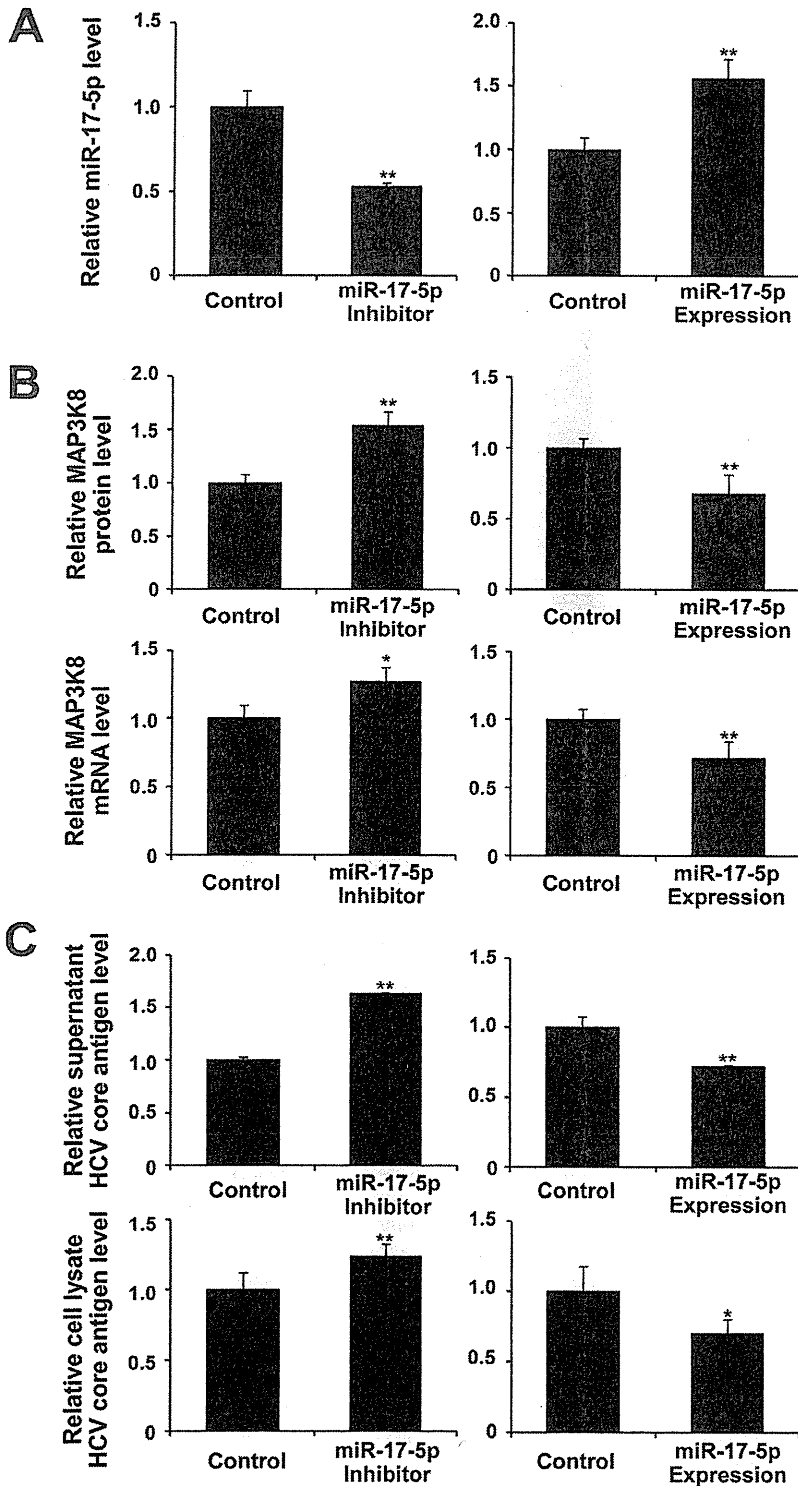

Figure 4. Expression and inhibition of hsa-miR-17-5p in Huh7.5.1 cells. (**A**) Functional suppression (left) and overexpression (right) plasmids of miR-17-5p. (**B**) Inhibition of miR-17-5p increased (left), whereas overexpression of miR-17-5p decreased MAP3K8 mRNA and protein expression levels (right). (**C**) HCV core antigen levels increased following miR-17-5p inhibition (left) and decreased by miR-17-5p overexpression (right) in both supernatant and cell lysate. Bars indicate the means of three independent experiments and the error bars indicate standard deviations. *$p<0.01$ and **$p<0.001$ compared with controls.

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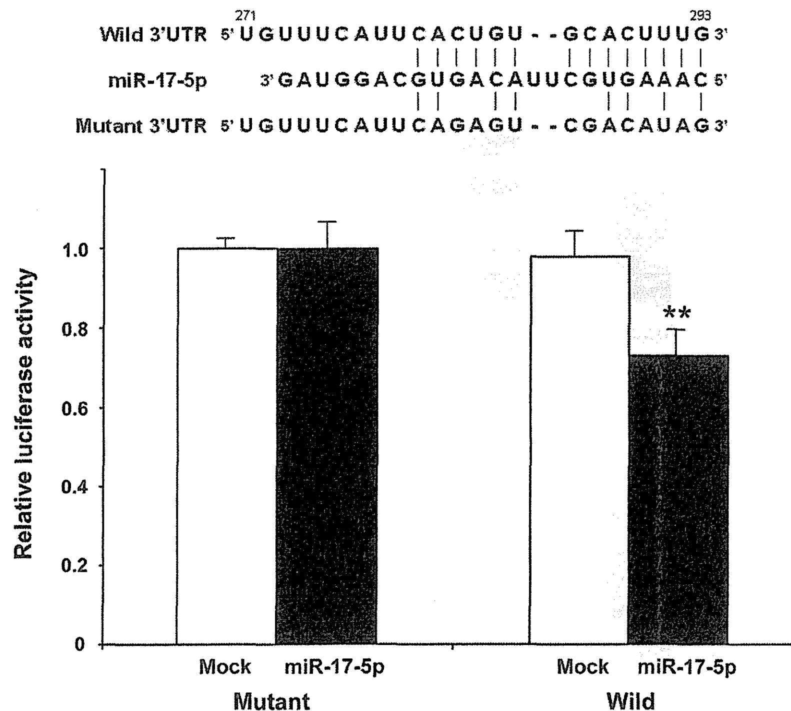

Figure 5. Luciferase reporter assay. miR-17-5p inhibited the luciferase activity of the wild-type MAP3K8 3′UTR construct (right), whereas no decrease in activity was observed in cells co-transfected with the mutant MAP3K8 3′UTR construct (left) or mock plus wild-type or mutant construct (right and left, respectively). Bars indicate the means of three independent experiments, and the error bars indicate standard deviations: $^{**}p<0.001$ compared with controls.
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type, patient race, treatment response definitions, study endpoints, and treatment regimens. This study analyzed patients with a homogeneous race and genotype (1b) who adhered to combination therapy and treatment for a specified duration.

MAP3K8, also known as cancer Osaka thyroid (cot) [22] or tumor progression locus 2 (tpl2) [23], was originally recognized as a proto-oncogenic protein. Toll-like receptors (TLRs) are innate immune sensors stimulated by specific microbial and viral components, including HCV. *In vitro* HCV infection directly induces TLR4 expression and activates human B cells to increase the production of IFN-β and IL-6 [24]. Peripheral blood mononuclear cells from HCV-infected individuals express higher TLR4 levels compared with uninfected controls [24]. In the IKK-NF-κB pathway, certain activated TLRs, including TLR4, induce inhibition of kappa B kinase (IKK)- catalyzed phosphorylation of nuclear factor kappa B (NF-κB) p105. Nonphosphorylated NF-κB p105 forms a stable, inactive complex with MAP3K8. Subsequent ubiquitination and proteasome-mediated processing of NF-κB-p105 to NF-κB-p50 releases MAP3K8, which activates the MAPK/ERK kinase (MEK)-extracellular signal-regulated kinase (ERK) pathway. MEK-ERK regulates the expression of pro- and anti-inflammatory mediators that lead to the production of various cytokines and chemokines in a stimulus- and cell/receptor type-specific manner (Fig. S3, Fig. S4) [25,26]. Indeed, MAP3K8 is an important and novel therapeutic target for inflammatory diseases [27]. MAP3K8 is involved in ERK signaling activation in hepatic Kupffer and stellate cells with being stimulated by TLR4 and TLR9, leading to ERK-dependent expression of the fibrogenic genes IL-1β and TIMP-1. Thus, MAP3K8 expression may contribute to liver fibrosis [28].

In addition, this study provided a novel insight into MAP3K8, which is involved in resistance to HCV treatment. The results of experiments in this study demonstrated the importance of MAP3K8 in HCV production. MAP3K8 knockdown by siRNA altered extracellular, but not intracellular, HCV core antigen levels. This result suggests that MAP3K8 might be involved in the release or assembly of HCV, does not exclude the possibility that MAP3K8 participates in intracellular HCV core production because miR-17-5p influenced both supernatant and cell-lysate HCV core antigen levels along with MAP3K8 mRNA and protein levels. If MAP3K8 limited viral release/assembly alone, intracellular HCV core antigen would accumulate following siRNA transfection. Conversely, MAP3K8 overexpression did not affect HCV production, probably because enough MAP3K8 may exist in the cells. This result is generally observed in other critical host factors (e.g. hVAP-33) involved in the HCV life cycle [29]. The above description [24–26] and *in silico* analyses (Fig. S3, Fig. S4) suggest that MAP3K8 might play a role in HCV production through a regulatory pathway and network (Fig. S5); however, the exact mechanism remains unknown and requires further investigation. It is important to note that there may be differences between the HCV genotype 1b- and 2a-derived strains/replicons. The 2a-derived JFH1 infection system is highly competent compared with other genotype-derived systems and allows steady inhibition and expression analyses [30]. Notably, this *in vitro* study focused on the correlation between MAP3K8 and miR-17-5p and their impact on HCV production; there may not be significant genotypic effect on MAP3K8 and miR-17-5p. Importantly, it is difficult to determine genotype-specific differences using different infection-competent systems.

The miR-17-92 polycistron, also known as the first oncomir, encodes six or seven miRNAs, including miR-17-5p [31,32], and is frequently overexpressed in several tumors [31,33]. In contrast, overexpression of miR-17-5p also leads to tumor suppression in breast cancer [34] and HeLa cells [32]. miR-17-5p may function as both a tumor suppressor and an oncogenic activator by targeting both pro- and anti-proliferative genes and by competing with each other in different cellular contexts, which are dependent on the expression of other transcriptional regulators [35]. Known targets of the miR-17-92 cluster primarily regulate cell cycle progression, apoptosis, and transcription factors [32,35]. Physiologically, this cluster is down-regulated during aging, and hematopoietic and lung differentiation. During HIV infection, suppression of this cluster by the virus is required for efficient viral replication [36]. Our results suggest that inhibition of miR-17-5p expression may be advantageous for HCV production. Interestingly, miR-17-5p overexpression in HeLa cells decreases the expression of the low-density lipoprotein (LDL) receptor (LDLR) and consequently induces reduced intracellular lipoprotein accumulation because of the impaired internalization [32]. LDLR is one of putative HCV receptors; however, its precise role remains controversial [37-40]. LDLR also aids the optimization of HCV replication, and the expression levels are stimulated by HCV infection. Decreased LDLR and lipoprotein uptake through LDLR may adversely affect the HCV life cycle because hepatocyte lipid metabolism pathways are required for HCV.

Bioinformatics and *in vitro* experiments showed that miR-17-5p expression levels were inversely correlated with MAP3K8 in response to anti-HCV treatment. miR-17-5p repressed HCV production by inhibiting MAP3K8 expression, whereas miR-17-5p expression was influenced by MAP3K8. The results also suggested a specific interaction between miR-17-5p and MAP3K8 3′UTR, which was previously validated by the luciferase reporter assay [35]. Taken together, MAP3K8 expression following HCV infection is negatively influenced by miR-17-5p at both the translational and transcriptional levels. This molecular interaction is a potential target for novel molecular therapeutics. However, a single miRNA can regulate the expression of multiple target mRNAs by imperfect base pairing [9,10]. Conversely, the expression of a single mRNA may be regulated by several miRNAs. Numerous mRNAs and miRNAs are key regulators in complicated pathophysiological networks (Fig. S3, Fig. S4). Therefore, it is important to note that the complex interaction between MAP3K8 and miR-17-5p may not be reflective of a correlation between their expression and viral load in our patient cohort.

Abundant hepatic miR-122 expression is essential for efficient HCV replication in cultured human hepatoma cells [11]. Suppression of miR-122 leads to a marked reduction and long-lasting suppression of HCV RNA in both sera and the livers of nonhuman primates with chronic HCV infection [41]. Paradoxically, this study showed significantly lower miRNA-122 (hsa-miR-122-5p) expression levels in null/partial responders than in SVRs/relapsers, independent of other factors. This finding is in agreement with the results of a previous study, which reported markedly low baseline miR-122 levels in poor responders [21]. Moreover, no positive correlation was observed between miR-122 expression and viral load. No convincing explanation exists for these paradoxical results. Re-analysis of registered miRNA microarray data [8] identified significantly low miR-122 levels, no change in miR-675-5p levels, and low (although not significant) miR-17-5p levels in null/partial responders. Most miR-122 target genes are involved in the lipid biogenesis pathway [42], and miR-122 antagonism induces a substantial decrease in plasma lipid levels. As described above, host lipid metabolism is vital to HCV [40], and may be related to the endogenous IFN response to HCV and IL28B SNPs [43]. However, we did not find a correlation between miR-122 expression and serum lipid levels nor identify miR-122 target genes, including lipid-related metabolic pathways, which could be considered key molecular signatures contributing to a null/partial response.

In conclusion, both global mRNA and miRNA expression profiling analyses increase our understanding of the molecular mechanisms that underlie refractory treatment responses and are even applicable to next-generation treatment. The results obtained in this study also aid the identification of novel features of known genes and target molecules for future therapeutic intervention.

Supporting Information

Figure S1 Hierarchical cluster analysis of mRNA expression using microarray analysis. Changes in mRNA expression levels are presented in graduated color patches from green (least expression) to red (most abundant expression).
(TIF)

Figure S2 Hierarchical cluster analysis of miRNA expression using microarray analysis. Changes in gene expression are presented in graduated color patches from blue (least expression) to red (most abundant expression).
(TIF)

Figure S3 Relationship between MAP3K8 (Tpl2/Cot) and related genes in underlying gene regulatory networks. MAP3K8 (Tpl2/Cot) was integrated by Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathways. MAP3K8 (Tpl2/Cot) was identified as an important node and considered to be a key regulator.
(TIF)

Figure S4 Gene networks for MAP3K8 and hsa-miR-17. MAP3K8 and hsa-miR-17 and array-independent/literature-based text-mining were integrated into the gene regulatory network analysis (Agilent Literature Search). The interaction data were visualized and analyzed by Cytoscape. MAP3K8 and its related mRNAs were associated with the miR-17 cluster family and its related miRNAs via IRF6, STAT3, AKT1, EPHB2, TIMP1, and VEGFA.
(TIF)

Figure S5 Postulated scheme for HCV replication regulated by MAP3K8 and hsa-miR-17-5p. IKK, inhibition of kappa B kinase; NF-κB, nuclear factor kappa B; MAP3K8, mitogen-activated protein kinase kinase kinase 8; MEK, MAPK/extracellular signal-regulated kinase.
(TIF)

Table S1 Comparison of baseline profiles between SVRs/relapsers and null/partial responders.
(DOC)

Data S1 List of gene probe sets up- and down-regulated in sustained virological responders (SVR) and relapsers compared with those in null responders.
(XLS)

Data S2 List of microRNA probe sets up- and down-regulated in sustained virological responders (SVR) and relapsers compared with those in null responders.
(XLS)

Data S3 List of all gene probe sets up- and down-regulated in sustained virological responders (SVR) and

relapsers compared with those in null responders, and gene signatures in previously reported references.
(XLS)

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Author Contributions

Conceived and designed the experiments: AT HA. Performed the experiments: AT HA K. Miyaguchi. Analyzed the data: AT K. Mogushi HA K. Miyaguchi TK TF HT. Contributed reagents/materials/analysis tools: AT KN HM TM TF. Wrote the paper: AT.

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Original Article

A 48-week telaprevir-based triple combination therapy improves sustained virological response rate in previous non-responders to peginterferon and ribavirin with genotype 1b chronic hepatitis C: A multicenter study

Noritomo Shimada,[1] Akihito Tsubota,[2] Masanori Atsukawa,[3] Hiroshi Abe,[4] Tatsuya Ide,[5] Koichi Takaguchi,[6] Yoshimichi Chuganji,[7] Hidenori Toyoda,[8] Kai Yoshizawa,[9] Makiko Ika,[1] Yoshiyuki Sato,[1] Keizo Kato,[1] Takashi Kumada,[8] Choitsu Sakamoto,[10] Yoshio Aizawa[4] and Michio Sata[5]

[1]*Division of Gastroenterology and Hepatology, Shinmatsudo Central General Hospital, Matsudo,* [2]*Institute of Clinical Medicine and Research, Jikei University School of Medicine, Kashiwa,* [3]*Division of Gastroenterology, Department of Internal Medicine, Nippon Medical School Chiba Hokusoh Hospital, Chiba,* [4]*Division of Gastroenterology and Hepatology, Department of Internal Medicine, Jikei University School of Medicine Katsushika Medical Center,* [7]*Department of Gastroenterology, Tokyo Metropolitan Bokutoh Hospital,* [9]*Department of Gastroenterology, Machida Municipal Hospital,* [10]*Division of Gastroenterology, Department of Internal Medicine, Nippon Medical School, Tokyo,* [5]*Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Kurume,* [6]*Department of Hepatology, Kagawa Prefectural Central Hospital, Takamatsu, and* [8]*Department of Gastroenterology, Ogaki Municipal Hospital, Ogaki, Japan*

Aim: The sustained virological response (SVR) rate of non-responders to peginterferon and ribavirin therapy (PR) is low for 24-week telaprevir-based triple combination therapy (T12PR24), compared to that of treatment-naïve patients or previous-treatment relapsers. This study investigated which characteristics of non-responders were associated with a better SVR rate to 48-week therapy (T12PR48).

Methods: A total of 103 Japanese non-responders with genotype 1b chronic hepatitis C received telaprevir-based therapy. Among them, 81 patients (50 partial and 31 null responders) received T12PR24 and 22 (seven partial and 15 null responders) who agreed to the extended therapy received T12PR48.

Results: Multivariate logistic regression analysis for SVR identified the interleukin-28B (*IL28B*) rs8099917 TT genotype ($P = 0.0005$, odds ratio [OR] = 10.38), extended rapid virological response ($P = 0.0008$, OR = 7.02), T12PR48 regimen ($P = 0.0016$, OR = 9.31) and previous partial responders ($P = 0.0022$, OR = 5.89). Among partial responders, the SVR rate did not differ significantly between T12PR48 (85.7%) and T12PR24 (70.0%). Among null responders, the SVR rate was significantly higher with T12PR48 than T12PR24 (66.7% vs 22.6%, $P = 0.0037$). Among patients with the *IL28B* non-TT genotype, the SVR rate was significantly higher with T12PR48 than T12PR24 (68.8% vs 37.7%, $P = 0.0288$). Moreover, among null responders with the non-TT genotype, the SVR rate was significantly higher with T12PR48 than T12PR24 (66.7% vs 9.1%, $P = 0.0009$).

Conclusion: T12PR48 improves the SVR rate in null responders, patients with the non-TT genotype, and null responders with a non-TT genotype.

Key words: 48-week therapy, chronic hepatitis C, non-responders, telaprevir

Correspondence: Dr Noritomo Shimada, Division of Gastroenterology and Hepatology, Shinmatsudo Central General Hospital, 1-380 Shinmatsudo, Matsudo, Chiba 270-0034, Japan. Email: noritomos@jcom.home.ne.jp
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INTRODUCTION

IN 2011, THE two first-generation direct-acting antiviral agents (DAA), telaprevir (TVR) and boceprevir, were approved for the treatment of chronic hepatitis C (CHC) patients with hepatitis C virus (HCV) genotype 1 in several countries. Triple combination therapy with

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TVR or boceprevir, peginterferon-α and ribavirin is the current standard of care for genotype 1 CHC patients.[1] TVR, a non-structural (NS)3/4A serine protease inhibitor, was approved in Japan and has been available since November 2011. At present, the treatment of CHC has entered a new era with the introduction of potent DAA.

Peginterferon-α and ribavirin combination therapy (PR) has been the standard of care for CHC patients infected with HCV genotype 1 over the last 10 years. However, only 40–53% of patients achieve sustained virological response (SVR) even when including an extended 72-week therapy.[2–8] Among Japanese CHC patients treated with PR, approximately 25–31% were non-responders, which was defined as serum HCV RNA never disappearing during PR.[5–8]

Meanwhile, in treatment-naïve genotype 1 CHC patients, TVR-based triple combination therapy for a shortened period of 24 weeks (i.e. telaprevir, peginterferon-α and ribavirin for 12 weeks followed by an additional 12 weeks PR; T12PR24) is reported to remarkably improve the SVR rate compared to PR alone.[9–11] In treatment-experienced patients, the outcomes of TVR-based therapy depend on their previous response to interferon-based therapy.[12–17] In patients with previous relapses, T12PR24 dramatically improved the SVR rate in clinical trials.[12–15,17–19] In Japan, the SVR rate of previous relapsers treated with T12PR24 was very high with more than approximately 90% in clinical practice.[20–24] On the other hand, in non-responders including partial and null responders to previous PR, the SVR rate with T12PR24 was only approximately 40%.[12,16,21–25] In particular, previous studies in Japan showed that the SVR rate of null responders treated with T12PR24 was extremely low at less than 20%.[16,22–25] Even when treated with a 48-week regimen of triple combination therapy with or without a 4-week PR lead-in regimen (pooled T12PR48), the SVR rate was lower in null responders than partial responders.[13] Based on these results, T12PR48 is recommended for non-responders to previous PR in the USA, Canada and the EU. However, in Japan, generally, T12PR24 is recommended because clinical trials were performed only in the T12PR24 regimen. Therefore, it is unclear whether T12PR48 improves the SVR rate of Japanese non-responders to PR compared to T12PR24.

Accordingly, this study investigated which characteristics of non-responders to previous PR are associated with the improvement of SVR rate with extended T12PR48.

METHODS

Patients

BETWEEN DECEMBER 2011 and March 2013, 456 consecutive Japanese genotype 1b-infected CHC patients received TVR-based triple combination therapy at the study hospitals. Among all patients, 103 non-responders to PR were enrolled in this multicenter study.

The inclusion criteria were as follows: (i) diagnosis of CHC; (ii) persistently positive sera for HCV RNA for more than 6 months determined by quantitative real-time polymerase chain reaction (PCR) method (COBAS AmpliPrep/COBAS TaqMan HCV Test; Roche Diagnostics, Tokyo, Japan); (iii) HCV genotype 1b confirmed by sequence analysis; (iv) non-responders to previous PR in whom HCV RNA never disappeared during PR after 24 weeks of therapy; (v) aged 18–75 years; and (vi) bodyweight of more than 35 kg at the time of entry into the study. The exclusion criteria were as follows: (i) decompensated cirrhosis; (ii) positive for hepatitis B surface antigen or antibodies against HIV; (iii) previous or current development of hepatocellular carcinoma; (iv) coexistence of other liver diseases such as autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, Wilson's disease and alcoholic liver disease; (v) renal disease or creatinine clearance of 50 mL/min or less at baseline; (vi) hemoglobin level of less than 12 g/dL, white blood cell count of less than 2000/μL, neutrophil count of less than 1500/μL and platelet count of less than 8.0×10^4/μL at baseline; (vii) depression, schizophrenia or history thereof, or history of suicide attempts; and (viii) pregnancy in progress or planned for either partner during the study period. Liver biopsy was performed in 80 of 103 (77.7%) patients within 12 months of enrollment. The presence or absence of cirrhosis was established according to the METAVIR score.[26] For the remaining 23 patients, the presence or absence of cirrhosis was determined by ultrasonography and/or computed tomography findings.

The patients were divided into two categories according to the Japan Society of Hepatology guidelines.[27] Partial responders were defined as having a decrease in HCV RNA of 2 $\log_{10}$ IU/mL or more from baseline at treatment week 12 but detectable at treatment week 24, and null responders were defined as having a decrease in HCV RNA of less than 2 $\log_{10}$ IU/mL at treatment week 12.

Treatments

Telaprevir (Telavic; Mitsubishi Tanabe Pharma, Osaka, Japan) was administrated every 8 h after meals

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(q8h, 500 or 750 mg) or every 12 h after meals (q12h, 750 or 1125 mg). The initial daily dose of TVR (1500 or 2250 mg/day) and administration intervals (q8h or q12h) were determined by each attending physician according to age, sex, bodyweight and hemoglobin level. Peginterferon-α-2b (PEG-Intron; MSD, Tokyo, Japan) was injected s.c. at a median dose of 1.5 μg/kg per week. Ribavirin (Rebetol; MSD) dose was adjusted according to bodyweight (600, 800 and 1000 mg for <60, ≥60 to <80, and ≥80 kg, respectively). In patients with hemoglobin level of less than 13 g/dL at the start of therapy, ribavirin dose was reduced by 200 mg in accordance with the general consensus statements.[28] Triple therapy was administrated for 12 weeks, followed by an additional 12 weeks of peginterferon-α-2b and ribavirin combination therapy (T12PR24) or 36 weeks of peginterferon-α-2b and ribavirin (T12PR48) in patients who agreed to the extended therapy. The administration of each drug was appropriately reduced or withdrawn if a serious adverse event occurred or was suspected to be developing during the course of treatment. Treatment was stopped for patients with HCV RNA of more than 3 $\log_{10}$ IU/mL at week 4, detectable HCV RNA at week 12 or a more than 2 $\log_{10}$ IU/mL increase in HCV RNA levels from the lowest level during therapy irrespective of adverse events because of the low likelihood of achieving an SVR and high likelihood of developing antiviral resistance.

Definitions of outcomes

Virological response was analyzed on an intent-to-treat basis. The successful end-point of treatment was SVR for patients showing undetectable HCV RNA for 24 weeks after treatment cessation. Relapse was defined as when HCV RNA levels became undetectable by the end-of-treatment but became positive during the follow-up period. Viral breakthrough (VBT) was defined as when HCV RNA became undetectable during the treatment period but then became positive before the end of the treatment period. Non-response was defined as when HCV RNA was detectable throughout the treatment period. Extended rapid virological response (eRVR) was defined as undetectable HCV RNA at both weeks 4 and 12 after starting treatment.

All patients provided written informed consent. The study protocol conformed to ethics guidelines established in adherence with the 2008 Declaration of Helsinki and was approved by the ethics committee of each participating institution.

Measurement of HCV RNA and amino acid substitution in the core and NS5A regions of HCV genotype 1b

Hepatitis C virus genotype was determined by direct sequencing followed by phylogenic analysis of the NS5B region.[29] The antiviral effects of therapy on HCV were assessed by measuring serum HCV RNA levels during treatment at least once every 4 weeks before, during and after therapy. HCV RNA levels 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.

Core amino acid substitution at position 70 was determined as described previously.[30] Core amino acid substitution at position 70 was defined as wild type (i.e. arginine) or mutant (i.e. glutamine or histidine). In addition, substitutions at amino acids 2290–2248 of the NS5A region (interferon-sensitivity determining region) were determined as described previously.[31] Amino acid substitutions in this region were defined as wild type (0 or 1) or non-wild type (≥2).

Single-nucleotide polymorphism genotyping

Genomic DNA was extracted from whole blood using the MagNA Pure LC and a DNA Isolation Kit (Roche Diagnostics). The genetic polymorphism near the interleukin-28B (*IL28B*) gene,[32,33] rs8099917, was genotyped by real-time PCR using the TaqMan SNP Genotyping Assays and the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The rs8099917 genotypes were classified as TT (major genotype) or non-TT (minor genotype: TG or GG).

Statistical analysis

Continuous variables are expressed as means and standard deviations. Continuous data were analyzed using the non-parametric Mann–Whitney *U*-test. Categorical data were analyzed using the χ^2-test with a Yates correction or Fisher's exact test. Univariate and multivariate logistic regression analyses were performed to identify factors that significantly contributed to SVR. Odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. All *P*-values for statistical tests were two-tailed. The levels of significance and marginal significance were set at $P < 0.05$ and $P < 0.15$, respectively. Variables showing statistical or marginal significance in univariate analysis were entered into multivariate logistic regression analyses to identify significant independent predictive factors of SVR. All statistical analyses

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were performed using SPSS version 17.0 (IBM-SPSS, Chicago, IL, USA). In this study, the adherence to each drug was excluded for the difference of treatment duration and the stopping rules.

RESULTS

Patient characteristics and treatment outcomes

PATIENT CHARACTERISTICS ARE summarized in Table 1. Of the 103 patients, 57 (55.3%) and 46 (44.7%) were partial and null responders, respectively. Partial responders had significantly higher platelet counts than null responders ($P = 0.0126$). α-Fetoprotein levels were significantly lower in partial responders than null responders ($P = 0.0202$). No other baseline factors differed significantly between groups.

Regarding treatment outcomes of TVR-based triple combination therapy, 58 patients (56.3%) achieved SVR, 23 (22.3%) showed relapse, 16 (15.5%) showed VBT and six (5.8%) showed non-response. Patients were stratified according to previous treatment response and regimen. Among the 50 partial responders treated with T12PR24, 35 (70.0%) achieved SVR, 12 (24.0%) showed relapse and three (6.0%) showed VBT. Among the seven partial responders treated with T12PR48, six (85.7%) achieved SVR and one (14.3%) showed VBT. Among all partial responders, the SVR rate was slightly higher with T12PR48 than T12PR24 (6/7 [85.7%] vs 35/50 patients [70.0%]), though not statistically significant ($P = 0.6763$) (Fig. 1).

Among the 31 null responders treated with T12PR24, seven patients (22.6%) achieved SVR, seven (22.6%) showed relapse, 11 (35.5%) showed VBT and six (19.4%) showed non-response. Among the remaining 15 patients treated with T12PR48, 10 (66.7%) achieved SVR, four (26.7%) showed relapse and one (6.7%) showed VBT. Among all null responders, the SVR rate was significantly higher with T12PR48 than T12PR24 (10/15 [66.7%] vs 7/31 patients [22.6%], $P = 0.0037$) (Fig. 1).

Besides treatment regimens, the SVR rate was significantly higher in partial responders than null responders (41/57 [71.9%] vs 17/46 patients [37.0%], $P = 0.0004$).

Predictive factors associated with SVR

The results of the univariate and multivariate analyses are shown in Table 2. In univariate analysis, the following

Table 1 Characteristics of patients

Variables	Partial responders	Null responders	*P*-value
No. of patients	57	46	
Sex (male/female) (male %)	37/20 (64.9%)	21/25 (45.7%)	0.0501
Age (years)	59.2 ± 8.0	56.9 ± 9.8	0.2575
Bodyweight (kg)	64.0 ± 12.4	62.5 ± 12.8	0.3740
Body mass index (kg/m^2)	23.6 ± 3.1	23.5 ± 3.3	0.6072
Absence or presence of cirrhosis (non-cirrhosis/cirrhosis) (cirrhosis %)	40/17 (29.8%)	27/19 (41.3%)	0.2245
rs 8099917 (TT/TG/GG) (TT %)	22/34/1 (38.6%)	12/32/2 (26.1%)	0.3353
White blood cells (/μL)	4835 ± 1461	4698 ± 1396	0.5886
Hemoglobin (g/dL)	14.1 ± 1.5	14.0 ± 1.5	0.6566
Platelets (×10^4/μL)	17.6 ± 6.3	14.8 ± 5.6	0.0126
Aspartate aminotransferase (IU/L)	53 ± 32	61 ± 29	0.0632
Alanine aminotransferase (IU/L)	58 ± 38	66 ± 41	0.1748
γ-Glutamyltransferase (IU/L)	70 ± 67	86 ± 83	0.1006
α-Fetoprotein (ng/mL)	16.2 ± 34.7	20.6 ± 28.6	0.0202
HCV RNA (log 10 IU/mL)	6.1 ± 1.2	6.6 ± 0.6	0.0513
Core amino acid substitution 70 (wild type/mutant type)	31/26 (54.4%)	18/28 (39.1%)	0.1233
ISDR of NS5A (wild type/non-wild type)	49/8 (86.0%)	43/3 (93.5%)	0.3647
Initial dose of peginterferon-α-2b (μg /kg)	1.5 ± 0.2	1.5 ± 0.2	0.2235
Initial dose of ribavirin (mg/kg)	11.0 ± 1.9	11.0 ± 1.9	0.7933
Initial daily dose of telaprevir (2250/1500 mg)	30/27	22/24	0.6277
Administration intervals of telaprevir (q8h/q12q)	30/27	29/17	0.2882

Data are expressed as numbers or means ± standard deviations.
HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; NS, non-structural.

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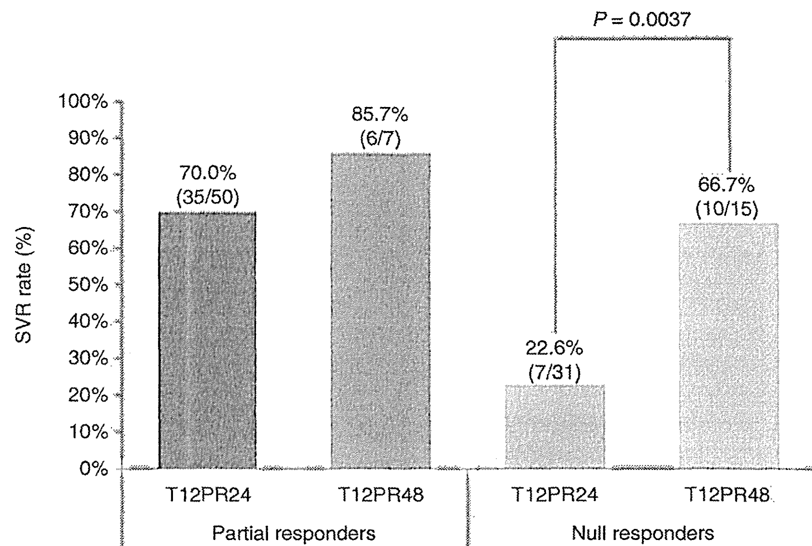

Figure 1 SVR rate according to response to previous peginterferon and ribavirin combination therapy and treatment regimen. SVR, sustained virological response; T12PR48, 12 weeks of triple combination therapy followed by 36 weeks of peginterferon and ribavirin combination therapy; T12PR24, 12 weeks of triple combination therapy followed by 12 weeks of peginterferon and ribavirin combination therapy.

factors were significantly associated with SVR: previous partial responders ($P = 0.0005$); *IL28B* TT genotype ($P = 0.0015$); wild-type core amino acid substitution at position 70 ($P = 0.0118$); eRVR ($P = 0.0002$); and higher white blood cell count ($P = 0.0418$), platelet count ($P = 0.0132$) and lower aspartate aminotransferase ($P = 0.0126$). Furthermore, in univariate analysis, the following factors were marginally associated with SVR: absence of cirrhosis ($P = 0.1220$); T12PR48 regimen ($P = 0.0858$); higher hemoglobin level ($P = 0.0813$), initial dose of peginterferon-α-2b ($P = 0.1237$) and initial daily dose of TVR ($P = 0.1411$); and lower alanine aminotransferase ($P = 0.1418$), γ-glutamyltransferase ($P = 0.0954$) and HCV RNA levels ($P = 0.0803$). The abovementioned variables were entered into the multivariate logistic regression analysis, and the following four independent factors were identified: *IL28B* TT genotype ($P = 0.0005$, OR = 10.38, 95% CI = 2.78–38.84), eRVR ($P = 0.0008$, OR = 7.02, 95% CI = 2.25–21.97), T12PR48 regimen ($P = 0.0016$, OR = 9.31, 95% CI = 2.32–37.38) and previous partial responders ($P = 0.0022$, OR = 5.89, 95% CI = 1.89–13.31) (Table 2).

SVR rate stratified by treatment regimen and *IL28B* genotype

Patients were subsequently stratified according to previous treatment response and treatment regimen. Among partial responders treated with T12PR24, the SVR rate was significantly higher in patients with the TT genotype than those with the non-TT genotype (17/19 [89.5%] vs 18/31 patients [58.1%], $P = 0.0262$). On the other hand, among those treated with T12PR48, the SVR rate did not differ significantly with respect to the *IL28B* genotype (3/3 [100%] vs 3/4 patients [75%], $P = 1.0000$). Among those with the non-TT genotype, the SVR rate was higher in T12PR48 than in T12PR24 (3/4 [75%] vs 18/31 patients [58.1%]) though not statistically significant ($P = 0.6350$).

Among null responders treated with T12PR24, the SVR rate was significantly higher in patients with the TT genotype than those with the non-TT genotype (5/9 [55.6%] vs 2/22 patients [9.1%], $P = 0.0118$). On the other hand, among those treated with T12PR48, the SVR rate was not significantly different between patients with the TT and non-TT genotype (2/3 [66.7%] vs 8/12 patients [66.7%], $P = 1.0000$). Among patients with the non-TT genotype, the SVR rate was significantly higher with T12PR48 than T12PR24 (8/12 [66.7%] vs 2/22 patients [9.1%], $P = 0.0009$) (Fig. 2).

Apart from previous treatment response, there was no significant difference in the SVR between patients with an *IL28B* TT genotype who received T12PR48 and T12PR24 (5/6 [83.3%] vs 22/28 patients [78.6%], $P = 1.0000$). In contrast, among patients with a non-TT genotype, the SVR rate was significantly higher with T12PR48 than T12PR24 (11/16 [68.8%] vs 20/53 patients [37.7%], $P = 0.0288$).

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Table 2 Factors associated with sustained virological response

Variable	Univariate			Multivariate		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Host-related factor						
Sex male vs female	1.46	0.66–3.20	0.3495			
Age (years)	0.99	0.95–1.03	0.6050			
Bodyweight (kg)	1.01	0.98–1.04	0.5460			
Body mass index (kg/m^2)	1.01	0.89–1.14	0.8802			
Cirrhosis absence vs presence	1.92	0.84–4.38	0.1220			
Partial responders vs null responders	4.37	1.90–10.04	0.0005	5.89	1.89–13.31	0.0022
rs8099917 TT vs non-TT	4.73	1.82–12.31	0.0015	10.38	2.78–38.84	0.0005
White blood cells (/μL)	1.00	1.00–1.00	0.0418			
Hemoglobin (g/dL)	1.27	0.97–1.66	0.0813			
Platelets (×10^4/μL)	1.09	1.02–1.17	0.0132			
Aspartate aminotransferase (IU/L)	0.98	0.97–1.00	0.0126			
Alanine aminotransferase (IU/L)	0.99	0.98–1.00	0.1418			
γ-Glutamyltransferase (IU/L)	1.00	0.99–1.00	0.0954			
α-Fetoprotein (ng/mL)	0.99	0.98–1.01	0.2280			
Virus-related factor						
HCV RNA ($\log_{10}$ IU/mL)	0.67	0.42–1.05	0.0803			
Core amino acid substitution 70 wild type vs mutant type	2.83	1.26–6.37	0.0118			
ISDR of NS5A non-wild type vs wild type	2.24	0.56–8.98	0.2551			
Treatment-response factor						
eRVR, + vs –	5.18	2.21–12.13	0.0002	7.02	2.25–21.97	0.0008
Treatment-related factor						
Initial dose of peginterferon-α-2b (μg/kg)	5.47	0.63–47.68	0.1237			
Initial dose of ribavirin (mg/kg)	1.13	0.92–1.39	0.2507			
Initial daily dose of telaprevir (2250/1500 mg)	1.81	0.82–3.97	0.1411			
Administration intervals of telaprevir (q8h/q12q)	0.70	0.32–1.54	0.3727			
T12PR48 vs T12PR24	2.48	0.88–6.97	0.0858	9.31	2.32–37.38	0.0016

CI, confidence interval; eRVR, extended rapid virological response; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; NS, non-structural; OR, odds ratio; T12PR24, 12 weeks of triple combination therapy followed by 12 weeks of peginterferon and ribavirin therapy; T12PR48, 12 weeks of triple combination therapy followed by 36 weeks of peginterferon and ribavirin therapy.

SVR rate stratified by eRVR according to treatment regimen and *IL28B* genotype

The SVR rates of the patients described above and depicted in Figure 2 were further analyzed after stratification by eRVR. Among partial responders with the *IL28B* TT genotype treated with T12PR24, there was no significant difference in the SVR rate between patients who achieved eRVR and those who did not (13/14 [92.9%] vs 4/5 patients [80.0%], $P = 0.4678$). Among patients with the non-TT genotype treated with T12PR24, patients who achieved eRVR tended to have a higher SVR rate than those who did not achieve eRVR, although the difference was not significant (16/24 [66.7%] vs 2/7 patients [28.6%], $P = 0.0994$). Among the patients with the TT genotype treated with T12PR48, all three patients who achieved eRVR exhibited SVR. Meanwhile, among patients with the non-TT genotype treated with T12PR48, the SVR rate did not differ significantly between those who achieved eRVR and those who did not (1/1 [100%] vs 2/3 patients [66.7%], $P = 1.0000$) (Fig. 3).

In addition, among null responders with the TT genotype treated with T12PR24, the SVR rate tended to be higher in patients who achieved eRVR than those who did not (3/3 [100%] vs 2/6 patients [33.3%], $P = 0.1667$). Among patients with the non-TT genotype treated with T12PR24, the SVR rate tended to be higher in patients who achieved eRVR than those who did not (2/8 [25.0%] vs 0/14 patients [0%], $P = 0.1212$). Among those with the TT genotype treated with T12PR48, two of three (66.7%) patients who did not

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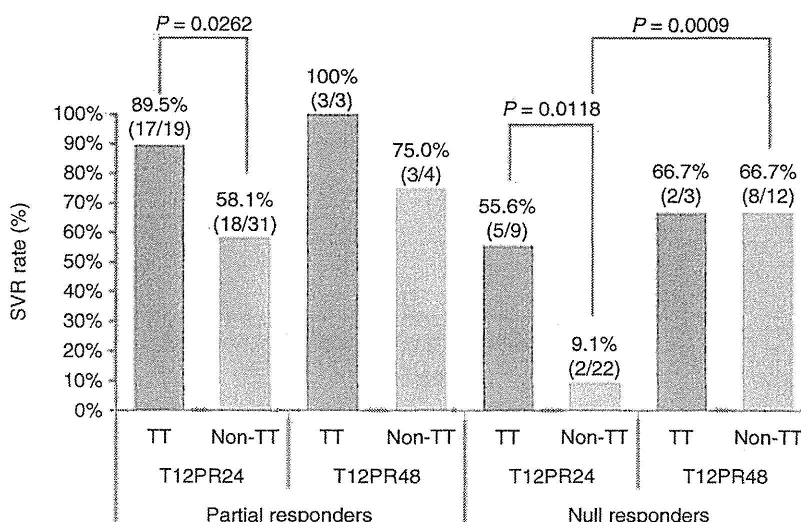

Figure 2 SVR rate stratified by treatment regimen and *IL28B* (rs8099917) genotype. IL, interleukin; SVR, sustained virological response; T12PR24, 12 weeks of triple combination therapy followed by 12 weeks of peginterferon and ribavirin combination; T12PR48, 12 weeks of triple combination therapy followed by 36 weeks of peginterferon and ribavirin combination therapy.

achieve eRVR achieved SVR. Among patients with the non-TT genotype treated with T12PR48, the SVR rate tended to be higher in patients who achieved eRVR than those who did not (6/8 [75.0%] vs 2/4 patients [50.0%], $P = 0.5475$) (Fig. 4).

DISCUSSION

THIS STUDY IS the first report indicating that T12PR48 regimen results in a significantly higher SVR rate for non-responders to previous PR than T12PR24 in Japan. Several reports showed that the SVR rate with T12PR24 for previous non-responders to PR was low, ranging 27–46%.[12,16,21–25] Furthermore, there was a remarkable difference in the SVR rate with T12PR24 between previous partial and null responders in Japan.[16,22–25] The REALIZE study revealed that the SVR rate of partial responders (56.7%) was superior to that of null responders (31.3%) even if null responders were treated with pooled T12PR48 (including T12PR48 and lead-in T12PR48).[13] Muir *et al.* reported the SVR rates in null responders with HCV genotype 1b treated with T12PR24 and T12PR48 were 12.5% (1/8) and 60% (6/10), respectively. Moreover, the SVR rate was 71.4% (5/7) in partial responders with genotype 1b treated with response-guided therapy, namely, patients who

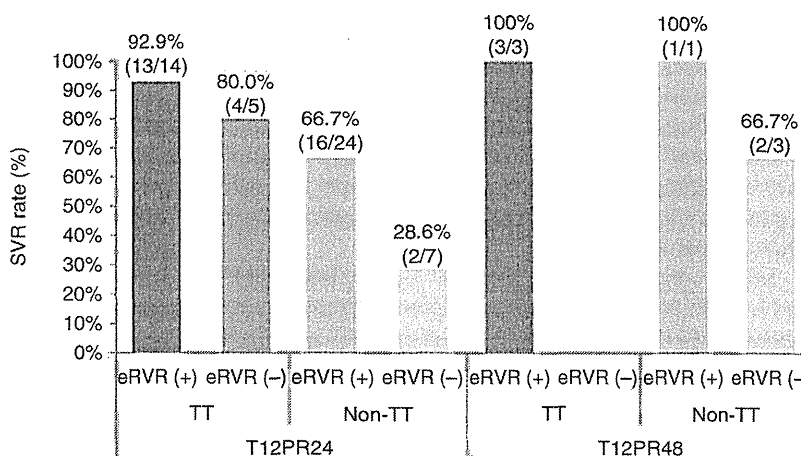

Figure 3 SVR rate in partial responders stratified by eRVR according to treatment regimen and *IL28B* genotype. eRVR, extended rapid virological response; IL, interleukin; SVR, sustained virological response; T12PR24, 12 weeks of triple combination therapy followed by 12 weeks of peginterferon and ribavirin combination; T12PR48, 12 weeks of triple combination therapy followed by 36 weeks of peginterferon and ribavirin combination therapy.

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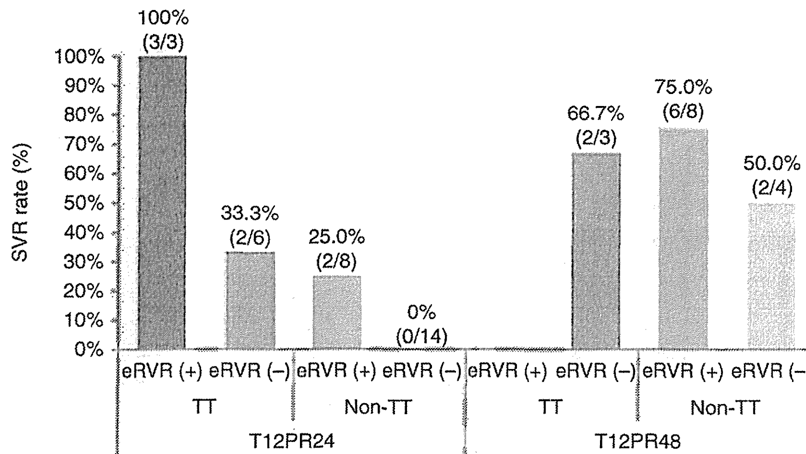

Figure 4 SVR rate in null responders stratified by eRVR according to treatment regimen and *IL28B* genotype. eRVR, extended rapid virological response; IL, interleukin; SVR, sustained virological response; T12PR24, 12 weeks of triple combination therapy followed by 12 weeks of peginterferon and ribavirin combination; T12PR48, 12 weeks of triple combination therapy followed by 36 weeks of peginterferon and ribavirin combination therapy.

achieved and did not achieve eRVR were treated with T12PR24 and T12PR48, respectively.[15] These results suggest that approximately 70% of partial responders may achieve SVR using response-guided therapy, but the SVR rate was extremely low in null responders treated with T12PR24. Although the study of Muir *et al.* was not a randomized controlled trial study, their results indicated that T12PR48 may improve the SVR rate in null responders to a greater extent than T12PR24.[15] The present study revealed the SVR rates of partial and null responders treated with T12PR24 were 70.0% and 22.6%, respectively. The lower SVR rate in null responders than partial responders is concordant with the results of the previous study on T12PR24.[15] To our knowledge, besides our previous reports,[22–24] only two studies performed in Japan have analyzed non-responders to PR classified as partial and null responders.[16,25] Akuta *et al.* reported that the SVR rates in partial and null responders treated with T12PR24 in clinical trials were 50% (4/8) and 0% (0/7), respectively.[16] Meanwhile, Ogawa *et al.* recently reported that the SVR rates in partial and null responders for CHC patients with advanced fibrosis (METAVIR score F3–4) treated with T12PR24 in clinical practice were 50% (9/18) and 16.7% (2/12), respectively.[25] Similarly, the SVR rate was lower in null responders than partial responders in the present study, when treated with T12PR24.

Genetic variations near the *IL28B* gene (rs8099917 and rs12979860) are strongly associated with treatment outcome of PR.[32–34] In addition, these genetic variations are also strong predictors of SVR with the T12PR24 regimen when including both treatment-naïve and treatment-experienced patients.[14,20–25,35] However, Pol *et al.* reported the *IL28B* genotype (rs12979860) has a limited and non-significant impact on SVR in treatment-experienced patients treated with T12PR48 regardless of relapsers, partial responders or null responders to previous PR.[19] In the present study, the SVR rate of partial responders with the TT genotype treated with T12PR24 was very high at approximately 90%, whereas that in patients with the non-TT genotype was significantly lower. Similarly, among null responders, the SVR rate was significantly lower in those with the non-TT genotype than those with the TT genotype. Ogawa *et al.* reported a similar trend that among previous partial and null responders treated with T12PR24, the SVR rate was lower in patients with the non-TT genotype than those with the TT genotype.[25] In contrast, the SVR rate did not differ significantly between either partial or null responders with the *IL28B* TT or non-TT genotype treated with T12PR48 in our study, although there were too few patients to make a meaningful comparison. Thus, the present and previous[25] results possibly indicated that *IL28B* genotype still affects SVR with T12PR24 but the impact of *IL28B* genotype is attenuated with T12PR48. Thus, the present results suggested that *IL28B* genotype is a strong pretreatment predictor of SVR in non-responders to previous PR treated with either T12PR24 or T12PR48. Nevertheless, the SVR rate was lower in null responders than partial responders treated with T12PR24 or T12PR48 even in patients had the same genotype. Therefore, the present results suggested that response to previous PR is a better predictor of SVR than *IL28B* genotype like the previous study.[19] However, in actual clinical practice, it may be impossible to differentiate between previous null and partial responders

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in some cases because of the absence of relevant data from medical records. Therefore, in such treatment-experienced patients, *IL28B* genotyping may have clinical utility by serving as a pretreatment marker of interferon responsiveness and treatment duration to guide physicians.

This study also demonstrated that eRVR is a useful on-treatment predictive factor associated with SVR. In particular, partial responders with the *IL28B* TT genotype who achieved eRVR showed an extremely high SVR rate, and all three null responders with the TT genotype who achieved eRVR showed SVR with T12PR24. However, the SVR rate was very low in partial responders with the non-TT genotype who failed to achieve eRVR; no null responders with a non-TT genotype who failed to achieve eRVR showed SVR with T12PR24. This study revealed T12PR48 is a useful strategy for null responders and patients with an unfavorable *IL28B* non-TT genotype. Partial responders with the non-TT genotype had a very high relapse rate except for patients who achieved SVR (11/13, 84.6%; data not shown) with T12PR24. Therefore, partial responders with the TT genotype should be treated with T12PR24 regardless of achievement of eRVR. Meanwhile, partial responders with the non-TT genotype should be treated with T12PR48 regardless of achievement of eRVR in order to increase the SVR rate by decreasing the relapse rate. Furthermore, excluding VBT and non-response, null responders should be treated with T12PR48 irrespective of *IL28B* genotype and achievement of eRVR. However, all three null responders with the TT genotype who achieved eRVR showed SVR. Therefore, these patients might be treated with T12PR24. This study may provide useful information for treatment selection on the basis of previous treatment response, *IL28B* genotype and eRVR.

This study has some limitations that should be mentioned. First, there were too few patients to conclusively determine the factors contributing to SVR; in particular, only 22 patients were treated with T12PR48. Second, this study was not a randomized controlled trial. Accordingly, a randomized controlled trial randomizing partial and null responders to receive T12PR24 or T12PR48 should be conducted to corroborate the present findings.

The second-generation DAA simeprevir, a once-daily oral NS3/4A protease inhibitor, was approved in Japan and has been marketed since December 2013, ahead of the rest of the world. Furthermore, the guideline for CHC with HCV genotype 1 by the Japan Society of Hepatology was recently updated.[36] Zeuzem *et al.* recently studied simeprevir-based triple therapy for treatment-experienced patients. The SVR rates of partial and null responders with HCV genotype 1b who received simeprevir (100 mg once daily) for 12 weeks plus PR for 48 weeks were 68% and 56%, respectively.[37] Therefore, prospective studies are required to confirm whether a 48-week regimen of simeprevir-based therapy improves the SVR rate as well as T12PR48 in clinical practice.

In conclusion, this multicenter study demonstrated that the T12PR48 regimen improves the SVR rate in Japanese genotype 1b CHC patients who were previous non-responders to PR. T12PR48 improved the SVR rate to a greater extent than T12PR24, especially in null responders, those with the *IL28B* non-TT genotype, and patients with the unfavorable non-TT genotype. Further large-scale prospective studies including a 48-week simeprevir-based triple combination therapy are required to confirm the present findings, individualize treatment and optimize therapeutics.

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Effect of fluvastatin on 24-week telaprevir-based combination therapy for HCV genotype 1b-infected chronic hepatitis C

Masanori Atsukawa[a], Akihito Tsubota[b], Noritomo Shimada[c], Chisa Kondo[a], Norio Itokawa[a], Ai Nakagawa[a], Takeshi Fukuda[d], Yoko Matsushita[d], Yoshiyuki Narahara[d], Yuji Osada[e], Hiroki Yamaguchi[f], Katsuhisa Nakatsuka[d], Katsuhiko Iwakiri[a], Chiaki Kawamoto[d], Choitsu Sakamoto[d]

[a]Division of Gastroenterology, Department of Internal Medicine, Nippon Medical School Chiba Hokusoh Hospital, Inzai, Chiba, Japan

[b]Institute of Clinical Medicine and Research (ICMR), Jikei University School of Medicine, Kashiwa, Chiba, Japan

[c]Division of Gastroenterology and Hepatology, Shinmatsudo Central General Hospital, Matsudo, Chiba, Japan

[d]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Nippon Medical School, Bunkyo-ku, Tokyo, Japan

[e]Division of Gastroenterology, Hakujikai Memorial Hospital, Adachi-ku, Tokyo, Japan

[f]Division of Hematology, Department of Internal Medicine, Nippon Medical School,

Bunkyo-ku, Tokyo, Japan

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Atsukawa M and Tsubota A contributed equally to the preparation of this manuscript.

Authors declare there was no conflict of interest.

Correspondence:Masanori Atsukawa, Division of Gastroenterology, Department of Internal Medicine, Nippon Medical School Chiba Hokusoh Hospital 1715, Kamakari, Inzai, Chiba, 270-1694, Japan.

Phone: +81-476-99-1111

E-mail: momogachi@yahoo.co.jp

Abstract

Objectives The addition of fluvastatin significantly improves sustained virological response (SVR) in pegylated-interferon and ribavirin (peg-IFN/RBV) combination therapy for patients infected with hepatitis C virus (HCV). However, the add-on effect on telaprevir-based triple combination therapy remains unknown. The aim of this study was to investigate the effect of fluvastatin on telaprevir-based combination therapy by conducting a prospective, open-label, randomized, controlled trial.

Patients and Methods Among 124 genotype 1b-infected chronic hepatitis C patients recruited, 116 eligible patients were randomly allocated to two study arms which they received 12weeks of telaprevir/peg-IFN/RBV followed by 12weeks of peg-IFN/RBV with or without 24weeksof fluvastatin (fluvastatin group and control group, respectively). Treatment outcomes and adverse effects were compared between the two groups.

Results The subjects consisted of 56 males and 60 females, with median age of 60 years (range, 28 to 71 years). Rapid virological response and end of treatment response rates were 87.9% (51/58) and 96.6% (56/58) in the control group, and75.9% (44/58) and

98.3% (57/58) in the fluvastatin group, respectively. SVR rates in the control group and fluvastatin group were 84.5% (49/58) and 81.0% (47/58), respectively; there was no significant difference (p=0.806). Stratified analysis showed that no factors associated with the SVR rate were found between the two groups. No adverse events were associated with fluvastatin.

Conclusion In this trial, administration of fluvastatin with telaprevir/peg-IFN/RBV was a safe combination. However, fluvastatin had no add-on effect on 24-week telaprevir-based combination therapy for chronic hepatitis C genotype 1b-infected patients.

Keywords: fluvastatin, telaprevir, pegylated interferon, ribavirin, chronic hepatitis C

Introduction

Pegylated interferon and ribavirin (peg-IFN/RBV) therapy, in combination with protease inhibitors such as telaprevir or boceprevir, is currently the standard of care for genotype 1b chronic hepatitis C. The combination improves treatment outcomes, and sustained viral response (SVR) can be achieved in more than 70% of patients [1-3], especially in more than 80% of patients with the IL28B major genotype [4-6]. At the same time, there are patients in whom SVR cannot be obtained; therefore, further development and modification of treatment drugs are needed.

Previously, we demonstrated in a prospective, randomized, controlled trial that the SVR rate for genotype 1b chronic hepatitis C can be significantly increased by combining fluvastatin, a statin that is a hydroxymethylglutaryl (HMG)-coenzyme A (CoA) reductase inhibitor, with peg-IFN/RBV therapy [7, 8]. An add-on effect with fluvastatin has especially been seen in male patients and patients with the IL28B major genotype.

Statins inhibit cholesterol synthesis by suppressing mevalonic acid synthesis from HMG-CoA by HMG-CoA reductase in the cholesterol biosynthesis pathway. Low density lipoprotein (LDL)-cholesterol was reported to decrease 20–30% when