Table 1. Baseline patients' characteristics

	Relapser (n = 43)	Partial responder (n = 39)	Null responder (n = 24)
Age, years	64 (44–75)	58 (33–77)	60 (30–69)
Male/female	18/25	19/20	12/12
Body mass index	22.2 (16.6–31.0)	23.4 (18.2–32.3)	22.2 (17.8–29.8)
Genotype 1/2	42/1	36/3	23/1
Viral load, log IU/ml	6.3 (3.7–7.2)	5.7 (1.2–7.2)	6.4 (3.7–7.5)
ALT, IU/ml	39 (15–189)	41 (12–379)	43 (18–275)
γ-GTP, IU/ml	24 (9–175)	51 (17–326)	39 (19–366)
WBC count, $/mm^3$	4,500 (1,900–8,300)	4,100 (2,000–9,400)	3,495 (1,700–7,100)
Platelet count, $\times 10^4/mm^3$	13.8 (7.6–24.9)	14.1 (4.5–28.6)	11.2 (4.5–29.3)
Hemoglobin, g/dl	13.8 (11.9–16.5)	13.3 (9.3–16.5)	12.4 (9.6–15.9)
LDL cholesterol, mg/dl	100 (42–167)	86 (32–128)	74 (15–122)
α-Fetoprotein, ng/ml	4 (1–48)	5.9 (1–30)	7.8 (2–108)
Core aa70, wild/non-wild	13/9	8/7	3/3
IL28B rs8099917 (TT/TG, GG)	21/5	12/7	3/7

Values are medians with ranges in parentheses or numbers.

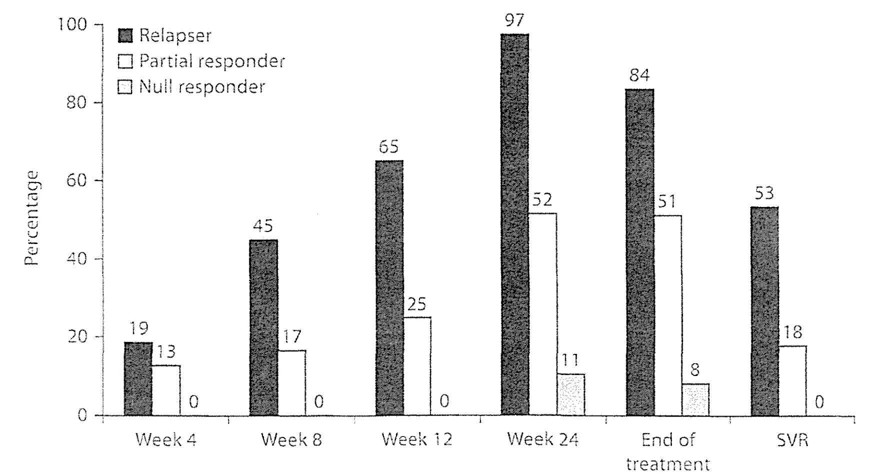

Fig. 1. Virological responses of PEG-IFNα-2a + RBV.

SVR rates in the relapsers, partial responders, and null responders were 53% (23/43), 18% (7/39), and 0% (0/24), respectively. The HCV RNA response rate in the relapsers was 65% at week 12 of the PEG-IFNα-2a + RBV therapy and 84% at the end of treatment (fig. 1). In contrast, the HCV RNA response rates in the non-responders were 15, 37, and 37% at week 12, week 24, and the end of retreatment, respectively. These rates were lower than those of the relapsers.

Examination of Previous Relapsers

The durations before an HCV RNA response was observed in the relapsers were ≤12 weeks in 65% (28/43) and 13–24 weeks in 26% (11/43) of the patients. In the 28 patients who achieved EVR, the SVR rates were 56% (9/16) and 92% (11/12) in the patients for whom retreatment commenced within 48 weeks and who received prolonged therapy, respectively; this difference was significant (p = 0.04).

Table 2. Factors associated to SVR

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p value	OR (95% CI)	p value
Response of previous treatment, relapsers/non-responders	9.20 (6.3–54)	<0.0001	–	n.s.
γ-GTP, <35/≥35 IU/l	2.92 (1.2–7.3)	0.0189	–	n.s.
Platelet counts, ≥12 × 10^4/<12 × 10^4/mm^3	2.96 (1.1–7.7)	0.0232	–	n.s.
Hemoglobin, ≥13/<13 g/dl	3.24 (1.2–8.8)	0.0179	–	n.s.
LDL cholesterol, ≥100/<100 mg/dl	4.61 (1.5–14)	0.0046	–	n.s.
α-Fetoprotein, <6/≥6 ng/ml	4.16 (1.5–11)	0.0112	–	n.s.
Treatment duration, prolonged/standard	3.32 (1.4–8.0)	0.0063	5.16 (1.13–23.6)	0.0343
HCV RNA at week 12, negative/positive	18.5 (6.3–54)	<0.0001	10.3 (1.65–64.7)	0.0022
PEG-IFN adherence, ≥80/<80%	4.80 (1.6–14)	0.0025	–	n.s.
Ribavirin adherence, ≥60/<60%	3.69 (1.2–12)	0.0214	–	n.s.

We also compared the RVR and EVR rates between initial treatment and retreatment among the 41 patients whose timing of HCV negativity was available (fig. 2). The rates of RVR and EVR were higher in the retreatment patients (2 and 44% in initial treatment, and 20 and 66% in retreatment, respectively).

Examination of Previous Non-Responders

Among the non-responders, an HCV RNA response after retreatment was achieved only in partial responders. Of these patients, 23% (9/39) achieved EVR, 23% (9/39) within 13–24 weeks, and 8% (3/37) after 25 weeks. In the 9 subjects who achieved EVR among partial responders, the SVR rate was 25% (1/4) during the retreatment within 48 weeks but was 60% (3/5) when the retreatment period was prolonged. No SVR was achieved in retreatment subjects with an HCV RNA response that required ≥25 weeks.

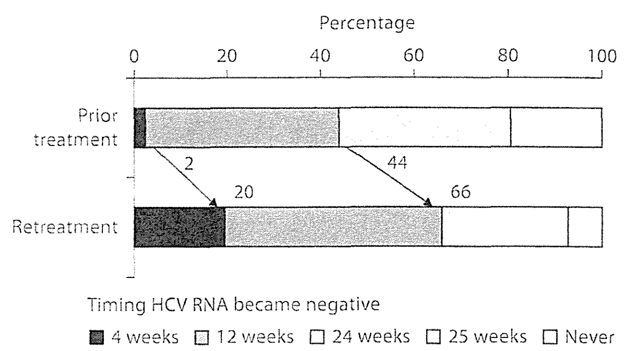

Fig. 2. Comparison of time to eradicate HCV RNA. Prior treatment: PEG-IFNα-2b + RBV combination treatment. Retreatment: PEG-IFNα-2a + RBV combination treatment.

Factors Associated with SVR

Analysis revealed that the SVR rate was significantly high in the following factors: relapsers; prolonged duration of retreatment; Hb level ≥13 g dl^{-1}; PLT ≥12 × 10^4/mm^3; α-fetoprotein (AFP) <6 ng ml^{-1}; low-density lipoprotein cholesterol (LDL-Chol) ≥100 mg dl^{-1}; γ-glutamyl transpeptidase (γ-GTP) ≥35 IU l^{-1}; adherence of PEG-IFN ≥80%; adherence of RBV ≥60%, and EVR. A multivariate analysis of SVR including the effect of initial treatment, duration of retreatment, Hb, PLT, AFP, LDL-Chol, γ-GTP, PEG-IFN adherence, RBV adherence, and EVR as variables showed that EVR and the period of retreatment were major factors contributing to SVR (table 2).

IL28B SNP Genotype

55 patients agreed to undergo a test for IL28B SNP genotyping. Among them, 36, 18, and 1 had the TT, TG, and GG genotypes, respectively. The patients with TT had significantly lower γ-GTP and AFP levels; moreover, relapse patients were more frequently found to have TT. Among the patients with TT, 40% (14/35) showed an HCV RNA response at week 12, 70% (21/29) at week 24, and 56% (20/36) at the end of treatment. Among the non-TT patients, 16% (3/19) showed an HCV RNA response at week 12, 44% (6/16) at week 24, and 42% (7/19) at the end of treatment (fig. 3).

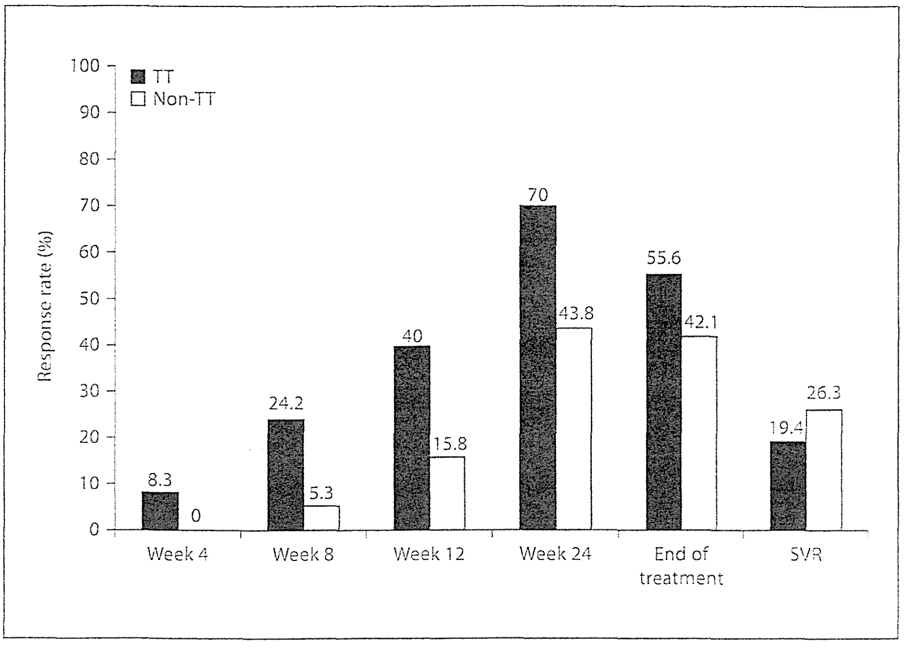

Fig. 3. Virological responses of PEG-IFNα-2a + RBV by IL28B genotype.

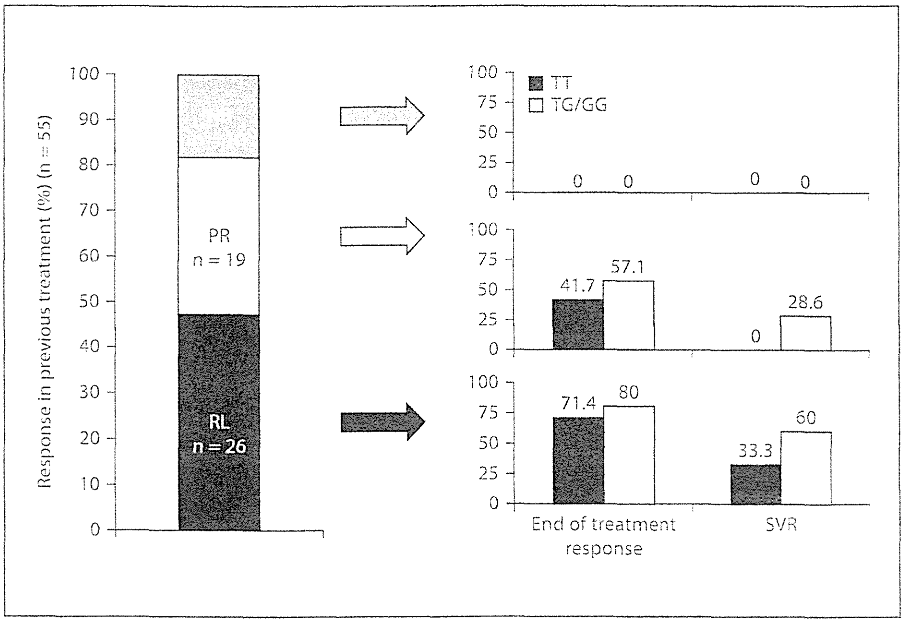

Fig. 4. Virological responses of PEG-IFNα-2a + RBV by IL28B genotype and response of prior treatment. NR = Null responders; PR = partial responders; RL = relapsers.

When we limited the analysis to relapsers, the EVR rates were 57% (12/21) and 40% (2/5) in the TT and non-TT patients, respectively. At week 24, the HCV RNA response rates were 90% (19/21) and 80% (4/5) in the TT and non-TT patients, respectively (fig. 3, 4).

Treatment Discontinuation

33 patients (31.1%) discontinued treatment during the study. 17 patients discontinued treatment due to lack of virological response, 5 due to personal reasons, 3 due to the incidence of hepatocellular carcinoma, and 8 (7.5%) due to adverse events. The reasons for the discontinuation of the 8 patients due to adverse events were anemia

(n = 2), fatigue (n = 2), depressed mental state (n = 1), unidentified fever (n = 1), heart failure (n = 1), and interstitial pneumonia (n = 1).

Discussion

We retrospectively examined the therapeutic efficacy and effective predictors after retreatment with PEG-IFNα-2a + RBV combination therapy in 106 CHC patients. These patients showed non-response or relapse after previous PEG-IFNα-2b + RBV combination therapy. SVR was achieved in 53% of the relapsers and 18% of the partial responders. In the relapsers, SVR was more likely to be achieved when HCV RNA became negative within 12 weeks after the commencement of therapy and the therapeutic effect was enhanced with prolonged administration. Among the non-responders, SVR was not achieved in cases without a ≥2 log decrease in HCV RNA during the previous therapy or in cases with an HCV RNA response after ≥25 weeks during the retreatment period. Similar to that observed in the relapsers, SVR in the non-responders was achieved after prolonged retreatment in patients who showed an HCV RNA response within 12 weeks.

Patients with genotype 1 who receive PEG-IFN + RBV therapy for the first time undergo response-guided therapy (RGT) [16], in which the therapeutic period is determined according to the timing of the HCV RNA response after the commencement of therapy. In RGT, PEG-IFN + RBV therapy is recommended for 48 weeks for patients who exhibit an HCV RNA response within 12 weeks after the commencement of therapy (i.e. complete early viral response, cEVR). However, the results of the present study suggest that prolonged administration is favorable for cEVR cases. Moreover, a different RGT is required in patients who receive retreatment and who have previously received PEG-IFN + RBV therapy.

We also examined the status of the IL28B genotype and found that the IL28B non-TT genotype is associated with factors that are unlikely to be related to the response including AFP levels, γ-GTP levels, and mutations in the core regions. However, when limiting the analysis to relapse patients, the rate of HCV RNA negativity did not differ among IL28B genotypes. This suggests that a prior response to PEG-IFN + RBV therapy is more important for predicting the response to retreatment than the IL28B genotype.

As stated earlier, some patients in Japan have not benefited from the launch of telaprevir, because CHC patients in Japan are older than those in Western countries [11, 12] and are often anemic. We believe that retreatment for elderly patients who cannot receive telaprevir-based therapy, especially relapsers and non-responders with a maximum virus decrease of ≥2 log during the previous therapy, should be considered after PEG-IFN + RBV therapy.

Disclosure Statement

The authors have no conflicts of interest to disclose.

References

1 Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J: Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975–982.

2 Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK: Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958–965.

3 Jensen DM, Marcellin P, Freilich B, Andreone P, Di Bisceglie A, Brandao-Mello CE, Reddy KR, Craxi A, Martin AO, Teuber G, Messinger D, Thommes JA, Tietz A: Retreatment of patients with chronic hepatitis C who do not respond to peginterferon-α2b: a randomized trial. Ann Intern Med 2009;150:528–540.

4 Poynard T, Colombo M, Bruix J, Schiff E, Terg R, Flamm S, Moreno-Otero R, Carrilho F, Schmidt W, Berg T, McGarrity T, Heathcote EJ, Goncales F, Diago M, Craxi A, Silva M, Bedossa P, Mukhopadhyay P, Griffel L, Burroughs M, Brass C, Albrecht J: Peginterferon alfa-2b and ribavirin: effective in patients with hepatitis C who failed interferon alfa/ribavirin therapy. Gastroenterology 2009;136:1618–1628 e1612.

5 Ghany MG, Strader DB, Thomas DL, Seeff LB: Diagnosis, management, and treatment of hepatitis C: an update. Hepatology 2009;49:1335–1374.

6 Kumada H, Okanoue T, Onji M, Moriwaki H, Izumi N, Tanaka E, Chayama K, Sakisaka S, Takehara T, Oketani M, Suzuki F, Toyota J, Nomura H, Yoshioka K, Seike M, Yotsuyanagi H, Ueno Y: Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis C virus infection for the fiscal year 2008 in Japan. Hepatol Res 2010;40:8–13.

7 McHutchison JG, Manns MP, Muir AJ, Terrault NA, Jacobson IM, Afdhal NH, Heathcote EJ, Zeuzem S, Reesink HW, Garg J, Bsharat M, George S, Kauffman RS, Adda N, Di Bisceglie AM: Telaprevir for previously treated chronic HCV infection. N Engl J Med;362:1292–1303.

8 Zeuzem S, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, Focaccia R, Younossi Z, Foster GR, Horban A, Ferenci P, Nevens F, Müllhaupt B, Pockros P, Terg R, Shouval D, van Hoek B, Weiland O, Van Heeswijk R, De Meyer S, Luo D, Boogaerts G, Polo R, Picchio G, Beumont M: Telaprevir for retreatment of HCV infection. N Engl J Med 2011;364:2417–2428.

9 Hayashi N, Okanoue T, Tsubouchi H, Toyota J, Chayama K, Kumada H: Efficacy and safety of telaprevir, a new protease inhibitor, for difficult-to-treat patients with genotype 1 chronic hepatitis C. J Viral Hepat 2012; 19:e134–e142.
10 Kumada H, Toyota J, Okanoue T, Chayama K, Tsubouchi H, Hayashi N: Telaprevir with peginterferon and ribavirin for treatment-naive patients chronically infected with HCV of genotype 1 in japan. J Hepatol 2012; 56:78–84.
11 Chung H, Ueda T, Kudo M: Changing trends in hepatitis c infection over the past 50 years in Japan. Intervirology 2010;53:39–43.
12 Tanaka Y, Kurbanov F, Mano S, Orito E, Vargas V, Esteban JI, Yuen MF, Lai CL, Kramvis A, Kew MC, Smuts HE, Netesov SV, Alter HJ, Mizokami M: Molecular tracing of the global hepatitis C virus epidemic predicts regional patterns of hepatocellular carcinoma mortality. Gastroenterology 2006;130:703–714.
13 Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Arase Y, Ikeda K, Kumada H: Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology 2005;48:372–380.
14 Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB: Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399–401.
15 Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M: Genome-wide association of IL28B with response to pegylated interferon-α and ribavirin therapy for chronic hepatitis C. Nat Genet 2009;41:1105–1109.
16 Lee SS, Ferenci P: Optimizing outcomes in patients with hepatitis C virus genotype 1 or 4. Antivir Ther 2008;13(suppl 1):9–16.

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Significance of a reduction in HCV RNA levels at 4 and 12 weeks in patients infected with HCV genotype 1b for the prediction of the outcome of combination therapy with peginterferon and ribavirin

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Abstract

Background

The importance of the reduction in hepatitis C virus (HCV) RNA levels 4 and 12 weeks after starting peginterferon (PEG-IFN) and ribavirin combination therapy has been reported to predict a sustained virologic response (SVR) in patients infected with HCV genotype 1. We conducted a multicenter study to validate this importance along with baseline predictive factors in this patient subpopulation.

Methods

A total of 516 patients with HCV genotype 1 and pretreatment HCV RNA levels ≥5.0 log_{10} IU/mL who completed response-guided therapy according to the AASLD guidelines were enrolled. The reduction in serum HCV RNA levels 4 and 12 weeks after starting therapy was measured using real-time PCR, and its value in predicting the likelihood of SVR was evaluated.

Results

The area under the receiver operating characteristics (ROC) curve was 0.852 for 4-week reduction and 0.826 for 12-week reduction of HCV RNA levels, respectively. When the cut-off is fixed at a 2.8-log_{10} reduction at 4 weeks and a 4.9-log_{10} reduction at 12 weeks on the basis of ROC analysis, the sensitivity and specificity for SVR were 80.9% and 77.9% at 4 weeks and were 89.0% and 67.2% at 12 weeks, respectively. These variables were independent factors associated with SVR in multivariate analysis. Among 99 patients who showed a delayed virologic response and completed 72-week extended regimen, the area under ROC curve was low: 0.516 for 4-week reduction and 0.482 for 12-week reduction of HCV RNA levels, respectively.

Conclusions

The reduction in HCV RNA levels 4 and 12 weeks after starting combination therapy is a strong independent predictor for SVR overall. These variables were not useful for predicting SVR in patients who showed a slow virologic response and experienced 72-week extended regimen

Keywords, Chronic hepatitis C, Peginterferon, Ribavirin, Reduction in HCV RNA levels, Four and twelve weeks, Baseline factors, Response-guided therapy, Extended treatment

Background

Many investigators have sought to identify factors that can predict the treatment outcome of peginterferon (PEG-IFN) and ribavirin combination therapy in patients infected with HCV genotype 1. Previous studies reported baseline host and viral factors that are associated with the treatment outcomes. The genetic polymorphisms near the *IL28B* gene (rs12979860 or rs8099917) reportedly constitute a host factor that is strongly associated with treatment outcome [1-5], and studies from Japan have reported that amino acid substitutions at residue 70 of the HCV core region and residues 2209–2248 of the NS5A region of HCV (i.e., interferon sensitivity-determining region, ISDR) are viral factors associated with treatment outcome in patients infected with HCV genotype 1 [6-10]. In addition to the baseline predictive factors, the response to HCV during therapy, i.e., the changes in serum HCV RNA levels after initiation of therapy, has also been shown to be an important predictor of treatment outcome [11-14]. Especially, the disappearance or the reduction in serum HCV RNA levels at 4 and 12 weeks after starting therapy have been reported to be important, therefore, rapid virologic response (RVR) or early virologic response (EVR) defined at 4 and 12 weeks after starting therapy, respectively, is a pivotal criteria in predicting treatment response [11-23].

There are adverse effects associated with PEG-IFN and ribavirin antiviral therapy, and the treatment course is costly. For these reasons, it is important to predict the likelihood that a patient will achieve SVR during early stages of therapy with high reliability, in order to prevent unnecessary treatment. This will become increasingly important with the emergence of new antiviral drugs against HCV [24-28]. In the present study, we conducted a multicenter cohort study to examine whether the reduction in HCV RNA levels 4 and 12 weeks after starting PEG-IFN and ribavirin combination therapy, along with baseline predictive factors, has any value in predicting SVR.

Methods

Patients, treatments, and evaluation of responses

The inclusion criteria for this multicentre study were (i) infection with HCV genotype 1 without co-infection with hepatitis B virus or human immunodeficiency virus; (ii) pretreatment HCV RNA levels ≥5.0 $\log_{10}$ IU/mL, based on a quantitative real-time PCR-based method (COBAS AmpliPrep / COBAS TaqMan HCV Test; Roche Molecular Systems: Pleasanton, CA, US.; lower limit of quantification, 1.6 $\log_{10}$ IU/ mL: lower limit of detection, 1.2 $\log_{10}$ IU/ mL) [29,30]; (iii) standard PEG-IFN and ribavirin therapy according to the American Association for the Study of the Liver Diseases (AASLD) guidelines [31] started between December 2004 and January 2010; (iv) completed treatment regimen of 48- or 72-week duration with virologic outcomes available for evaluation; and (v) 100% medication adherence for both PEG-IFN and ribavirin during the initial 4 weeks of therapy and 80% or more throughout the treatment period. With regard to inclusion criterion (i), this study did not include any patients infected with HCV genotype 1a because this genotype is usually not found in the Japanese general population. With regard to criterion (ii), we focused on patients with pretreatment HCV RNA level ≥5.0 $\log_{10}$ IU/mL because the use of ribavirin along with PEG-IFN is not allowed by Japanese National Medical Insurance System for patients with pretreatment HCV RNA levels <5.0 $\log_{10}$ IU/mL. With regard to criterion (iv), the treatment duration was determined based on the response-guided therapy according to AASLD guidelines. Patients in whom serum HCV RNA disappeared until 12 weeks after starting therapy (complete EVR) underwent 48-week treatment regimen. Patients in whom serum HCV RNA disappeared after 12 weeks but until 24 weeks after starting therapy (delayed virologic response) underwent 72-week extended treatment regimen. Patients whose treatment was discontinued due to the presence of serum HCV RNA at 24 weeks of therapy (partial responders or null responders as per the AASLD guidelines), or due to viral breakthrough were also included in the study.

A total of 808 patients underwent the combination therapy with PEG-IFN and ribavirin between December 2004 and January 2010 in one of the following five Liver Centers: Musashino Red Cross Hospital, Kurume University Hospital, Ogaki Municipal Hospital, Shinmatsudo Central General Hospital, and Kagawa Prefectural Central Hospital. For 126 patients, the treatment regimen consisted of weekly PEG-IFN alpha-2a (Pegasys, Chugai Pharmaceutical, Tokyo, Japan) and daily ribavirin (Copegus, Chugai Pharmaceutical). The other 682 patients were treated with weekly PEG-IFN alpha-2b (Pegintron, MSD Co., Tokyo, Japan) and daily ribavirin (Rebetol, MSD Co.). We excluded patients who had been treated with PEG-IFN alpha-2a and ribavirin in order to avoid the influence of PEG-IFN subtype on the association between viral dynamics and treatment outcome. In 682 patients who received PEG-IFN alpha-2b, 516 patients fulfilled the eligibility criteria and were included for analysis (Figure 1). The doses of PEG-IFN alpfa-2b and ribavirin were adjusted based on the patient's body weight. Patients ≤ 45 kg were given 60 µg of PEG-IFN alpha-2b weekly, those > 45 kg and ≤ 60 kg were given 80 µg, those > 60 kg and ≤ 75 kg were given 100 µg, those > 75 kg and ≤ 90 kg were given 120 µg, and those > 90 kg were given 150 µg. Patients ≤ 60 kg were given 600 mg of ribavirin daily, those > 60 kg and ≤ 80 kg were given 800 mg, and those > 80 kg were given 1000 mg per day. Dose modifications of PEG-IFN or ribavirin were based on the manufacturer's recommendations.

Figure 1 Schematic representation of the study patients

SVR was defined as undetectable serum HCV RNA 24 weeks after the end of therapy. A patient was considered to have relapsed when serum HCV RNA levels became detectable between the end of treatment and 24 weeks after completion of therapy, although serum HCV RNA levels were undetectable at the end of therapy. A non-response was defined as detectable serum HCV RNA at 24 weeks after initiation of therapy (i.e., null response or partial non-response according to the AASLD guidelines). RVR was defined as undetectable serum HCV RNA 4 weeks after starting therapy. EVR was defined as the disappearance or a decrease in serum HCV RNA levels by at least 2 $\log_{10}$ at 12 weeks after starting therapy. Patients were considered to have a complete EVR if the serum HCV RNA levels were undetectable 12 weeks after starting therapy and a partial EVR if the serum HCV RNA levels were detectable but had decreased by at least 2 $\log_{10}$ at 12 weeks of therapy. A non-EVR was defined as a lack of a decrease of HCV RNA by more than 2 $\log_{10}$ at 12 weeks when compared to pretreatment levels. Patients were considered to have a delayed virologic response if serum HCV RNA levels became undetectable after 12 weeks but until 24 weeks on treatment.

The study protocol was in compliance with the Helsinki Declaration and was approved by the ethics committee of each participating institution, i.e., the ethics committee of Musashino Red Cross Hospital, the ethics committee of Kurume University Hospital, the ethics committee of Ogaki Municipal Hospital, the ethics committee of Shinmatsudo Central General Hospital, and the ethics committee of Kagawa Prefectural Central Hospital. Prior to initiating the study, written informed consent was obtained from each patient to use their clinical and laboratory data and to analyze stored serum samples.

Measurements of serum HCV RNA levels, amino acid substitution at residue 70 in the HCV core, amino acid sequence of HCV NS5A-ISDR, and genetic polymorphisms near the IL28B gene

After a patient gave informed consent, serum samples were obtained during the patient's regular hospital visits, just prior to beginning treatment, and every 4 weeks during the treatment period and the 24-week follow-up period after treatment. Serum samples were stored at −80°C until they were analyzed. HCV RNA levels were measured using a quantitative real-time PCR-based method (COBAS AmpliPrep/ COBAS TaqMan HCV Test) [29,30]. The reduction in HCV RNA 4 and 12 weeks after initiation of therapy was calculated. When calculating the decrease in serum HCV RNA, HCV RNA level was defined as 0 when HCV RNA was undetectable.

Amino acid 70 of the HCV core region and the amino acid sequence of ISDR region (residues 2209–2248 of the NS5A region) were analyzed by direct nucleotide sequencing of each region as previously reported [6,7]. The following PCR primer pairs were used for direct sequencing of the HCV core region:

5′-GCCATAGTGGTCTGCGGAAC-3′ (outer, sense primer),
5′-GGAGCAGTCCTTCGTGACATG-3′ (outer, antisense primer),
5′-GCTAGCCGAGTAGTGTT-3′ (inner, sense primer), and
5′-GGAGCAGTCCTTCGTGACATG-3′ (inner, antisense primer).

The following PCR primers were used for direct sequencing of ISDR:

5′-TTCCACTACGTGACGGGCAT-3′ (outer, sense primer),
5′-CCCGTCCATGTGTAGGACAT-3′ (outer, antisense primer),
5′-GGGTCACAGCTCCCTGTGAGCC-3′ (inner, sense primer), and
5′-GAGGGTTGTAATCCGGGCGTGC-3′ (inner, antisense primer).

When evaluating ISDR, HCV was defined as wild-type when there were 0 or 1 amino acid substitutions in residues 2209–2248 as compared with the HCV-J strain [32], and as non-wild-type when there was more than 1 substitutions.

Genotyping of rs 8099917 polymorphisms near the *IL28B* gene was performed using the TaqMan SNP assay (Applied Biosystems, Carlsbad, CA) according to the manufacturer's guidelines. A pre-designed and functionally tested probe was used for rs8099917 (C__11710096_10, Applied Biosystems). Genetic polymorphism of rs8099917 reportedly corresponds to rs12979860 in more than 99% of individuals of Japanese ethnicity [33]. The TT genotype of rs8099917 corresponds to the CC genotype of rs12979860, the GG genotype of rs8099917 corresponds to the TT genotype of rs12979860, and the TG heterozygous genotype of rs8099917 corresponds to the CT of rs12979860.

Statistical analyses

Quantitative values are reported as medians and ranges. Differences in percentages between groups were analyzed with the chi-square test. Differences in mean quantitative values were analyzed by the Mann–Whitney U test. The receiver-operating characteristics (ROC) analyses were performed to determine the cut-offs of the reduction in HCV RNA levels at 4 and 12 weeks after starting therapy to evaluate the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy for predicting SVR. Univariate and multivariate analyses using a logistic regression model were performed to identify factors that predict SVR. The factors that are potentially associated with SVR were included in the analyses, i.e., age, sex, body mass index (BMI), serum alanine aminotransferase activity, serum gamma-glutamyl transpeptidase level, total-cholesterol levels, neutrophil count, hemoglobin, platelet count, grade of activity and fibrosis of the liver, pretreatment HCV RNA levels, reduction in HCV RNA levels 4 and 12 weeks after starting therapy, amino acid substitution at residue 70 in the HCV core (arginine vs. glutamine or histidine), amino acid mutations in ISDR (non-wild-type vs. wild-type), and genetic polymorphisms near the *IL28B* gene (rs8099917, genotype TT vs. genotype TG or GG). Data analyses were performed using StatFlex statistical software, version 6 (Artech Co., Ltd., Osaka, Japan). All p values were two-tailed, and $p < 0.05$ was considered statistically significant.

Results

Patient characteristics and treatment outcome

The characteristics of the patients are shown in Table 1. Genotyping of rs8099917 near the *IL28B* gene was performed in 396 patients. Amino acid substitutions at residue 70 in the HCV core region were measured in 361 patients. Amino acid sequences in the ISDR were evaluated in 416 patients. Among 516 patients who were included in the analysis, treatment was completed at 48 weeks in 268 patients who underwent the standard regimen because they showed complete EVR. Treatment was extended from 48 weeks to 72 weeks in 99 patients who yielded delayed virologic response. Treatment was discontinued until 48 weeks in 149 patients because serum HCV RNA remained positive 24 weeks after starting therapy (partial response or null response), or because patients experienced viral breakthrough during therapy.

Table 1 Characteristics of study patients

Age (years), median (range)	60.0 (20.0–80.0)
Sex (male/female) (%)	245 (47.5)/ 271 (52.5)
Body weight (kg), median (range)	58.0 (36.35–107.6)
BMI, median (range)	22.7 (15.8–37.0)
Prior treatment for HCV (no/yes) (%)	359 (69.6)/ 157 (30.4)
Initial dose of PEG-IFN (μg), median (range)	80.0 (40.0–150.0)
Initial dose of ribavirin (mg), median (range)	600 (400–1000)
Pretreatment HCV RNA levels ($\log^{10}$ IU/mL), median (range)	6.1 (5.0–7.7)
Platelet count ($\times 10^3$/μL)	161 (43–352)
Hemoglobin (g/dL)	13.9 (9.7–17.9)
Neutrophil count (/μL)	2489 (578–7480)
Alanine aminotransferase (IU/L)	47 (10–485)
LDL-cholesterol (mg/dL)	99 (25–226)
Total-cholesterol (mg/dL)	171 (29–325)
γ-glutamyl transpeptidase (IU/L)	34.5 (7.0–579)
Alfa fetoprotein (ng/mL)	5.0 (0.8–584)
Fibrosis score (F1/F2/F3/F4) (%)	208(45.9)/139(30.7)/69(15.2)/37(8.2)
Activity score (A1/A2/A3/A4) (%)	258(56.1)/178(38.7)/24(5.2)/0(0)
Genetic polymorphisms of rs8099917 (TT/GG or TG) (%)	288 (72.7)/ 108(27.3)
Amino acid at residue 70 of HCV core (arginine/glutamine or histidine) (%)	242 (67.0)/ 119 (33.0)
Amino acid sequence of ISDR (non-wild-type/wild-type) (%)	110 (26.4)/ 306 (73.6)

BMI, body mass index; *HCV*, hepatitis C virus; *PEG-IFN*, peginterferon; *ISDR*, interferon sensitivity-determining region.
(N = 516)\

As a final outcome, 272 patients (52.7%) achieved SVR, 90 patients (17.5%) relapsed, and 128 patients (24.8%) had a non-response (48 patients with partial response and 80 patients with null-response). Viral breakthrough was observed in 26 patients (5.0%). The rate of SVR was 79.9% (214 of 268 patients) among patients with complete EVR in whom treatment was completed at 48 weeks and 58.6% (58 of 99 patients) among patients with delayed virologic response who underwent the extended 72-week regimen.

Baseline factors affecting SVR in all patients who underwent response-guided therapy according to AASLD guidelines

In all patients who underwent treatment according to the AASLD guidelines, the rate of SVR was significantly higher in patients with the TT genotype of rs8099917 near the *IL28B* gene (179 of 288 patients [62.3%] with TT genotype vs. 15 of 108 patients [13.9%] with TG/GG genotype, $p < 0.0001$). In addition, SVR rate was significantly higher in patients with HCV with arginine at residue 70 in the HCV core region (145 of 242 patients [59.9%] with arginine vs. 34 of 119 patients [28.6%] with glutamine or histidine, $p < 0.0001$). SVR was significantly higher in patients with HCV with non-wild type ISDR (75 of 110 patients [68.2%] with non-wild-type ISDR vs. 139 of 306 patients [45.4%] with wild-type ISDR, $p < 0.0001$). SVR was significantly higher in patients with pretreatment HCV RNA levels <6.0 $\log_{10}$ IU/mL (127 of 199 patients [63.8%] with pretreatment HCV levels <6.0 $\log_{10}$ IU/mL vs. 145 of 317 patients [45.7%] with pretreatment HCV RNA levels ≥6.0 $\log_{10}$ IU/mL, $p < 0.0001$).

Association between reduction of serum HCV RNA levels 4 and 12 weeks after starting therapy and SVR in all patients who underwent response-guided therapy according to the AASLD guidelines

The ROC analysis was performed in 516 patients who underwent the response-guided therapy according to the AASLD guidelines in order to evaluate the association between the reduction in serum HCV RNA levels 4 and 12 weeks after starting therapy and SVR (Figure 2). The area under the ROC curve was 0.852 and the best cut-off was calculated as 2.8 $\log_{10}$ IU/mL, when evaluated with the reduction of serum HCV RNA levels 4 weeks after starting therapy. The rate of SVR was significantly higher in patients with greater than 2.8-$\log_{10}$ reduction at 4 weeks (220 of 274 patients [80.3%] with > 2.8-$\log_{10}$ reduction vs. 52 of 242 patients [21.5%] with ≤ 2.8-$\log_{10}$ reduction, $p < 0.0001$). The sensitivity, specificity, PPV, NPV, and accuracy were 80.9%, 77.9%, 80.3%, 78.5%, and 79.5%, respectively, at this cut-off level. When evaluated with the reduction of serum HCV RNA levels 12 weeks after starting therapy, the area under the ROC curve was 0.826 and the best cut-off was calculated as 4.9 $\log_{10}$ IU/mL. The rate of SVR was significantly higher in patients with greater than 4.9-$\log_{10}$ reduction at 12 weeks (242 of 321 patients [75.4%] with > 4.9-$\log_{10}$ reduction vs. 30 of 194 patients [15.5%] with ≤ 4.9-$\log_{10}$ reduction, $p < 0.0001$). The sensitivity, specificity, PPV, NPV, and accuracy were 89.0%, 67.2%, 75.4%, 84.5%, and 78.7%, respectively, at this cut-off level.

Figure 2 The receiver operating characteristics (ROC) analysis for the prediction of the sustained virologic response to combination therapy with peginterferon alpha-2b and ribavirin according to the reduction in serum HCV RNA levels in all patients who underwent response-guided therapy based on the AASLD guidelines. A) According to the reduction in serum HCV RNA levels 4 weeks after starting therapy. The area under the ROC curve was 0.852. B) According to the reduction in serum HCV RNA levels 12 weeks after starting therapy. The area under the ROC curve was 0.826

A multivariate analysis showed that the reductions in serum HCV RNA levels at 4 and 12 weeks after starting therapy were independent factors associated with SVR, along with pretreatment HCV RNA levels, platelet counts, polymorphisms of rs8099917 near the *IL28B* gene, and amino acid mutations in the HCV NS5A-ISDR (Table 2).

Table 2 Univariate and multivariate analyses for sustained virologic response to the combination therapy with peginterferon and ribavirin in patients who underwent response guided therapy according to the AASLD guidelines

	Univariate analysis	Multivariate analysis*	Odds ratio (95% confidence interval)
Age (years)	< 0.001	N.S.	
Sex (male/female)	0.005	N.S.	
BMI, median (range)	N.S.		
Prior treatment for HCV (no/yes)	N.S.		
Pretreatment HCV RNA levels ($\log_{10}$ IU/mL), (≤6.0 vs. 6.0<)	0.015	0.013	2.235 (1.189-4.203)
Platelet count ($\times 10^3/\mu L$)	< 0.001	0.011	1.007 (1.002-1.013)
Hemoglobin (g/dL)	0.002	N.S.	
Neutrophil count (/μL)	0.003	N.S.	
Alanine aminotransferase (IU/L)	N.S.		
Total-cholesterol (mg/dL)	0.001	N.S.	
γ-glutamyl transpeptidase (IU/L)	0.014	N.S.	
Fibrosis score (F1 or F2/F3 or F4)	< 0.001	N.S.	
Activity score (A1 or A2/A3 or A4)	0.002	N.S.	
Genetic polymorphisms of rs8099917 (TT/GG or TG)	< 0.001	< 0.001	5.782 (2.298-14.552)
Amino acid at residue 70 of HCV core (arginine/glutamine or histidine)	< 0.001	N.S.	
Amino acid sequence of ISDR (non-wild-type/wild-type)	< 0.001	0.038	2.077 (1.041-4.147)
Reduction of HCV RNA [Pre - 4 week] ($\log_{10}$ IU/mL), (≤2.8 vs. 2.8<)	< 0.001	< 0.001	3.911 (1.935-7.908)
Reduction of HCV RNA [Pre - 12 week] ($\log_{10}$ IU/mL), (≤4.9 vs. 4.9<)	< 0.001	0.013	2.578 (1.220-5.448)

*Multivariate analysis was performed on 314 patients in whom all variables were available.
(N = 516)

Association between reduction of serum HCV RNA levels 4 and 12 weeks after starting therapy and SVR in patients with delayed virologic response who underwent an extended 72-week regimen according to response-guided therapy

The ROC analysis was performed in 99 patients with delayed virologic response who underwent an extended 72-week treatment regimen according to the response-guided therapy of the AASLD guidelines to evaluate the association between reduction in serum HCV RNA levels 4 and 12 weeks after starting therapy and SVR (Figure 3). The area under the ROC curve was 0.516 and the best cut-off was calculated as 2.3 $\log_{10}$ IU/mL, when evaluated with the reduction of serum HCV RNA levels 4 weeks after starting therapy. There was no significant difference in the rate of SVR according to the reduction at 4 weeks (21 of 33 patients [63.6%] with > 2.3-$\log_{10}$ reduction vs. 37 of 66 patients [56.1%] with ≤ 2.3-$\log_{10}$ reduction, $p = 0.6120$). The area under the ROC curve was 0.482 and the best cut-off was calculated as 5.1 $\log_{10}$ IU/mL, when evaluated with the reduction of serum HCV RNA levels 12 weeks after starting therapy. There was no significant difference in the rate of SVR according to the reduction at 12 weeks (24 of 42 patients [57.1%] with > 5.1-$\log_{10}$ reduction vs. 34 of 57 patients [59.6%] with ≤ 5.1-$\log_{10}$ reduction, $p = 0.9634$).

Figure 3 The receiver operating characteristics (ROC) analysis for the prediction of the sustained virologic response to combination therapy with peginterferon alpha-2b and ribavirin according to the reduction in serum HCV RNA levels in patients with delayed virologic response who underwent an extended 72-week regimen according to response-guided therapy. A) According to the reduction in serum HCV RNA levels 4 weeks after starting therapy. The area under the ROC curve was 0.516. B) According to the reduction in serum HCV RNA levels 12 weeks after starting therapy. The area under the ROC curve was 0.482

Discussion

Several previous studies have reported that patients who achieved RVR, in whom serum HCV RNA levels become undetectable 4 weeks after starting the therapy, had a high likelihood of achieving SVR [15-18]. However, there are relatively few patients infected with treatment-resistant HCV genotype 1 who achieve RVR. A considerable percentage of patients achieve SVR even without RVR. Therefore, RVR has high specificity but low sensitivity for predicting SVR. Previous studies from Asia evaluated the predictive value of the degree of reduction in serum HCV RNA levels 4 weeks after starting therapy, in addition to RVR [19-21]. However, the number of patients in these studies was small and the analyses were not sufficient to form reliable conclusions.

In the present study, we evaluated the ability of a decrease in serum HCV RNA levels 4 weeks after starting therapy to predict the likelihood of SVR as a final outcome in Japanese patients infected with HCV genotype 1b, based on the data from a large, multi-institution study. The ROC analyses showed that a reduction in serum HCV RNA levels 4 week after starting therapy was strongly associated with SVR, and its predictive value was higher than that of a reduction in serum HCV RNA levels 12 weeks after starting therapy, with higher area under the ROC curve and accuracy. Multivariate analyses including baseline factors that were associated with SVR revealed that the reductions of HCV RNA level at both 4 and 12 weeks after starting therapy were independent factors associated with SVR, and the reduction at 4 weeks had a second strongest impact for SVR, following genetic polymorphisms of rs8099917 near *IL28B* gene.

The important novelty from this study is that the reductions of HCV RNA level 4 and 12 weeks after starting therapy had no predictive value for SVR when focusing on patients who showed delayed virologic response and underwent the extended 72-week treatment regimen according to the response-guided therapy. This was in contrast to the prediction for SVR in all patients who underwent response-guided therapy. The impact of the reduction of HCV RNA level on the prediction of SVR would decline by the selection of patients based on the delayed virologic response. There were also no baseline factors that were associated with SVR in patients who underwent the extended 72-week treatment (data not shown). Prolonged treatment duration may relieve delayed virologic responders from unfavorable conditions. Further studies will be, therefore, needed to identify predictive factors for SVR in patients with delayed virologic response who underwent the 72-week treatment regimen.

There are several limitations to this study. The data were based on Japanese patients infected with HCV genotype 1b. Therefore, these results should be confirmed in patients of other ethnicities and patients infected with HCV genotype 1a. In addition, the value of the reduction in HCV RNA levels 4 and 12 weeks after starting therapy as predictors of SVR should be evaluated in patients who underwent therapy with PEG-IFN alpha 2a and ribavirin to determine the best cut-off levels with that regimen. Statistically, there were many missing data. We performed complete case analysis without the imputation of missing data for multivariate analysis. Although comparison between cases with and without missing data did not show statistically significant differences for cases characteristics, we cannot rule out that the condition of data missing completely at random does not hold. Furthermore, this resulted in the decrease in the number of patients analyzed in multivariate analysis and might have substantially caused the reduction of statistical power, altering the value of non-significant results. In addition, the study did not perform internal validation. The use of hold-out method or split-group validation was difficult because of the number of study patients. Therefore, the validation in another larger study patients will be required in the future for confirming the results of this study.

Conclusions

A reduction in HCV RNA levels 4 and 12 weeks after starting therapy indicated likelihoods that patients will achieve SVR as a final outcome of combination therapy for HCV infection when patients underwent the response-guided therapy according to the AASLD guidelines. These reductions in serum HCV RNA levels were not predictive for SVR when focusing on patients who showed delayed virologic response and underwent the extended 72-week regimen.

Abbreviations

HCV, Hepatitis C virus; PEG-IFN, Peginterferon; SVR, Sustained virologic response; ROC, Receiver operating characteristics; ISDR, Interferon sensitivity-determining region; RVR, Rapid virologic response; EVR, Early virologic response; AASLD, American Association for the Study of the Liver Diseases; BMI, Body mass index; PPV, Positive predictive value; NPV, Negative predictive value

Competing interests

The authors declare the following matters.
The authors have not received reimbursements, fees, funding, or salary from an organization that may in any way gain or lose financially from the publication of this manuscript, neither now nor in the future.
The authors have no stocks or shares in an organization that may in any way gain or lose financially from the publication of this manuscript, neither now nor in the future.
The authors are currently applying no patents relating to the content of the manuscript. We have not received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript.
The authors do not have any other financial competing interests.
There are no non-financial competing interests to declare in relation to this manuscript.
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Authors' contributions

Study design: HT, TK, NS, KT, TI, MS, HG, KM, and NI. Treatment of patients and data acquisition: HT, TK, NS, KT, TI, MS, and NI. Data analyses: HG and KM. Manuscript preparation: HT. Read and approval of the final manuscript: HT, TK, NS, KT, TI, MS, HG, KM, and NI. All authors read and approved the final manuscript.

References

1. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB: **Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance.** *Nature* 2009, **461**:399–401.

2. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J: **IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy.** *Nat Genet* 2009, **41**:1100–1104.

3. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M: **Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C.** *Nat Genet* 2009, **41**:1105–1109.

4. McCarthy JJ, Li JH, Thompson A, Suchindran S, Lao XQ, Patel K, Tillmann HL, Muir AJ, McHutchison JG: **Replicated association between an IL28B gene variant and a sustained response to pegylated interferon and ribavirin.** *Gastroenterology* 2010, **138**:2307–2314.

5. Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, Bochud M, Battegay M, Bernasconi E, Borovicka J, Colombo S, Cerny A, Dufour JF, Furrer H, Günthard HF, Heim M, Hirschel B, Malinverni R, Moradpour D, Müllhaupt B, Witteck A, Beckmann JS, Berg T,

Bergmann S, Negro F, Telenti A, Bochud PY, Swiss Hepatitis C Cohort Study; Swiss HIV Cohort Study: **Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study.** *Gastroenterology* 2010, **138**:1338–1345.

6. Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C: **Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection.** *N Engl J Med* 1996, **334**:77–81.

7. Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Kobayashi M, Arase Y, Ikeda K, Kumada H: **Association of amino acid substitution pattern in core protein of hepatitis C virus genotype1b high viral load and non-virological response to interferon-ribavirin combination therapy.** *Intervirology* 2005, **48**:372–380.

8. Donlin MJ, Cannon NA, Yao E, Li J, Wahed A, Taylor MW, Belle SH, Di Bisceglie AM, Aurora R, Tavis JE: **Pretreatment sequence diversity differences in the full-length hepatitis C virus open reading frame correlate with early response to therapy.** *J Virol* 2007, **81**:8211–8224.

9. Maekawa S, Enomoto N: **Viral factors influencing the response to the combination therapy of peginterferon plus ribavirin in chronic hepatitis C.** *J Gastroenterol* 2009, **44**:1009–1015.

10. Hayes NC, Kobayashi M, Akuta N, Suzuki F, Kumada H, Abe H, Miki D, Imamura M, Ochi H, Kamatani N, Nakamura Y, Chayama K: **HCV substitutions and IL28B polymorphisms on outcome of peg-interferon plus ribavirin combination therapy.** *Gut* 2011, **60**:261–267.

11. Zeuzem S, Herrmann E, Lee JH, Fricke J, Neumann AU, Modi M, Colucci G, Roth WK: **Viral kinetics in patients with chronic hepatitis C treated with standard or peginterferon alpha2a.** *Gastroenterology* 2001, **120**:1438–1447.

12. Buti M, Sanchez-Avila F, Lurie Y, Stalgis C, Valdes A, Martell M, Esteban R: **Viral kinetics in genotype 1 chronic hepatitis C patients during therapy with 2 different doses of peginterferon alfa-2b plus ribavirin.** *Hepatology* 2002, **35**:930–936.

13. Berg T, Sarrazin C, Herrmann E, Hinrichsen H, Gerlach T, Zachoval R, Wiedenmann B, Hopf U, Zeuzem S: **Prediction of treatment outcome in patients with chronic hepatitis C: significance of baseline parameters and viral dynamics during therapy.** *Hepatology* 2003, **37**:600–609.

14. Lee SS, Ferenci P: **Optimizing outcomes in patients with hepatitis C virus genotype 1 or 4.** *Antiviral Res* 2008, **13**:S9–S16.

15. Martinez-Bauer E, Crespo J, Romero-Gomez M, Moreno-Otero R, Sola R, Tesei N, Pons F, Forns X, Sanchez-Tapias JM: **Development and validation of two models for early prediction of response to therapy in genotype 1 chronic hepatitis C.** *Hepatology* 2006, **43**:72–80.