Table 3. Comparison of Vitamin D Concentrations Between the Control and Vitamin D Groups After Four Week of Alfacalcidol at Starting PEG-IFN/Ribavirin Combination Therapy [a]

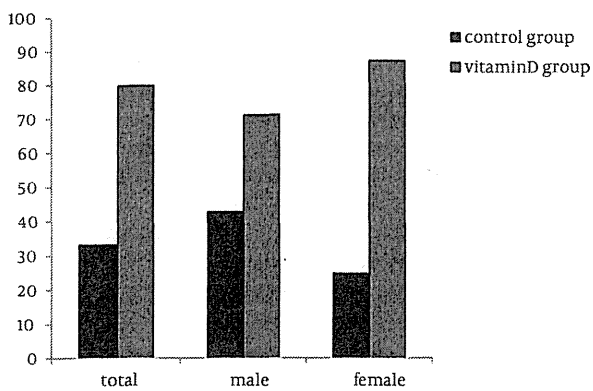
Variable	Vitamin D group (post [b])	Control group (pre [b])	P value [c]	difference
25 (OH) D3	27 (7-36)	25 (11-40)	0.371	2 (± 12)
1alpha, 25 (OH) 2D3	67 (30-136)	63 (23-97)	0.563	0 (± 44)

[a] Continuous variables are represented as median (range). Differences are represented as mean (± SD).
[b] Pre, baseline; post, after 4weeks of alfacalcidol.
[c] Mann-Whitney Test was performed to compare variables between two groups.

Serum vitamin D concentration was measured in the 30 study patients. Serum 25 (OH) D3 was 27 (13-40) ng/mL in males, and 20 (11-37) ng/mL in female (P = 0.004). Thus, the serum 25 (OH) D3 concentrations were significantly lower in females.

The SVR rates in the vitamin D and control groups were 80.0% (12/15) versus 33.3% (5/15) (P = 0.012, bootstrap method), respectively (Figure 2). Five (33.3%) control group patients discontinued treatment and were included in the analysis (intention-to-treat analysis). The reasons for discontinuance were: depression in 1, anorexia in 2, and poor response to treatment (NVR) in 2. Two vitamin D group patients who showed NVR to previous combination treatment discontinued due to poor response to treatment (NVR) regardless of the addition of alfacalcidol. No adverse events due to the addition of alfacalcidol were observed in the vitamin D group. Adverse events such as anemia, neutropenia, skin rash and eruption, gastrointestinal disorders including nausea and anorexia, and psychiatric disorders including insomnia were similar and mild between the two groups. Next, 13 patients in the vitamin D group and 10 patients in the control group completed the therapy as scheduled and were subjected to analysis (per protocol). The SVR rate was numerically higher (92.3%, 12/13) in the vitamin D group than the control group (50.0%, 5/10) (P = 0.055).

Figure 2. Comparison of the Rate of SVR Between Control Group and Vitamin D Group

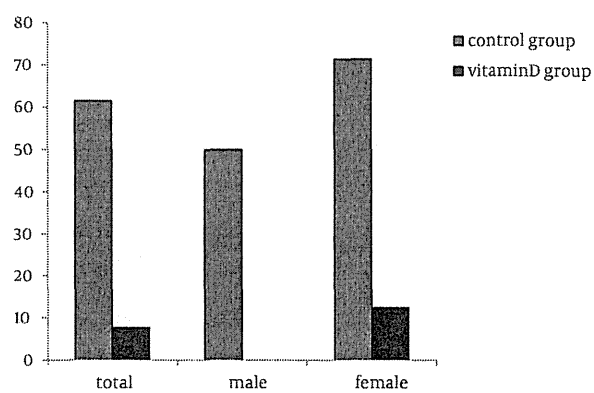


The results were analyzed by the Fisher's exact test, adopting the bootstrap method (10,000 times).

In the male patients, the rate of SVR in the vitamin D group was numerically higher than the control group, but the difference was not statistically significant (P = 0.415, bootstrap method) (Figure 2). Interestingly, in the females, the SVR rate was significantly higher in the vitamin D group (87.5%, 7/8) than the control group (25.0%, 2/8) (P = 0.020, bootstrap method) (Figure 2).

In the control group, relapse was shown after obtaining ETR in 8 patients, including 5 women (62.5%). The median serum 25 (OH) D3 concentration in these patients with relapse was 18 (11-29) ng/mL, lower than the median concentration for all patients. In the vitamin D group treated with alfacalcidol supplementation, a significant decrease was shown in the relapse rate after ETR had been obtained (7.7% vs. 61.5%, P = 0.004, bootstrap method) (Figure 3). Especially, in females, the relapse in the control group was shown in 71.4% (5/7), whereas in the vitamin D group the relapse rate was decreased in 12.5% (1/8) (P = 0.030, bootstrap method) (Figure 3).

Figure 3. Comparison of the Rate of Relapse Between Control Group and Vitamin D Group



The results were analyzed by the Fisher's exact test, adopting the bootstrap method (10,000 times).

In the vitamin D group, serum 1alpha, 25 (OH) 2D3 concentration numerically increased after administration of alfacalcidol for 4weeks, although the increase was not statistically significant (63.0 pg/mL and 67.0 pg/mL, respectively; P > 0.999). The lead-in duration of 4 weeks may be too short to observe the elevation of serum 1alpha,

25 (OH) 2D3 concentration (Table 2). Moreover, serum 25 (OH) D3 concentration did not apparently increase (22.0 ng/mL and 27.0 ng/mL, respectively; P = 0.352). One possible explanation for this finding is that alfacalcidol is directly metabolized into its active form 1alpha, 25 (OH) 2D3 in the liver (Table 2).

The rates of total adherence to PEG-IFN were not significantly different between the vitamin D (mean 95.4%, range 70%-100%) and control groups (95.8%, 60%-100%) (P = 0.427). The median dose of total adherence to ribavirin was 8.95 mg/kg/day (range, 3.95-13.04 mg/kg/day) in the vitamin D group, and 9.05 mg/kg/day (3.92-12.31 mg/kg/day) in the control group (P > 0.999).

5. Discussion

The present study is the first to analyze the effects of PEG-IFN/ ribavirin therapy combined with alfacalcidol as vitamin D source on elderly patients with chronic hepatitis C infection of high-viral-load genotype 1b. Although there have been some reports on the outcome of combination therapy with vitamin D (18, 24), our report focuses on the treatment response of elderly subjects, who were 65-78 years of age (median, 70 years). Moreover, in other studies patients were given vitamin D3 as vitamin D supplementation, in this study patients were given alfacalcidol [1alpha (OH)D3] as vitamin D source. Alfacalcidol is metabolized rapidly to 1, 25(OH) 2D3 in the liver. 1, 25 (OH) 2D3 is known to be the active form of vitamin D3 and mediates biological activities.

Elderly patients are likely to have low serum vitamin D levels caused by aging and long disease duration. In fact, serum vitamin D levels have been reported to decrease with age and the progression of liver fibrosis (19). Holick et al. defined ≥ 30 ng/mL of serum 25 (OH) D3 as sufficiency, 21–29 ng/mL as insufficiency, and ≤ 20 ng/mL as deficiency (25). In the present study, the serum 25 (OH) D3 level was deficient or insufficient in 86.7% of the study elderly patients with chronic hepatitis C according to this definition, and sufficient in only a small percentage (13.3%).

Several recent reports have documented the direct anti-HCV effects of vitamin D. In an in vitro study using Huh7.5 cells, the addition of vitamin D and its metabolite 1α, 25 (OH) 2D3 reduced secretion of HCV from hepatocytes into the cell medium; this effect was synergistically enhanced by IFN administration (26). In another in vitro study using HCV-JFH1, 25 (OH) D3 decreased the amount of HCV core protein by suppressing the formation of HCV particles (27).

The results of this study suggest that the addition of alfacalcidol could potentially suppress relapse in patients with low serum vitamin D concentration, particularly elderly females. Since treatment-induced anemia occurs more frequently and more severely in the elderly than younger people (28), the total dose of ribavirin may be reduced in elderly subjects. As mentioned earlier, the reduction of total dose of ribavirin is an important factor contributing to viral relapse after the completion of PEG-IFN/ribavirin therapy (29). The results suggest that the addition of alfacalcidol as vitamin D source may potentially improve the therapeutic outcome of such elderly patients who showed relapse after previous combination treatment due to reduced drug adherence.

This study also showed that alfacalcidol combination therapy had some limitations. In elderly patients with the difficult-to-treat IL28B SNP minor genotype or who showed NVR to previous combination treatment, even the addition of vitamin D had little effect on the improvement of the SVR rate. Two patients with pretreatment non-response by IFN therapy in the vitamin D group also showed non-virological response. Moreover, SVR rate among patients with IL28B minor genotype in the vitamin D group was 33.3% (1 of 3). Alternatively, long-term IFN therapy with low doses is recommended to prevent the development of hepatocellular carcinoma (30-32).

As for gender difference, the average serum 25 (OH) D3 concentration of female elderly patients was significantly lower than male elderly patients (median; 20 ng/mL versus 27 ng/mL) in this study. It has been reported that females of age 55 years and over have lower serum 25 (OH) D3 concentration than females younger than 55 years, and males do not differ in serum 25 (OH) D3 concentration between the two age groups (19). The addition of vitamin D may be beneficial to female elderly patients with low serum vitamin D concentration, because Japanese elderly patients have poor response to the conventional combination therapy (33, 34). By extension, the addition of vitamin D for patients with low serum vitamin D concentration is expected to further improving the outcome of PEG-IFN/ ribavirin therapy in combination with protease inhibitors such as telaprevir.

The present study had some limitations; these included a small number of subjects and lack of a randomized controlled design. Although sample size was too small, patients were matched for gender, age and IL28B genotype for the control group. As a result, strong predictors for PEG-IFN/ribavirin combination therapy such as IL28B genotype (21-23) and core amino acid 70 substitutions (35) were similar in the two groups. Moreover, analysis was performed, adopting the bootstrap method. Next, the serum vitamin D concentration was measured at the start of therapy in various seasons. It has been reported that the serum vitamin D concentration is higher in summer and autumn than winter and spring.

In this study, alfacalcidol was orally administered at 1 μg/day as a vitamin D supplement. Since it is directly metabolized into its active form 1alpha, 25 (OH) 2D3 in the liver, 25(OH) D3 levels do not increase theoretically (36), as also described in this study. Therefore, the level of 1alpha, 25 (OH) 2D3 in the liver may be an important factor in the achievement of SVR, although the serum concentration was not significant in this study. In fact, it has been pointed out that 1alpha, 25 (OH) 2D3 is impor-

tant for the suppression of HCV (26). However, we did not confirm whether this dose was sufficient for the combination therapy. In this stratified analysis, serum 25 (OH) D3 concentration might be a useful indicator to predict the SVR and relapse rates. The reason may be that the half-life of serum 25 (OH) D3 is longer, hence more stable than 1alpha, 25 (OH) 2D3, and consequently maintains a high concentration in serum. Accordingly, further investigation is required to examine whether vitamin D or its metabolites exert efficacy in vivo, because there is no clinical study comparing the efficacy among vitamin D forms and its metabolites.

In conclusion, the present study demonstrated that PEG-IFN/ ribavirin therapy combined with alfacalcidol may be effective and safe in elderly patients with chronic hepatitis C of high-viral-load HCV genotype 1b. In particular, female elderly patients with low serum vitamin D concentration, who are less likely to respond virologically to PEG-IFN/ ribavirin, may benefit from the combination of PEG-IFN/ ribavirin and alfacalcidol through its effect in reducing the relapse rate and consequently improving the SVR rate.

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Authors' Contribution

All authors have contributed towards the assessment of patients, collection of laboratory data, and making decision for treatment. Dr. Akihito Tsubota contributed to writing the manuscript and critical revision.

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No potential conflict of interest relevant to this study was reported.

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Clinical Trial Perspective

CLINICAL INVESTIGATION

Sustained and rapid virological responses in hepatitis C clinical trials

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Direct-acting antivirals (DAAs) in combination with pegylated IFN-α and ribavirin drastically improve the rates of rapid virological response (RVR) and sustained virological response (SVR) in the treatment of chronic hepatitis C virus infection, specifically in difficult-to-cure hepatitis C virus genotype 1. At present, RVR is an important milestone highly predictive of SVR. Response-guided therapy based on RVR is important to shorten the treatment duration whilst preserving the greatly improved SVR rate, given that DAA-based treatments are costly and could produce serious adverse events and antiviral-resistant variants. Because strong factors other than RVR independently influence SVR, more highly personalized treatments should be developed through a combination of several robust factors. The advent of more potent DAAs may change the concept of RVR and SVR.

Keywords: chronic hepatitis C virus infection • direct-acting antivirals • pegylated IFNα • response-guided therapy • ribavirin

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According to the WHO, approximately 170 million people are chronically infected with hepatitis C virus (HCV) worldwide and are at risk of developing cirrhosis and life-threatening complications, including portal hypertension, hepatic failure, and hepatocellular carcinoma. More than 350,000 people die from HCV-related liver diseases every year. Antiviral therapy for chronic hepatitis C can lead to a sustained virological response (SVR), defined as an undetectable serum HCV RNA level (using a qualitative polymerase chain reaction assay) 24 weeks after treatment cessation, which provides short- and long-term clinical benefits by improving quality of life, lessening hepatic fibrosis, and reducing the incidence of hepatocellular carcinoma and liver disease-related mortality [1–8]. Over the past decade, pegylated IFN-α (peg-IFNα)-2a or -2b in combination with weight-based doses of ribavirin (RBV) has been used as the standard-of-care treatment for chronic hepatitis C, leading to improvement in the overall SVR rate from <20% to >60%: 40–60% of difficult-to-cure HCV genotype 1/4-infected patients who are treated with 48-week treatment, and 70–90% of easy-to-cure HCV genotype 2/3-infected patients who are treated with 24-week treatment [9–15]. However, more than 50% of patients infected with HCV genotype 1, the most prevalent genotype worldwide, fail to eradicate HCV with dual combination of peg-IFNα and RBV (peg-IFNα/RBV). Efforts to improve the treatment outcomes have focused on the development of antiviral therapy specifically and directly targeted to HCV, especially HCV genotype 1.

Numerous novel therapeutic approaches are being developed and assessed [16–18]. Direct-acting antivirals (DAAs) directly inhibit specific replication processes in the HCV life cycle, targeting the HCV polyproteins including the nonstructural 3/4A (NS3/4A) protease, NS5A phosphoprotein, and NS5B polymerase. The NS3/4A serine protease is required for RNA replication and virion assembly. The first-generation NS3/4A serine protease inhibitors (PIs), boceprevir (BOC) and/or telaprevir

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(TVR), have been approved for use by each government organization in several European and North American countries and in Japan in 2011. When used in combination with peg-IFNα/RBV for HCV genotype 1 infection, Phase II and III clinical trials have proven that both PIs greatly improve viral response rates in both treatment-naive patients and patients who have had virological failure on previous treatment [19–29]. Furthermore, in Japan, where TVR, but not BOC, is available by medical insurance and subvention, a post-marketing investigation of 10,000 subjects showed that TVR-based combination therapy for 24 weeks yielded an SVR rate of approximately 90% in treatment-naive patients and patients who had virological relapse after previous peg-IFNα/RBV treatment [30]. High rates of early viral suppression and low rates of relapse suggested that treatment duration could potentially be shortened to 24 weeks in patients who achieve a rapid virological response (RVR, defined as an undetectable serum HCV RNA level at week 4 of treatment) or extended RVR (eRVR, defined as an undetectable serum HCV RNA level at both weeks 4 and 12 of treatment) [19–22,24]. RVR has been used as the most important on-treatment milestone to shorten treatment duration from the era of dual combination therapy with peg-IFNα/RBV [14,15,31–36].

The treatment strategy for chronic HCV infection is personalized on the basis of strong predictors of SVR to IFN-based therapy, such as HCV genotype [9–12,15,37,38], the initial virological response to treatment [32,39–41], and previous treatment response (treatment-naive, or previous relapse or no virological response) [21,23,27–29,42]. This manuscript addresses the impact of newly available DAAs on RVR and SVR in the combination therapy for chronic HCV infection and discusses the potential of response-guided therapy (RGT) based on RVR in DAA-based combination therapy to explore more optimal and highly personalized therapeutic strategies.

Contribution of factors to SVR

Identification of factors highly predictive of SVR, including host-, virus-, and treatment-related and on-treatment components, can tailor treatment to individual needs, helping to make decisions regarding which treatment regimens are suitable or whether treatment should be initiated, continued or stopped. Personalized medicine determined by using robust factors can reduce unnecessary physical/economic burdens and social loss on the patient without adversely affecting treatment outcomes.

A number of host-related factors, such as older age, African–American or Hispanic race, presence of advanced fibrosis or cirrhosis, overweight, obesity, insulin resistance, diabetes, hepatic steatosis, and low levels of hemoglobin, platelet count, and cholesterol, have been reported to decrease the likelihood of SVR to IFN-based therapy [9,11,43–53]. Among these factors, single nucleotide polymorphisms (SNPs) near the IL 28B (*IL28B*) gene, which resides on chromosome 19 and encodes *IL28B* or IFN-lambda-3, have a stronger impact on treatment response to peg-IFNα/RBV combination alone and TVR-based triple combination therapy in HCV genotype 1-infected patients [42,53–57]. Patients with favorable genotypes at the *IL28B* SNPs (such as rs12979860 genotype CC and rs8099917 genotype TT) are more likely to achieve SVR than those with unfavorable genotypes (rs12979860 CT or TT and rs8099917 TG or GG).

HCV genotype (two and three rather than one and four), pretreatment viral load (low rather than high), and initial virological response are significantly strong independent predictors of SVR to IFN-based therapy [9,11,12,32,37–41,45,51–53,58–64]. Thus, easy-to-cure genotype-infected patients with favorable factors have a greater chance to achieve SVR with abbreviated treatment duration and/or less potent treatment. Conversely, for difficult-to-cure genotype-infected patients with unfavorable factors, more intensive therapy is recommended including the use of DAAs or a longer treatment duration. Strictly speaking, HCV genotypes can be ranked in a decreasing order of susceptibility to IFN-based therapy as follows: genotypes 2, 3, 4, and 1 [15]. Moreover, genotypes 1b and 2a are likely to respond better to IFN-based therapy than 1a and 2b, respectively [33,65]. Of note, TVR-resistant variants and viral breakthrough occur more frequently in 1a than in 1b in TVR-based treatment [19,66,67]. For instance, the substitution of R with K at amino acid position 155 of the NS3 protease region (R155K) or V36M, which is frequently related to TVR resistance, requires only one nucleotide substitution in 1a, whereas two substitutions are required in 1b. Similarly, the emergence of the BOC-resistant mutant differs between 1a and 1b [68]. When anti-HCV treatment is initiated or treatment outcomes are interpreted,the HCV sub-genotype as well as the HCV genotype should be taken into consideration.

A virological response at critical time points or HCV kinetics during the early phase of treatment are closely associated with SVR or non-SVR [32,33,39,41]. Absence of an early virological response at week 12 of treatment is the best negative predictor of non-SVR. Conversely, RVR is an important milestone highly predictive of SVR and one of the strongest independent on-treatment predictors [15,32–34,42,53,57,62,69,70]. Patients with RVR can have an SVR rate as high as 80–90% when treated for 48 weeks, although RVR is achieved in a small percentage of HCV genotype 1-infected patients (<20%) who receive peg-IFNα/RBV alone [15,31–34,53,69,71–73]. The addition of TVR greatly improves the RVR rate to 66–84% in treatment-naive patients, and patients with RVR show an SVR rate of approximately ≥90% [19,20,22,24,28]. In contrast, the probability of SVR is

<5% in patients with a minimal fall in a viral load of <1 log_{10} from the baseline level at treatment week 4, when peg-IFNα/RBV are combined with DAAs [19]. With advances in antiviral treatment, RVR has become a more important milestone for tailoring treatment regimens and predicting SVR.

Although early viral kinetics are influenced by various factors, RVR is an independent predictor of SVR, irrespective of other strong predictors including HCV genotype and the *IL28B* SNP [15,42,53,57]. The proportion of patients achieving RVR with peg-IFNα/RBV alone varies greatly among HCV genotypes (16% in 1, 71% in 2, 60% in 3 and 38% in 4) [15]. Importantly, the probability of SVR is consistently high across HCV genotypes (88% in 1, 86% in 2, 86% in 3 and 100% in 4). With regard to race and host genetic variations, Caucasians and/or patients with the favorable *IL28B* genotype are more likely to achieve RVR with peg-IFNα/RBV alone than African–American and/or those with an unfavorable genotype [74]. The RVR rates appear to differ among Caucasians, East Asians, and African Americans with the same favorable *IL28B* genotype CC (28%, 19% and 12%, respectively) [53,74]. Although the racial and genetic disparities are apparent, patients with RVR appear to have consistently high SVR rates, irrespective of the *IL28B* genotype and race. Taken together, these findings highlight the accepted notion that RVR is strongly linked to a high likelihood of SVR and the most reliable milestone in RGT across HCV genotypes, *IL28B* SNP genotypes, and races.

Among patients who have failed to achieve SVR with previous IFN-based therapy, previous virological response has an impact on SVR with retreatment. Patients who did not have a virological response to previous treatment have a limited chance of successful outcome with retreatment [6,33,34,75–77]. The addition of DAA to peg-IFNα/RBV apparently increases the SVR rates in patients who had a previous virological relapse, which is defined as an undetectable HCV RNA level at the end of treatment, but re-emergent HCV RNA thereafter (designated as previous relapsers) [21,23,27]. Phase III studies conducted in Japan showed that the SVR rate for previous relapsers was 88–93% [29,42]. Previous relapsers are one of the most suitable candidates for DAA-based treatment, followed by patients with a partial response to previous treatment, which is defined as a decline of ≥2 log10 IU/mL in viral load at 12 weeks of treatment but with constantly detectable HCV RNA during treatment [78]. Patients with a null response to previous treatment experience little benefit from TVR-based triple combination therapy [21,23]; a null response is defined as a decline of <2 log10 IU/ml in viral load at 12 weeks of treatment [78].

Clinical trials for treatment-naive patient

The PROVE 1 trial was a randomized, double-blind, placebo-controlled Phase IIb trial [19]. Treatment-naive HCV genotype 1-infected patients were randomly assigned to one of the three TVR groups or to the control group. The control group received peg-IFNα-2a (180 µg per week) and RBV (1000 or 1200 mg/day for body weight) for 48 weeks, plus TVR-matched placebo for the first 12 weeks (PR48 group, 75 patients). The TVR groups received TVR (1250 mg on day 1 and 750 mg every 8 h thereafter) for 12 weeks, as well as peg-IFNα-2a/RBV (at the same doses as in the PR48 group) for the same 12 weeks (T12PR12 group, exploratory 17 patients) or for 24 weeks (T12PR24 group, 79 patients) or 48 weeks (T12PR48 group, 79 patients). The RVR rates (Figure 1) were much higher in the T12PR24 (81%) and T12PR48 (81%) groups than in the PR48 (control) group (11%). The SVR rates (Figure 1) were 61% in the T12PR24 group and 67% in the T12PR48 group compared with 41% in the PR48 group. In another Phase IIb trial (PROVE 2) of 323 treatment-naive HCV genotype 1-infected patients

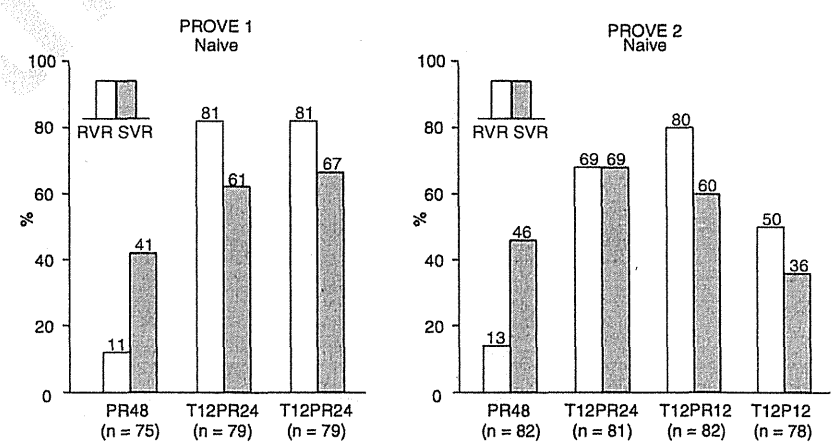

Figure 1. Rapid virological response and sustained virological response rates for treatment-naive HCV genotype 1 patients in randomized controlled Phase II trials (PROVE 1 and PROVE 2).

RVR: Rapid virological response; SVR: Sustained virological response.
Data taken from [19,20].

[20], the RVR rates (Figure 1) were 69% in the T12PR24 group 80% in the T12PR12 group, and 50% in the T12P12 group (that did not receive RBV) compared with 13% in the PR48 (control) group ($P < 0.001$ for each). The SVR rate (Figure 1) was significantly higher in the T12PR24 group (69%) than in the PR48 group (46%). The SVR rate was not significantly higher in the T12PR12 or T12P12 group than in the PR48 group, although the rates were significantly different between the T12PR12 and T12P12 groups (60 versus 36%), suggesting that RBV is required as an essential component in TVR-based combination therapy. Taken together, the two Phase IIb trials indicated that the addition of TVR greatly increases the RVR rate, resulting in a shortened duration of treatment from 48 to 24 weeks in most treatment-naive patients. The 24-week treatment duration is sufficient in patients who achieve RVR. Overall, the 12-week duration lowers the SVR rate, but may be sufficient for a certain subpopulation of patients with favorable robust factors. From Japan, a Phase III study for treatment-naive patients infected exclusively with HCV genotype 1b showed that RVR was 84% and SVR was 73% with the 24-week treatment regimen [28]. When treatment outcomes are compared between trials conducted in the west and east, the distribution of HCV genotype (1a versus 1b), *IL28B* SNP genotype, and race should be taken into consideration. To clarify the variations, multi-national/-racial trials are required on a worldwide scale.

RGT

The degree of viral load decay and rapidity of virological response during the first 12 weeks of peg-IFNα/RBV treatment can predict the likelihood of achieving SVR [11,15,31,32,36,38–41,58,72]. The time points usually used to decide whether treatment should be shortened, stopped or continued/extended are treatment weeks 4, 12 and 24 [14,32]. A dynamic modification of treatment duration based on the virological response is known as RGT. As described above, RVR is a critical milestone for RGT with SVR rate maintenance. When RVR is achieved, the treatment duration of 48 weeks can be shortened to 24 weeks with peg-IFNα/RBV dual therapy alone [12,33,34] for genotype 1 or 4 or TVR-based triple combination [19,20] for genotype 1. In patients who achieved RVR with 24-week peg-IFNα/RBV alone, the SVR rates were 79–89% for genotype 1 and 86–87% for genotype 4 [33,34,63,73,75,79].

The current recommendation for genotype 2 or 3 advocates a 24-week treatment course [12,31,45,59,61,80]. Patients with RVR have a high probability of SVR

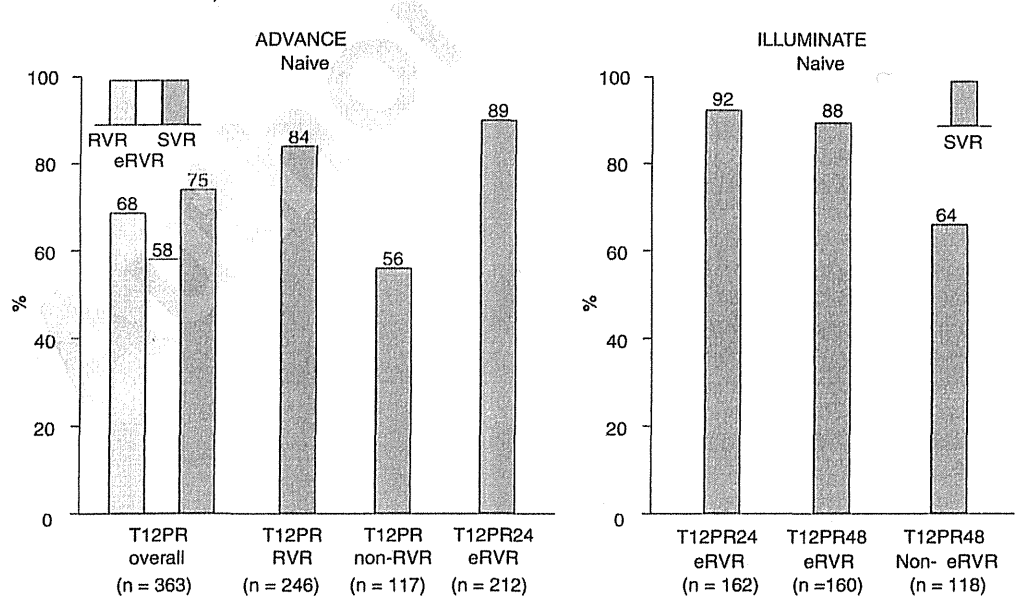

Figure 2. Rapid virological response, extended rapid virological response and sustained virological response rates for treatment-naive HCV genotype 1 patients in randomized controlled Phase III trials for response-guided therapy (ADVANCE and ILLUMINATE).

eRVR: Extended RVR; RVR: Rapid virological response; SVR: Sustained virological response.

Data taken from [22,24].

despite the shortened treatment duration from 24 to between 12 and 16 weeks [45,61,62,80–82], but the risk of relapse increases with abbreviated treatment, resulting in the reduction of the SVR rates [62,80,83]. Conversely, there is little information on the most suitable duration of treatment for genotype 2 or 3-infected patients who do not achieve RVR [65]. To shorten the treatment duration, whether RVR is appropriate for the decision needs to be verified, because the susceptibility to IFN-based therapy apparently differs between genotypes, subgenotypes, or baseline viral loads within an identical genotype. Genotype 2 or 3-infected patients may benefit from the ongoing development of DAAs, although there are limited data for the use of DAAs in such patients [84,85].

In a randomized, double-blind, placebo-controlled Phase III trial (ADVANCE) [22], 1088 treatment-naive HCV genotype 1-infected patients were randomly assigned to one of the three groups:

- TVR combined with peg-IFNα-2a/RBV for 12 weeks (T12PR group), followed by peg-IFNα–2a/RBV alone for 12 weeks if eRVR was achieved or for 36 weeks if HCV RNA was detectable at either time point;
- 8-week TVR and 4-week placebo with peg-IFNα-2a/RBV (T8PR group), followed by 12 or 36 weeks of peg-IFNα-2a/RBV on the basis of the same criteria; or 12-week placebo with 48-week peg-IFNα-2a/RBV (PR group).

The SVR rates were significantly higher in the T12PR (75%; Figure 2) or T8PR (69%) group than in the PR group (44%). The RVR and eRVR rates in the T12PR group were 68 and 58%, respectively (Figure 2). The SVR rates were 84 and 56% in the T12PR with and without RVR, respectively (Figure 2). The SVR rate of 89% in the T12PR24 with eRVR was the highest among all subgroups (Figure 2). Taken together, the 24-week treatment duration is sufficient for treatment-naive patients who achieved eRVR. A longer duration of peg-IFNα/RBV therapy is indicated for patients who do not achieve eRVR.

In an open-label, randomized, Phase III noninferiority trial (ILLUMINATE) [24], treatment-naive HCV genotype 1-infected patients who had eRVR were randomly assigned to the T12PR24 or T12PR48 group. Of 540 patients, 72% had RVR and 65% had eRVR; the overall SVR rate was 72%. Among the 322 patients with eRVR, 92% in the T12PR24 group and 88% in the T12PR48 group achieved SVR (Figure 2). A total of 118 patients without eRVR were assigned to the T12PR48 group and 64% achieved SVR (Figure 2). This study also showed that the 24-week treatment duration is sufficient for patients who achieve eRVR, even if they have refractory factors such as high viral loads, bridging fibrosis or African race. Collectively, triple combination therapy yielded an RVR rate of 68–81% and an eRVR rate of 58–65% in treatment-naive HCV genotype 1-infected patients (Figures 1 & 2), and the 24-week treatment course for patients with eRVR generated an SVR rate of 89–92% (Figure 2). RGT based on eRVR permits a shorter treatment duration while preserving high SVR rates, improves the overall tolerability, and reduces exposure to unnecessary medication. However, there were a small number of cirrhotic patients in the clinical trials. Cirrhotic patients may not comply with RGT and should receive treatment for 48 weeks [78]. Treatment should be stopped if HCV RNA levels are >1000 IU/ml at week 4 or 12 of treatment and/or detectable at week 24 of treatment.

Clinical trials for treatment-experienced patients

In a Phase II study for previously treated HCV genotype 1-infected patients (PROVE 3) [21], 453 patients who had failed to achieve SVR with previous peg-IFNα-2a/RBV therapy were randomly assigned to one of four treatment groups. The SVR rates in the three TVR groups (51% in the T12PR24 group, 53% in the T24PR48 group and 24% in the T24P24 group) were significantly higher than the rate in the PR48 (control) group (14%; Figure 3). The RVR rates were 61, 50 and 47% in the TVR groups, respectively, and 0% in the control group (Figure 3). The SVR and RVR rates were higher in previous relapsers than in previous nonresponders. The SVR rates were similar between the T12PR24 and T24PR48 groups (Figure 3), and treatment discontinuation because of adverse events was less common in the T12PR24 group than in the T24PR48 group. Therefore, the T12PR24 regimen appeared to provide a better risk–benefit profile. The higher termination rates and the lower relapse rates in the T24PR48 group suggest that an optimal retreatment regimen may consist of a 12-week treatment duration with TVR combined with a longer duration with peg-IFNα-2a/RBV. Of note, the SVR rates in previous relapsers were 69% in the T12PR24 group and 76% in the T24PR48 group. Furthermore, in Phase III studies from Japan, the SVR rates in relapsers were 88–93% with the T12PR24 regimen [29,42]. Relapsers appear to be the most suitable for the 24-week treatment regimen. In contrast, previous non-responders were less likely to achieve SVR, with the rates of 39% in the T12PR24 group and 38% in the T24PR48 group. However, these SVR rates were more than four times the rate in the control group (9%).

In a Phase III study for previously treated HCV genotype 1-infected patients (REALIZE) [23], 663 patients were randomly assigned to one of the three groups: the T12PR48 group, the lead-in T12PR48 group, which received 4 weeks of peg-IFNα/RBV

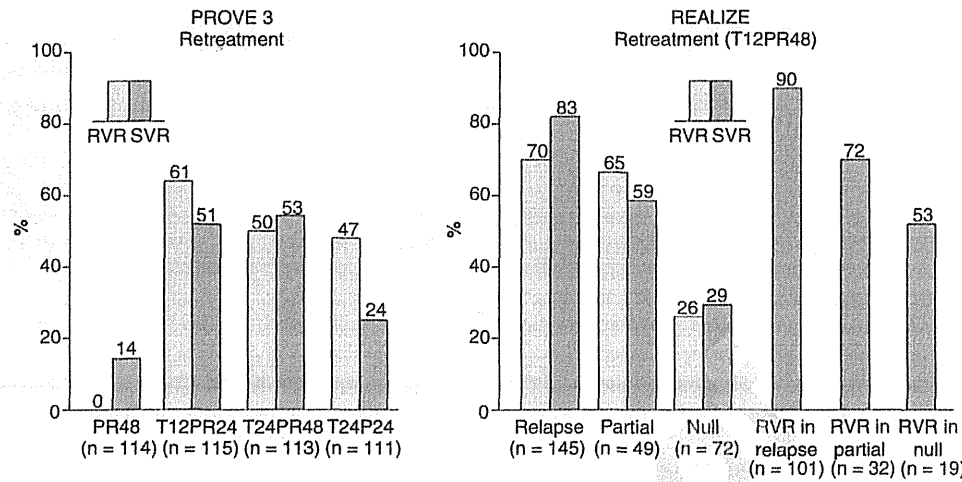

Figure 3. Rapid virological response and sustained virological response rates for treatment-experienced HCV genotype 1 patients in randomized controlled Phase II and III trials (PROVE 3 and REALIZE).
RVR: Rapid virological response; SVR: Sustained virological response.
Data taken from [21,23].

followed by 12 weeks of TVR and peg-IFNα/RBV for a total of 48 weeks, and the PR48 (control) group. The SVR rates were significantly higher in the two TVR groups than in the control group among patients with relapse (83% in the T12PR48 group, 88% in the lead-in T12PR48 group and 24% in the PR48 group), partial response (59, 54 and 15%, respectively), and null response (29, 33 and 5%, respectively). Figure 3 shows the RVR and SVR rates in the T12PR48 group alone. Among patients with RVR in the T12PR48 group, the SVR rates were 90% in relapsers, 72% in partial responders and 53% in null responders. When no virological response to previous treatment was categorized into partial and null responses, viral response rates with the T12PR48 regimen were apparently different between the partial and null responders. Of note, even among patients with RVR, the SVR rates were influenced by the previous treatment response (Figure 3). These results suggest that there may be independent factors (other than RVR) associated with the final treatment outcome. To more accurately predict or completely attain SVR, other variables (such as previous treatment response, cirrhosis and the *IL28B* SNP) may be better used for RGT in combination with RVR. Unfortunately, the REALIZE study was not a randomized-controlled trial to compare the treatment duration of 24 weeks versus 48 weeks for patients with RVR. Therefore, RGT for treatment-experienced patients can be considered for relapsers, may be considered for partial responders, but cannot be recommended for null responders [78].

Impact of the *IL28B* SNP

The favorable *IL28B* SNP genotype (rs12979860 CC) significantly increases viral response rates during the first 12 weeks of treatment, as well as the SVR rate, in peg-IFNα/RBV combination alone for HCV genotype 1-infected patients [74]. Among patients with RVR, however, *IL28B* genotype is not associated with SVR [74,86]. In treatment-naive HCV genotype 1-infected Caucasian patients (a part of the ADVANCE trial, available for 454/1,088 [42%] participants), the addition of TVR greatly increased RVR, eRVR, and SVR rates across all *IL28B* genotypes (Figure 4) [22]. Although patients with favorable *IL28B* CC still had higher SVR rates in each treatment arm (Figure 4), the largest increasing rates were observed in those with unfavorable CT or TT, suggesting that this closeness in the SVR rate between *IL28B* genotypes lowers the significance of *IL28B* SNP as a predictor. In another study using a part of the REALIZE cohort, the RVR rates were numerically higher in genotype CC than in CT/TT [84]. Previous relapsers achieved RVR rates of 77–82% regardless of the *IL28B* genotype. Previous partial responders and null responders somewhat differed according to the *IL28B* genotype (88% [CC], 66% [CT] and 64% [TT] in partial responders; and 50, 33, and 34%, respectively, in null responders). However, SVR rates were similar across all *IL28B* genotypes (85, 85 and 88% in relapsers; 63, 58, and 71% in partial responders; and 40, 29, and 31% in null responders, respectively) [87]. In the REALIZE study, only data of 48-week treatment regimens were available and did not include 24-week regimens. Several studies from Japan reported different

results; both RVR and *IL28B* were independent factors significantly associated with SVR in the 24-week regimen for treatment-naive and -experienced patients [42,57]. Another independent cohort in Japan showed similar results [30]. In Japan, the SVR rates were 90–97% in patients with the favorable *IL28B* SNP (rs8099917) genotype TT versus 56% in those with unfavorable genotype TG/GG and 89–92% in those with RVR versus 35–55% in those without RVR. The *IL28B* SNP and RVR were prominently significant in treatment-naive patients, neither was significant in previous relapsers, and *IL28B* alone was significant in previous partial responders. Taken together, the addition of TVR appears to alter or attenuate the impact of *IL28B* SNPs on SVR. In treatment-naive patients and previous relapsers, however, the *IL28B* SNP genotype can certainly identify those with a high likelihood of SVR through a shortened treatment duration. The CONCISE interim analysis suggested that non-cirrhotic *IL28B* CC patients with RVR could shorten the treatment duration to 12 weeks [88]. In treatment-experienced patients, the impact of the *IL28B* SNP genotype is limited and less informative for SVR once early viral response (such as RVR) is known. More potent DAA regimens will further attenuate the importance of the *IL28B* SNP genotype as a determinant of the likelihood of a response.

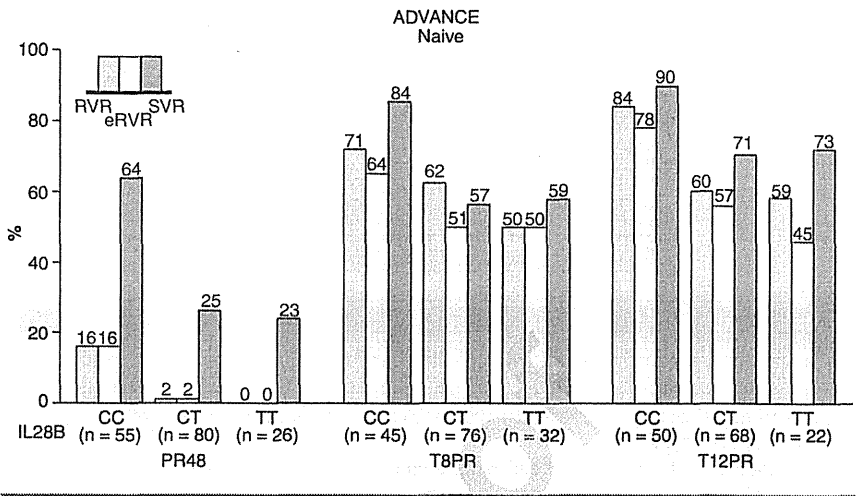

Figure 4. Rapid virological response, extended rapid virological response and sustained virological response rates according to the IL28B SNP genotype (rs12979860) in the ADVANCE study.

eRVR: Extended RVR; RVR: Rapid virological response; SVR: Sustained virological response.
Data taken from [22,92].

Changing concept of RVR & SVR

The remarkable development of DAAs may change the concept of RVR and SVR. More recent Phase II and III studies showed that sofosbuvir, an NS5B polymerase inhibitor, in combination with peg-IFNα-2a/RBV for only 12 weeks generated RVR rates of 94–99% and an SVR rate of approximately 90% in treatment-naive patients mainly infected with HCV genotype 1 [85,89]. More potent DAAs will increase the RVR rate up to almost 100%. RVR could no longer be an important milestone predictive of SVR or RGT. US FDA has recently approved SVR at 12 weeks after cessation of treatment (SVR12) as an end point of treatment outcome [90]. The previously approved SVR was designated as SVR24. However, a small minority of patients who achieve SVR12 appear to have virological relapse thereafter and fail to achieve SVR24. For the time being, the conventional concept will be used until a new concept is acceptable for the next-generation of treatment.

Future perspective

SVR indicates a permanent eradication of HCV from individuals because HCV seldom reappears in patients who achieve SVR. In peg-IFNα/RBV combination alone and DAA-based combination therapy for chronic HCV infection, SVR is closely associated with several robust pretreatment and on-treatment predictors, that is HCV genotype, pre-existence of cirrhosis, treatment-naive or virological response to previous treatment, *IL28B* SNP genotype, and early viral kinetics including RVR or non-RVR. Until date, RVR has been a critical on-treatment milestone of RGT. However, more potent DAAs in combination with peg-IFNα/RBV or DAA combinations without IFN may attenuate the importance of RVR because more potent anti-HCV therapy greatly increases the RVR rate up to almost 100%. Currently, there is no perfect variable or model for prediction of SVR with individual treatment tailoring. To develop more optimal and highly personalized treatment strategies, RVR should be used in combination with other robust predictors or currently unidentified factors. Alternatively, a viral response during the extremely early phase (e.g., day 2, week 1 or week 2) of treatment may be required to develop much shorter treatment durations (<12 weeks) with the advent of more potent DAAs. It will be important to reconsider the value of the currently identified robust predictors.

The next wave of DAAs are appearing in Phase I–III trials, such as second-generation NS3/4A PIs, NS5A inhibitors, and NS5B polymerase inhibitors (nucleos[t]

ide inhibitors and nonnucleos[t]ide inhibitors), and represents amazing progress in the management of difficult-to-cure patients, such as prior null responders, with promising results [16–18,85,89,91,92]. These exciting developments emphasize the importance of thoughtful use of TVR or BOC, closely following the recommended regimens and stopping rules, so as to not negatively influence the possibility of treatment when the next-generation DAAs become available. Patients with a cluster of difficult-to-cure features might benefit from awaiting for the next-generation of treatments. In the near future, these ceaseless efforts will relieve a large number of HCV-infected patients worldwide.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

Executive summary

- *Treatment regimens for chronic HCV infection are tailored according to independent robust factors predictive of sustained* virological response (SVR) (HCV genotype, treatment combined with or without direct-acting antivirals (DAAs), treatment-naive or viral response to previous treatment, early viral kinetics such as rapid virological response(RVR), *IL28B* SNP, and presence or absence of cirrhosis).
- The addition of telaprevir, one of the approved NS3/4A PIs, substantially increases RVR, eRVR, and SVR rates in combination with peg-IFNα/ribavirin for treatment-naive and -experienced patients infected with difficult-to-cure HCV genotype 1.
- RVR and/or eRVR are further important milestones of response-guided therapy.
- Among treatment-experienced patients who achieve RVR, SVR rates may differ according to a previous treatment response or presence of cirrhosis.
- Previous relapsers are the most suitable for the 24-week treatment regimen.
- The importance of *IL28B* SNP is attenuated or less informative for SVR in treatment-experienced patients but still controversial.
- In treatment-naïve patients, *IL28B* SNP can certainly identify those with a high likelihood of achieving SVR with RGT.
- With the advent of more potent DAAs, even difficult-to-cure patients will achieve SVR with a further shorter treatment duration. More potent DAAs may attenuate the importance of RVR and change the concept of RVR and SVR. We should reconsider how valuable the currently identified robust predictors are, including RVR.

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▪▪ **Notable treatment outcomes in 12-week regimens of sofosbuvir, an NS5B polymerase inhibitor, combined with peg-IFNα-2α/ribavirin for treatment-naive patients with hepatitis C virus genotype 1, 4, 5, or 6, and IFN-free sofosbuvir/ribavirin combination for those with genotype 2 or 3.**

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Baseline factors and very early viral response (week 1) for predicting sustained virological response in telaprevir-based triple combination therapy for Japanese genotype 1b chronic hepatitis C patients: a multicenter study

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Abstract
Background Genetic polymorphisms near *Interleukin 28B* (*IL28B*) (rs8099917) and a rapid virological response (RVR) have been reported as predictors for a sustained virological response (SVR) to telaprevir (TVR)-based triple combination therapy. However, the association between SVR and viral kinetics earlier than week 4 after initiation of therapy remains unclear. Thus, we evaluated the SVR prediction ability of baseline factors and reduced hepatitis C virus (HCV) RNA levels at week 1 after the initiation of TVR-based therapy in Japanese genotype-1b chronic hepatitis C (CHC) patients.

Methods A total of 156 Japanese CHC patients received a 24-week regimen of TVR-based therapy. Baseline factors and reduction in HCV RNA levels at weeks 1 and 4 after the initiation of therapy were analyzed for SVR prediction.
Results Multiple logistic regression analysis for SVR in TVR-based therapy identified the *IL28B* TT genotype, a reduction of $\geq$4.7 $\log_{10}$IU/mL in HCV RNA levels at week 1, RVR, and treatment-naïve/relapse. Whereas the SVR rate was higher than 90 % regardless of the reduction in HCV RNA levels at week 1 in patients with the TT genotype, a reduction of $\geq$4.7 $\log_{10}$IU/mL in HCV RNA levels at week 1 was the strongest predictor of SVR in patients with the non-TT genotype, as determined by multiple logistic regression analysis ($P = 0.0043$).
Conclusions The *IL28B* TT genotype is the most important baseline factor for predicting SVR, and a $\geq$4.7 $\log_{10}$IU/mL reduction in HCV RNA at week 1 is a useful very early on-treatment predictor of SVR, especially in the non-TT genotype.

Keywords Chronic hepatitis C · Reduction in HCV RNA at week 1 · Telaprevir · *IL28B*

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Introduction

In 2011, the first-generation direct-acting antiviral agents telaprevir (TVR) and boceprevir (BOC) were approved for treatment of chronic hepatitis C (CHC) patients with hepatitis C virus (HCV) genotype 1 in the United States (US), Canada, and the European Union (EU). Triple combination therapy with TVR or BOC, PEG-interferon (PEG-IFN), and ribavirin (RBV) is the current standard of care for genotype 1 CHC patients [1]. In Japan, TVR, which is a nonstructural (NS) 3/4A serine protease inhibitor, was

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approved in September 2011 and has been marketed since November 2011. In treatment-naïve genotype 1 CHC patients, TVR-based triple combination therapy for a shortened period was reported to remarkably improve the rate of sustained virological response (SVR) compared with PEG-IFN and RBV alone [2–4]. In treatment-experienced patients, the effect of TVR-based triple combination therapy reportedly depends on the response to PEG-IFN and RBV combination therapy [5–16].

Pivotal genome-wide association studies have found that genetic variations near the interleukin 28B (*IL28B*) gene (rs8099917 and rs12979860) are strongly associated with the treatment outcome of PEG-IFN and RBV combination therapy [17–19]. We previously confirmed that the *IL28B* single-nucleotide polymorphism (SNP) genotype was the strongest factor contributing to SVR in PEG-IFN and RBV combination therapy [20–23]. These genetic variations appear to be strong predictors of SVR to a 24-week regimen of TVR-based triple therapy, as well as PEG-IFN and ribavirin combination therapy [7, 11, 14–16, 24].

Two guidelines for treatment of genotype 1 CHC patients, which were based on the results of the clinical trials of a 24-week regimen of TVR-based triple therapy for Japanese patients [4, 10], provided recommendations for patient selection for TVR-based therapy [25]. Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis published by the Ministry of Health, Labour and Welfare of Japan, and Japan Society of Hepatology guidelines provided recommendations of a 24-week regimen of TVR-based triple therapy for Japanese genotype 1 CHC patients. These recommendations are based on baseline factors, including patient's age, sex, *IL28B* genotype, core amino acid substitution at position 70, previous treatment history and response, stage of fibrosis, viral load, and baseline hemoglobin level [25, 26].

In addition to the baseline predictive factors, changes in HCV RNA levels after the start of therapy are predictive for treatment outcomes. A rapid virological response (RVR), defined as undetectable serum HCV RNA at week 4 after the start of therapy, and an extended rapid virological response, defined as undetectable serum HCV RNA at both weeks 4 and 12, were also reported as significant predictors of TVR-based treatment outcome [7, 11, 15, 16, 27]. However, the association between SVR and viral kinetics earlier than 4 weeks after initiating TVR-based triple combination therapy remains unclear. RVR was achieved in only approximately 3–11 % of cases receiving PEG-IFN and RBV combination therapy [2, 3, 6, 27, 28]. In contrast, RVR was achieved in approximately 61–84 % of cases receiving TVR-based triple combination therapy [2–6, 8, 10, 11, 15, 16, 27, 29]. It is therefore important to determine whether viral kinetics earlier than week 4 after the start of therapy is predictive for SVR in TVR-based triple combination therapy?

TVR-based triple combination therapy remarkably improves the SVR rate in CHC patients with the difficult-to-treat HCV genotype 1. However, some patients still fail to achieve SVR. Adverse events occurred more frequently and were more severe in patients treated with TVR-based therapy than in those treated with PEG-IFN and RBV alone [2–6]. Additionally, TVR-based therapy is expensive. In clinical practice, the determination of predictive factors of successful treatment outcome as early as possible is necessary for preventing unnecessary treatment in addition to physical and economic burden. Thus, in this prospective, multicenter study, we evaluated the clinical relevance of baseline predictors and the reduction in HCV RNA levels at week 1 after starting therapy for predicting SVR in a 24-week regimen of TVR-based triple combination therapy for genotype 1b CHC patients.

Methods

Patients, treatment, and definition of outcomes

Between December 2011 and September 2012, 156 Japanese genotype 1b monoinfected CHC patients were enrolled in this multicenter study at Shinmatsudo Central General Hospital, Kurume University School of Medicine, Kagawa Prefectural Central Hospital, Jikei University School of Medicine Kashiwa Hospital, and Ogaki Municipal Hospital. The inclusion criteria for the study included persistently positive sera for HCV RNA for > 6 months as determined using the quantitative real-time PCR method (COBAS AmpliPrep/COBAS TaqMan HCV test, Roche Diagnostics, Tokyo, Japan), HCV RNA $\geq$5.0 $\log_{10}$IU/mL in treatment-naïve patients, age of 18–75 years, and body weight >35 kg at the time of entry into the study. Exclusion criteria were: (1) decompensated cirrhosis; (2) positive for hepatitis B surface antigen or antibodies 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 $\leq$ 50 mL/min at baseline; (6) hemoglobin level < 12 g/dL, white blood cell count < 2000/μL, neutrophil count < 1500/μL, and platelet count < 8.0 × 10^4/μL at baseline; (7) depression, schizophrenia or its history, or history of suicide attempts, (8) pregnancy in progress or planned for either partner during the study period. For 114 of 156 (73.1 %) patients, liver biopsy was conducted within 12 months of enrollment. The presence or absence of cirrhosis was established according to the Metavir score

Table 1 Patient profiles

Number of patients	156
Sex (male/female)	78/78
Age (years)	58.4 ± 10.3
Body weight (kg)	61.8 ± 12.8
Body mass index (kg/m^2)	23.7 ± 3.5
Absence or presence of cirrhosis (non-cirrhosis/cirrhosis)	120/36
Response to previous treatment (treatment-naïve/relapsers/partial responders/null responders)	78/50/14/14
rs8099917 (TT/TG/GG)	106/48/2
Core amino acid substitution 70 (wild-type/mutant-type)	97/59
ISDR of NS5A (wild-type/non-wild-type)	138/18
White blood cells (/μL)	4972 ± 1542
Hemoglobin (g/dL)	14.2 ± 1.4
Platelets (×10^4/μL)	17.1 ± 5.6
Aspartate aminotransferase I (U/L)	54 ± 36
Alanine aminotransferase I (U/L)	60 ± 50
Gamma-glutamyl-transpeptidase I (U/L)	59 ± 68
Albumin (g/dL)	4.2 ± 0.3
Total cholesterol (mg/dL)	173 ± 31
Low-density lipoprotein cholesterol (mg/dL)	103 ± 28
Alpha-fetoprotein (ng/mL)	10.9 ± 20.7
HCV RNA ($\log_{10}$IU/mL)	6.4 ± 0.9
Initial dose of PEG-IFN (μg/kg)	1.5 ± 0.2
Initial dose of ribavirin (mg/kg)	11.2 ± 1.6
Initial daily dose of telaprevir (1500/2250 mg)	84/72
Administration intervals of telaprevir (q8/q12 h)	96/60

Data are expressed as numbers or mean ± standard deviation

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

[30]. For the remaining 42 patients, the presence or absence of cirrhosis was evaluated using ultrasonography and/or computed tomography findings.

Patient profiles are shown in Table 1. 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 (≥2 $\log_{10}$IU/mL reduction in HCV RNA at week 12 but never undetectable), or null responders (< 2 $\log_{10}$IU/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, RBV, and TVR triple therapy. TVR (Telavic; Mitsubishi Tanabe Pharma, Osaka, Japan) was administered every 8 h after meals (q8 h) at 500 or 750 mg, or every 12 h after meals (q12 h) at 750 or 1125 mg. The initial daily dose of TVR (1500 or 2250 mg per day) and administration intervals (q8 or q12 h) were determined by each attending physician according to age, sex, 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 per week. The RBV (Rebetol, MSD, Tokyo, Japan) dose was adjusted by body weight (600 mg for < 60 kg; 800 mg for ≥60 to <80 kg; and 1000 mg for ≥80 kg; in the case of hemoglobin < 13 g/dL at start of therapy, the RBV dose was reduced by 200 mg), based on the guidelines of the Ministry of Health, Labor and Welfare of Japan, and 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 RBV combination therapy (T12PR24). Administration of each drug was appropriately reduced or withdrawn when a serious adverse event was suspected to be developing or if a serious adverse event occurred during the course of treatment. Regardless of adverse events, treatment was stopped for patients who had HCV RNA > 3 $\log_{10}$IU/mL at week 4 or detectable HCV RNA at week 12, or those showing a > 2 $\log_{10}$IU/mL increase in HCV RNA levels from the lowest level during therapy, because of the low likelihood of achieving SVR and the high risk of developing antiviral resistance.

Adherence to PEG-IFN was calculated based on the initial weekly dose, and that to RBV was calculated based on the initial daily dose. Adherence to TVR was defined as 100 % when 2250 mg was given each day for 12 weeks, which is the recommended daily dose.

The virological response was analyzed on an intent-to-treat basis. The successful endpoint of treatment was SVR for patients showing undetectable HCV RNA for 24 weeks after cessation of treatment. Patients were defined as relapse when HCV RNA levels became undetectable until the end of treatment, but became positive during the follow-up period. Patients were defined as at viral breakthrough when HCV RNA became undetectable during the treatment period, but then became positive again before the end of the treatment period. Patients were defined as non-response when HCV RNA was detectable throughout the treatment period. Furthermore, RVR was defined as undetectable HCV RNA at week 4 after 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 each participating institution.

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

HCV genotype was determined by direct sequencing followed by phylogenic analysis of the NS5B region [31]. The

antiviral effects of the therapy on HCV were assessed by measuring serum HCV RNA levels. In this study, HCV RNA levels were evaluated at baseline; weeks 1, 4, 8, 12, 16, 20, and 24 during treatment; and once every 4 weeks after cessation of treatment. HCV RNA levels were determined using the COBAS AmpliPrep/CABAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). 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 according to a previously described method [32, 33]. Core amino acid substitution at position 70 was defined as wild-type (arginine) or mutant-type (glutamine or histidine). Additionally, substitutions at amino acids 2290–2248 of the NS5A region [interferon-sensitivity determining region (ISDR)] were determined using a previously described method [34]. 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). The genetic polymorphism rs8099917, near the *IL28B* gene [17, 18], was genotyped by real-time detection PCR using the TaqMan SNP Genotyping Assays and the 7500Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The rs8099917 genotypes were classified into 2 categories, including TT (major genotype) and non-TT genotype (minor genotype: TG or GG).

Statistical analysis

Continuous variables are expressed as the mean and standard deviation. Categorical data were analyzed using the Chi-squared test and Fisher's exact test, while continuous data were analyzed using the non-parametric Mann–Whitney *U* test. Univariate and multiple logistic regression analyses were used to identify factors that significantly contributed to SVR. The odds ratios (OR) and 95 % confidence intervals (95 % CI) were also calculated. All *P* values for statistical tests were 2-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 analyses to identify significant independent predictive factors of SVR.

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 SVR. Statistical analysis was performed using SPSS version 17.0 (IBM-SPSS, Chicago, IL, USA).

Results

Characteristics of patients and treatment outcome

Table 1 summarizes the characteristics of the patients. In total, 78 patients (50.0 %) were treatment-naïve, and 78 patients (50.0 %) were treatment-experienced with PEG-IFN and RBV. The *IL28B* TT genotype was present in 67.9 % (106 of 156) of the patients. The proportion of patients with cirrhosis was 23.1 % (36 of 156). In total, 72 patients (46.1 %) were treated with TVR at 2250 mg/day, and 84 patients (53.9 %) were treated with TVR at 1500 mg/day. In terms of dosing schedule, 96 patients (61.5 %) were treated q8 h, and 60 patients (38.5 %) were treated q12 h.

Regarding treatment outcomes, 125 patients (80.1 %) achieved SVR; 14 patients (9.0 %) relapsed. 12 patients (7.7 %) showed viral breakthrough, and the remaining five patients (3.2 %) showed non-response. For the *IL28B* SNP genotypes, among the 106 patients with the TT genotype, 102 (96.2 %) achieved an SVR, and one (0.9 %) relapsed; two (1.9 %) showed viral breakthrough, and one (0.9 %) showed non-response Among the 50 patients with the non-TT genotype, 23 (46.0 %) achieved an SVR; 13 (26.0 %) relapsed. Ten (20.0 %) showed viral breakthrough, and four (8.0 %) showed non-response. Thus, the SVR rate was significantly higher in patients with the TT genotype than in those with the non-TT genotype [102 of 106 patients (96.2 %) vs. 23 of 50 (46.0 %), $P < 0.0001$] (Fig. 1). According to previous treatment response, among the 78 treatment-naïve patients, 66 (84.6 %) achieved an SVR; five (6.4 %) relapsed. Five (6.4 %) showed viral breakthrough, and two (2.6 %) showed non-response. Among the 50 relapsers, 48 (96.0 %) achieved an SVR; one (2.0 %) relapsed, and one (2.0 %) showed viral breakthrough. Among the 14 partial responders, eight (57.1 %)

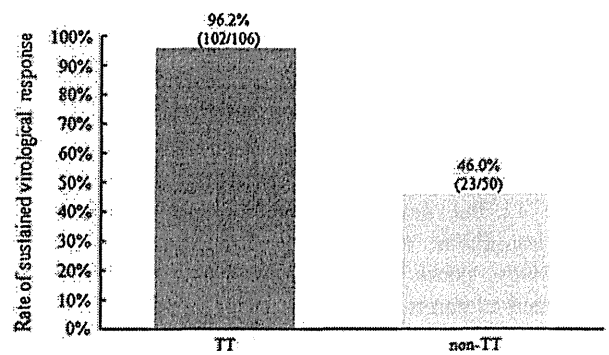

Fig. 1 Rate of sustained virological response according to the *IL28B* (rs8099917) genotype. The rate of sustained virological response was significantly higher in patients with the TT genotype than in those with the non-TT genotype ($P < 0.0001$)

achieved an SVR; four (28.6 %) relapsed, and two (14.3 %) showed viral breakthrough. Among the 14 null responders, three (21.4 %) achieved an SVR; four (28.6 %) relapsed. Four (28.6 %) showed viral breakthrough, and three (21.4 %) showed non-response. The SVR rate was significantly different across the four categories of previous treatment response ($P < 0.0001$). In particular, the SVR rate was significantly lower in non-responders than in treatment-naïve patients or relapsers [114 of 128 patients (89.1 %) vs. 11 of 28 patients (39.3 %), $P < 0.0001$].

Six patients stopped triple therapy before 12 weeks. The reasons were loss of appetite in three patients, severe anemia in one patient, systemic skin flare in one patient, and viral breakthrough in one patient. Among the six patients, five (83.3 %) with the IL28B TT genotype achieved an SVR, and one (16.7 %) with the non-TT genotype who showed viral breakthrough did not achieve an SVR.

Association between reduced serum HCV RNA levels at week 1 after starting therapy and SVR

ROC curve analysis was performed in 156 patients, to evaluate the association between reduced serum HCV RNA levels at week 1 after starting therapy and SVR. The area under the ROC curve was 0.754, and the best cut-off value was calculated as 4.7 $\log_{10}$IU/mL (Fig. 2). The SVR rate was significantly higher in patients with a reduction of $\geq$4.7 $\log_{10}$IU/mL at week 1 than in those with a reduction of <4.7 $\log_{10}$IU/mL [65 of 68 patients (95.6 %) with $\geq$4.7 $\log_{10}$IU/mL vs. 60 of 88 patients (68.2 %) with <4.7 $\log_{10}$IU/mL, $P < 0.0001$]. All four patients with the TT genotype who failed to show an SVR had a reduction of

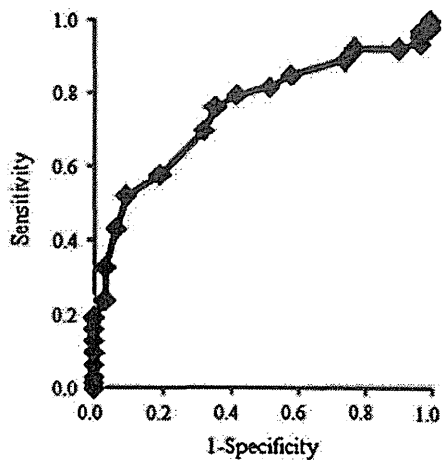

Fig. 2 Receiver operating characteristic (ROC) analysis for prediction of a sustained virological response according to the reduction in serum HCV RNA levels at week 1 after the start of therapy. The area under the ROC curve was 0.754

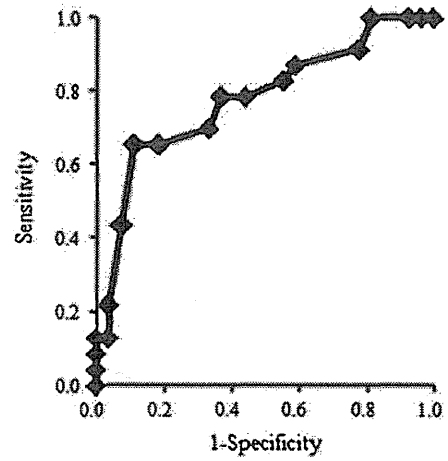

Fig. 3 Receiver operating characteristics (ROC) analysis for prediction of a sustained virological response in the *IL28B* (rs8099917) non-TT genotype according to the reduction in serum HCV RNA levels at week 1 after the start of therapy. The area under the ROC curve was 0.777

<4.7 $\log_{10}$IU/mL at week 1 (4.1 $\log_{10}$IU/mL in treatment-naïve patient, 3.8 $\log_{10}$IU/mL in partial responder, 3.7 $\log_{10}$IU/mL in null responder, and 4.6 $\log_{10}$IU/mL in null responder, respectively).

Patients with the *IL28B* TT genotype presented an extremely high SVR rate. Therefore, the ROC analysis focused on 50 patients with the *IL28B* non-TT genotype. The area under the ROC curve was 0.777, and the best cut-off value was calculated as 4.7 $\log_{10}$IU/mL, which was similar to the value calculated for all patients (Fig. 3). The SVR rate was significantly higher in patients with a reduction of $\geq$4.7 $\log_{10}$IU/mL at week 1 than in those with a reduction of <4.7 $\log_{10}$IU/mL [15 of 18 patients (83.3 %) with a reduction of $\geq$4.7 $\log_{10}$IU/mL vs. 8 of 32 patients (25.0 %) with a reduction of <4.7 $\log_{10}$IU/mL, $P = 0.0001$].

Predictive factors associated with SVR

According to the univariate analysis, the following factors were associated with SVR: treatment-naïve patients or relapsers ($P < 0.0001$); *IL28B* TT genotype ($P < 0.0001$); higher white blood cell count ($P = 0.0098$), platelet count ($P = 0.0299$), total cholesterol level ($P = 0.0467$), and low-density lipoprotein cholesterol level ($P = 0.0080$); lower gamma glutamyl transpeptidase level ($P = 0.0014$) and alpha-fetoprotein level ($P = 0.0175$); core amino acid substitution at position 70 of the wild-type ($P = 0.0010$); achievement of RVR ($P < 0.0001$); and reduction of $\geq$4.7 $\log_{10}$IU/mL in HCV RNA levels at week 1 ($P = 0.0003$). Multiple logistic regression analysis identified the following four independent factors: *IL28B* TT