6.90 \times 10^{-5}$), pre-existence of cirrhosis ($P = 3.99 \times 10^{-3}$), prior treatment response ($P = 0.0126$), and rapid virological response ($P = 0.0239$).

Conclusions: The *IL28B* single nucleotide polymorphism still remained informative as a predictor of SVR to 24-week telaprevir-based triple combination therapy for East Asian patients infected with hepatitis C virus genotype 1b.

Introduction

Telaprevir is an orally bioavailable peptidomimetic inhibitor of the hepatitis C virus (HCV) non-structural (NS) 3/4A serine protease.[1] Phases 2 and 3 studies conducted in the United States, Europe, and Japan have proved that this direct-acting antiviral agent (DAA) combined with pegylated interferon (peg-IFN) alpha-2a or -2b plus ribavirin (RBV) substantially increases the rate of sustained virological response (SVR) in treatment-naïve and previously treated patients infected with HCV genotype 1, albeit with higher rates of discontinuation because of adverse events, compared with peg-IFN alpha/RBV combination alone.[2–10] Based on the proved efficacy and safety, the telaprevir-based triple therapy for chronic hepatitis C (CH-C) with HCV genotype 1 has been approved by each government organization.[11–13] Since November 2011, in Japan, patients have been receiving 12-week telaprevir in combination with 24-week peg-IFN alpha-2b/RBV by utilizing the government subvention.

Numerous studies have reported that various host-, virus-, and treatment-related factors are associated with the outcome of peg-IFN alpha/RBV combination therapy for CH-C.[14–19] Some of these factors appear to contribute to SVR in telaprevir-based triple combination therapy,[4–8,10] although these correlations between them remain controversial and do not reach a definitive conclusion. Recently, 48-week telaprevir-based triple combination therapy for predominantly Caucasian cohort was reported to attenuate the value of single nucleotide polymorphisms (SNPs) nearby the interleukin 28B (*IL28B*) gene,[20] which is one of the strongest

pretreatment predictors of peg-IFN alpha/RBV treatment outcome.[17,19,21,22] It is conceivable that more potent antiviral treatment very highly increases the SVR rate, resulting in deflating or obviating the value of various factors as a predictor of the previous-generation treatment.

The aim of this study was to clarify which or how factors (including *IL28B* SNPs) could have an impact on SVR in 24-week triple combination therapy with telaprevir/peg-IFN alpha-2b/RBV for East Asian patients infected with HCV genotype 1b alone.

Methods

Study design and assessment. Between December 2011 and June 2012, 140 Asian patients (137 Japanese, 2 Korean, and 1 Chinese) chronically infected with HCV genotype 1b were enrolled in this study at seven specialty hospitals. Patients received subcutaneous peg-IFN alpha-2b (PegIntron; MSD, Tokyo, Japan) at a dose of 1.5 μg/kg once weekly and oral RBV (Rebetol; MSD) at a dose of 600–1000 mg twice daily; the dose was adjusted according to body weight (600 mg for weight ≤ 60 kg, 800 mg for weight > 60 to ≤ 80 kg, and 1000 mg for weight > 80 kg), and oral telaprevir (TELAVIC; Mitsubishi Tanabe Pharma, Osaka, Japan) at a dose of 750 mg every 8 or 12 h after meal. The treatment duration lasted 24 weeks: the triple combination therapy for the first 12 weeks followed by peg-IFN/RBV alone for the subsequent 12 weeks (T12PR24). After the completion or discontinuation of treatment, patients were followed for at least 24 weeks. Leading inclusion criteria were CH-C that were diagnosed by laboratory, virology, and histology; HCV genotype 1b confirmed by the conventional polymerase chain reaction (PCR)-based method; age between 20 and 75 years; and hemoglobin concentration ≥ 11 g/dL. Leading exclusion criteria were decompensated cirrhosis; liver cancer or other malignancy; other forms of liver disease, such as alcoholic liver disease, autoimmune hepatitis, primary biliary cirrhosis, and hemochromatosis; white blood cell count $< 2.0 \times 10^3/\mu L$; neutrophil count $< 1.5 \times 10^3/\mu L$; platelet count $< 8.0 \times 10^4/\mu L$; abnormal hemoglobin disease; coexisting uncontrolled or serious medical or psychiatric illness; therapy with any other antiviral or immunomodulatory agent administered within the previous 24 weeks; concurrent treatment with any contraindicating drugs; positive for hepatitis B surface antigen or human immunodeficiency virus; hypersensitivity to pegylated IFN, RBV, or telaprevir; and pregnancy or lactation. On-treatment dose reduction, modification, and discontinuation of peg-IFN, RBV, or telaprevir followed the criteria and procedures according to the proper usage guideline for telaprevir[13] or patient condition to reduce or avoid adverse effects and treatment discontinuation. The use of growth factors (erythropoietin and granulocyte colony-stimulating factor) was prohibited in this study. When serum HCV RNA level was decreased by < 2.0 logs from the baseline at 12 weeks of treatment in naïve patients or when qualitative HCV RNA was detectable at 12 weeks of treatment in prior relapsers and non-virological responders (NVRs), treatment was recommended to discontinue prematurely. The study protocol was conducted in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines, and was approved by the Institutional Review Boards of all participating sites. Written informed consent was acquired from each individual.

Clinical examination and laboratory data were assessed at least twice weekly during the first week, every week between 2 and 12 weeks of treatment, and thereafter every 4 weeks until 24 weeks post-treatment. Virological data were assessed by monitoring serum HCV RNA levels every 4 weeks during and off treatment until 24 weeks post-treatment. Pre-existence of cirrhosis was determined by using percutaneous liver biopsy or ultrasonography, and/or computed tomography. Serum HCV RNA loads were measured, and the presence or absence of serum HCV RNA was determined by using a quantitative PCR assay (COBAS AmpliPrep/COBAS TaqMan HCV Test, Roche Molecular Systems, Pleasanton, CA, USA).

The primary end-point was SVR defined as undetectable serum HCV RNA at 24 weeks post-treatment. Relapse was defined as undetectable serum HCV RNA at the end of treatment but detectable viremia during the follow-up period. Non-virological response (NVR) was defined as persistent viremia throughout the treatment. Patients with each response were termed sustained virological responders (SVRs), relapsers, and NVRs, respectively. Rapid virological response (RVR) and extended RVR (eRVR) were defined as undetectable serum HCV RNA at 4 weeks of treatment and at both 4 and 12 weeks of treatment, respectively. Viral breakthrough was defined as undetectable serum HCV RNA after treatment but reappearance of serum HCV RNA during the treatment, or as an increase in the HCV RNA level of $\geq 1.0 \log_{10}$ IU/mL from the lowest value during the treatment period. NVR was further divided into partial response and null response: partial response was defined as viral load decline from the baseline level was $\geq 2.0 \log_{10}$ IU/mL at 12 weeks of treatment, but viremia was persistently detectable during treatment; null response was defined as the viral decline of $< 2.0 \log_{10}$ IU/mL at 12 weeks of treatment and persistent viremia during treatment.

Viral and host-related markers. HCV genotype, substitutions at amino acid positions 70 and 91 (core 70 and core 91, respectively)[23] of the HCV core region, and the number of amino acid substitutions within the interferon sensitivity determining region (2209–2248)[24] of the HCV NS5A region was determined by using direct sequencing of PCR products for the corresponding regions after reverse transcription of extracted RNA from sera.

Genomic DNA was extracted from whole blood using the MagNA Pure LC and the DNA Isolation Kit (Roche Molecular Systems). SNPs, rs8099917 and rs12979860 nearby the *IL28B* gene,[17,21,22] and rs1127354 at the *ITPA* gene,[25] were determined by real-time detection PCR using the TaqMan SNP Genotyping Assays and the 7500 Real-Time PCR System (Life Technologies, Carlsbad, CA, USA).

Statistical analysis. Pearson or Mantel–Haenszel chi-square test, Fisher's exact test, or Mann–Whitney test was used to compare frequencies in categorical data or differences in continuous data between groups, respectively. Possible variables contributing to SVR included baseline and on-treatment features (Table 1). Variables that reached statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) in bivariate comparisons were subsequently entered into multiple logistic regression analysis using forward and backward stepwise selection method to identify

Table 1 Baseline and on-treatment profiles of SVR and non-SVR patients

Variable	SVR (n = 112)	Non-SVR (n = 25)	P-value
Demographic feature			
Age (year)	20–73 (59)	40–68 (61)	0.366
Gender (female/male)	68/44	13/12	0.423
Body weight (kg)	40.1–101.6 (57.4)	40.5–83.8 (59.1)	0.788
Body mass index	15.9–34.3 (22.6)	15.1–31.9 (24.5)	0.511
Laboratory data			
AST (IU/L)	13–215 (37)	22–139 (58)	3.37×10^{-3}
ALT (IU/L)	13–305 (37)	20–201 (58)	0.0335
GGT (IU/L)	11–339 (36)	14–296 (61)	4.59×10^{-3}
Albumin (g/dL)	3.0–4.8 (4.2)	3.1–5.0 (4.0)	0.329
WBC count ($\times 10^3/\mu L$)	2.0–8.7 (4.9)	2.7–7.9 (4.4)	0.0549
Hemoglobin (g/dL)	11.3–17.2 (13.9)	11.1–16.3 (13.3)	0.198
Platelet count ($\times 10^4/\mu L$)	8.9–40.7 (16.9)	8.7–22.8 (14.5)	0.0421
LDL-cholesterol (mg/dL)	45–194 (97)	51–146 (85)	0.0183
Liver disease stage			
Non-cirrhosis/cirrhosis	89/23	11/14	3.05×10^{-4}
Virology			
Viral load ($\log_{10}$ IU/mL)	4.1–7.7 (6.5)	5.8–7.6 (6.5)	0.575
Core 70 (wild/mutant)	80/32	10/15	2.76×10^{-3}
Core 91 (wild/mutant)	79/33	14/11	0.159
ISDR (0–1/2 ≤)	91/21	22/3	0.422
SNPs			
IL28B rs8099917 (TT/TG/GG)	84/27/1	3/20/2	3.29×10^{-9}
IL28B rs12979860 (CC/CT/TT)	84/26/2	3/19/3	3.29×10^{-9}
ITPA rs1127354 (CC/CA/AA)	92/19/1	23/2/0	0.225
Previous treatment			
Naïve/relapse/NVR	53/50/9	7/4/14	4.13×10^{-8}
On-treatment			
Telaprevir (2250 mg/1500 mg)	60/52	13/12	0.887
Peg-IFN adherence (%)	20.8–100 (100)	25.0–100 (100)	0.772
RBV adherence (%)	16.7–100 (69.0)	19.4–100 (68.4)	0.523
Telaprevir adherence (%)	22.2–100 (66.8)	33.7–100 (66.7)	0.740
RVR (yes/no)	96/16	12/13	2.99×10^{-5}
eRVR (yes/no)	96/16	12/13	2.99×10^{-5}

Continuous data expressed as range (median).
ALT, alanine transaminase; AST, aspartate transaminase; eRVR, extended rapid virological response; GGT, gamma glutamyl transpeptidase; ISDR, interferon sensitivity determining region; LDL, low-density lipoprotein; peg-IFN, pegylated interferon; RBV, ribavirin; RVR, rapid virological response; SVR, sustained virological response; WBC, white blood cell.

significantly independent factors associated with SVR. P values of < 0.05 denoted the presence of a statistically significant difference. All data analyses were performed using the SPSS statistical package for Windows, version 17.0 (IBM-SPSS, Chicago, IL, USA).

Results

Treatment outcome. Of 140 entries for the treatment, 137 were subjected to the analysis, and three were excluded because of treatment cessation within the first week (two because of personal reason and one because of systemic skin flare). Of the 137 patients, 112 (82%) achieved SVR. The remaining 25 (18%) patients were classified into non-SVR: 16 relapsed, 8 had viral breakthrough (6 of 8 once showed undetectable HCV RNA during treatment), and 1 showed partial response. Table 1 summarizes differences in baseline and on-treatment features between SVR and non-SVR groups. All drugs were discontinued due to adverse events in six patients (four SVRs and two relapsers). Telaprevir alone was stopped in nine patients (six SVRs and three relapsers). Adverse effects were similar to those reported in previous studies.[2–13]

Pretreatment and on-treatment factors associated with SVR. Multiple logistic regression analysis identified three independent pretreatment variables that were significantly associated with SVR (Table 2): *IL28B* SNP rs8099917 genotype (GT/TT *vs* TT, $P = 5.04 \times 10^{-5}$), pre-existence of cirrhosis (presence *vs* absence, $P = 2.42 \times 10^{-3}$), and prior treatment response (NVR *vs* naïve/relapse, $P = 2.22 \times 10^{-3}$). Next, on-treatment variables were also entered into the multiple logistic regression analysis, which identified four significantly independent variables (Table 3): *IL28B* SNP rs8099917 genotype ($P = 6.90 \times 10^{-5}$), pre-existence of cirrhosis ($P = 3.99 \times 10^{-3}$), prior treatment response