

and 40 IU/L. Recently, Kumada *et al.*^{71,72} demonstrated that the cumulative incidence of hepatocarcinogenesis increased in parallel with the increase in ALT average integration value in CH-C even in patients with normal ALT levels. In a community-based study, an elevated ALT level (>35 IU/L) was shown to be a significant risk factor of HCC development.⁷³

Recommendation 7: To prevent the occurrence of HCC, levels of serum ALT should be controlled at below 30 IU/l. (Level 3, Grade A.)

IFN administration

More than two decades have passed since IFN began to be used to treat CH-C patients. Nowadays, more than 70% of HCV-infected people can be cured by the combination therapy of PEG-IFN plus RBV. However, even in patients who were cured of HCV infection and attained an SVR, the occurrence of HCC may be reported long after completion of IFN therapy. The risk factor of HCC occurrence after IFN therapy is a combination of advanced hepatic fibrosis score before therapy, older age and male sex.⁷⁴⁻⁷⁶ Bruno *et al.*⁷⁵ reported that annual incidence of HCC occurrence in liver cirrhosis after attaining SVR was 0.66%, which was one-third of the incidence of HCC in liver cirrhosis without a virological response (non-SVR).

Recommendation 8: Surveillance is required for the occurrence of HCC in patients with CH-C and liver cirrhosis. Even if IFN-based therapy is successful in attaining SVR, screening for the detection of HCC by computed tomography (CT), magnetic resonance imaging or ultrasonography and measurement of the serum tumor markers should be carried out routinely, especially for patients with advanced hepatic fibrosis, older age and male sex, because they are at high risk for the occurrence of HCC. (Level 2b, Grade A.)

Indication of IFN therapy for CH-C

Interferon-based therapy is used to treat chronic HCV-infected patients worldwide and PEG-IFN plus RBV is the first choice indication for CH-C patients. Because IFN and RBV have a variety of adverse effects including depression and thyroid dysfunction, "who and how" to treat should be determined with caution. The AALSD practice guideline advocates that treatment decision should be individualized based on the severity of liver disease, the potential for serious side-effects, the likelihood of treatment response, the presence of comorbid condition and the patient's readiness for treatment.¹

Recommendation 9: Treatment decision of IFN therapy for CH-C should be individualized based on the body/

mental condition, probability of successful therapy and prolonged survival, and likelihood of provoking serious adverse effects. Scores of hepatic stage/grade should be considered as well. For aged patients, in whom HCV infection is regarded as the major determinant of survival, IFN-based therapy should be considered with caution. (Level 3, Grade A.)

PEG-IFN AND RBV COMBINATION THERAPY

Factors associated with virological response to PEG-IFN and RBV combination therapy

TREATMENT WITH PEG-IFN- α -2A or -2b together with RBV has been evaluated in two nationwide phase III registration trials in Japan.^{77,78} In one trial, which determined efficacy of PEG-IFN α -2b and RBV,⁷⁹ the SVR rate to 48-week combination therapy was 48% (121/254) in patients with HCV genotype 1b and a high viral load (≥ 100 KIU/mL). Another trial using PEG-IFN- α -2a and RBV demonstrated an SVR rate to 48-week combination therapy of 59% (57/96) in patients with HCV genotype 1b and a high viral load (≥ 100 KIU/mL).⁸⁰ Based on these results, the currently recommended standard therapy for the patients with CH-C in Japan is the combination of a PEG-IFN together with RBV, except for the treatment naïve patients with a low viral load for whom a PEG-IFN monotherapy is recommended.

These clinical trials identified the following factors that are associated with non-SVR in patients with HCV genotype 1b and a high viral load; (i) older patients; (ii) non-responders to previous IFN therapy; (iii) advanced fibrosis; (iv) female sex; and (v) poor adherence below 80%. In marked contrast to the data from Europe and the USA, the SVR rate in Japanese female patients is lower than that in the male patients. Several community-based retrospective studies in Japan also demonstrated that female patients, especially older female patients, are more difficult to treat compared with other patients.^{81,82} Other factors associated with virological response reported from Japan include the low-density lipoprotein cholesterol level,⁸³ α -fetoprotein (AFP) level,⁸³ whole-body insulin sensitivity index,⁸⁴ single nucleotide polymorphisms of MAPKAPK3,⁸⁵ RIG-I/IPS-1 ratio,⁸⁶ Th1/Th2 ratio⁴⁵ and PKR response.⁸⁷ Association between viral mutations and treatment response is discussed in depth above.

Recommendation 10: Predictors associated with a non-SVR to PEG-IFN and RBV include: (i) age older than 60 years, particularly older women; (ii) advanced fibro-

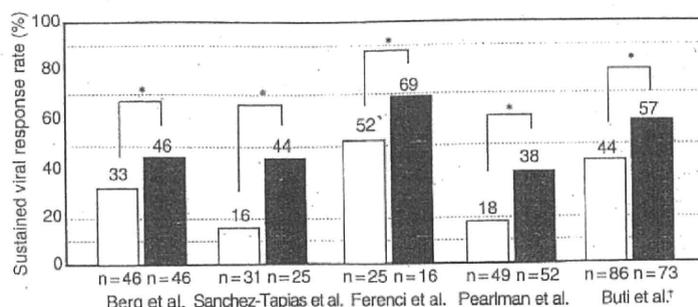


Figure 2 Comparison of sustained virological response rate between 48-week (open column) and 72-week (closed column) treatment with pegylated interferon and ribavirin in patients with partial early virological responder, which is defined as ≥ 2 log reduction in hepatitis C virus (HCV) RNA level compared to baseline HCV RNA level but detectable HCV RNA at treatment week 12. *Statistical significance between two treatment groups. †Comparison in patients with $\geq 80\%$ adherence is shown.

sis; (iii) non-responder to previous IFN therapy; and (iv) poor adherence below 80%. (Level 2a, Grade B.)

Response-guided therapy for patients with HCV genotype 1

Measuring the rate of viral clearance from serum is helpful in predicting the likelihood of a response to PEG-IFN and RBV, and useful for determining the optimal duration of therapy. In two nationwide registration trials conducted in Japan,^{77,78} the SVR rate was high, from 76–100% in patients whose HCV RNA was cleared rapidly from serum by week 4, and 71–73% in patients who achieved undetectable HCV RNA from week 5 to week 12. In contrast, the SVR rate in patients with late clearance of HCV RNA from week 13 to week 24 was low at 29–36%. No patients without clearance of HCV RNA by week 24 achieved SVR. It should be noted that time point of HCV clearance was determined by measurement of serum HCV RNA utilizing the Amplicor HCV method in these trials.

Recommendation 11: Measuring the time of viral clearance from serum is helpful in predicting the likelihood of a response to PEG-IFN and RBV. Measurement of HCV RNA is recommended at weeks 4, 12 and 24. (Level 1, Grade A.)

As mentioned above, patients whose HCV RNA measured by Amplicor HCV had not cleared by week 24 were unable to achieve SVR with 48-week standard PEG-IFN and RBV therapy. However, in a retrospective study conducted in 52 patients without HCV RNA clearance from serum by week 24, the rate of ALT normalization 6 months after the completion of therapy (so-called biochemical response) was 56% (5/9) and 62% (8/13) of

patients achieved ALT normalization up to 2 years after the completion of therapy (sustained biochemical response).⁸⁸ Therefore, the proposal that recommends a continuation of PEG-IFN and RBV therapy for 48 weeks in biochemical responders at week 24 even without HCV clearance has been accepted widely in Japan. This proposal is in marked contrast to the AASLD practice guideline,¹ in which treatment discontinuation is strongly recommended in patients whose HCV RNA remains positive at week 24.

Recommendation 12: It is impossible to achieve SVR in patients without HCV RNA clearance by week 24 measured by Amplicor HCV. (Level 1, Grade A.) However, it is recommended to continue the therapy for 48 weeks even in patients without HCV RNA clearance by week 24 if ALT normalizes at week 24, because a sustained biochemical response can be obtained in these patients. (Level 4, Grade C.)

The strategy of extending therapy in patients with delayed virological responses, defined as clearance of HCV RNA between weeks 12 and 24, was evaluated in five studies.^{89–93} These results cannot be compared directly with each other because of the heterogeneous study populations, differences in the baseline characteristics and the different regimens utilized amongst them. Nevertheless, the results showed a trend toward a higher SVR rate by extending therapy from 48 to 72 weeks in patients with delayed virological response (Fig. 2).^{89–93}

In Japan, a randomized controlled trial was conducted in 113 patients with HCV genotype 1b and a high viral load, comparing a 48-week treatment group and extended treatment group where patients were treated for an additional 44 weeks after clearance of

HCV RNA from serum. In this trial, the SVR rate was 36% in the 48-week treatment group and 53% in the extended treatment group, and the SVR rate was significantly higher in patients in the extended treatment group who became HCV RNA-negative during the period week 16–24 (9% vs 78%, $P=0.005$).⁹⁴ In addition, in a case-control study matched for age, sex and the timing of HCV RNA clearance from serum, the SVR rate was high at 62% in the 72-week treatment group ($n=65$) compared to 33% in the 48-week treatment group ($n=130$), and the extended treatment was particularly effective in patients with HCV core mutations at aa70 and aa91 as well as patients with wild type of ISDR sequence.⁷⁹ Accordingly, 72-week extended treatment is recommended for patients who are slow to clear of HCV RNA between weeks 12 and 24.

Currently, HCV RNA clearance from serum is determined by real-time PCR detection, although most of former studies utilized the Amplicor HCV method for this purpose. Because real-time PCR is highly sensitive, it should be reevaluated in terms of who gains benefit from extended therapy. Currently, there is no sufficient evidence to determine this. Nevertheless, substantial number of community-based Japanese study using real-time PCR detection suggested that SVR could be obtained by 72-week treatment if HCV RNA became undetectable by week 36. Accordingly, when determining the timing of HCV RNA clearance using real-time PCR detection, 72-week treatment could be recommended for patients who achieve HCV RNA clearance between weeks 12 and 36.

Recommendation 13: 72-week extended therapy should be considered for patients with HCV genotype 1 who have delayed HCV RNA clearance from serum between weeks 12 and 24. (Level 2a, Grade B.)

Recommendation 14: When using a real-time detection-PCR method for measurement of HCV RNA, SVR can be obtained by 72-week extended treatment in patients who have achieved HCV RNA clearance by week 36. (Level 2b, Grade C.)

Response-guided therapy for patients with HCV genotype 2

Six trials have evaluated a shortening of the duration of therapy from 24 weeks to 12–16 weeks for patients with chronic HCV genotype 2 and 3.^{80,95–99} Although the data from some of these trials suggest that patients with genotype 2 and 3 infection who achieve viral clearance from serum by week 4 can shorten their treatment duration to 12–16 week,^{80,95,99} the benefit of a shortening the duration of therapy remains controversial.⁹⁶ In a recent

study by Mangia *et al.*, the factors associated with relapse after shorter duration of therapy are identified as age over 45 years, pre-treatment platelet count of less than $140 \times 10^9/L$, and body mass index over 30 kg/m^2 ,¹⁰⁰ suggesting shortening the duration of therapy can be considered only in particular patients without predictors associated with relapse. Because most Japanese patients have risk factors for relapse such as older age and advanced fibrosis, shortening the duration of the therapy is not generally recommended for Japanese patients with genotype 2, even if they achieve viral clearance by week 4.

PEG-IFN and RBV combination therapy in patients with compensated cirrhosis

In the early Western registration trials, patients with HCV-related compensated cirrhosis did achieve SVR but at lower rates than did those without cirrhosis.^{101–103} Subsequently, there was one treatment study that focused exclusively on patients with compensated cirrhosis.¹⁰⁴ In this study, 124 patients with compensated cirrhosis were assigned randomly to an RBV 1000/1200-mg (standard dose) group and 600/800-mg (low dose) group to determine the efficacy of PEG-IFN and RBV combination therapy. The SVR was achieved in 52% of patients who received the standard RBV dose and in 38% of those treated with the low dose. Serious adverse events developed in 14% and 18% of recipients of the standard and low RBV doses, respectively, while dose reduction was necessary in 78% and 57% of the two groups, respectively. HCV genotype 2/3 and platelet count over $150 \times 10^9/L$ were identified as factors contributing to SVR. Thus, patients with HCV-related compensated cirrhosis can be treated successfully with PEG-IFN and RBV but careful observation is needed because of an anticipated higher rate of adverse effects. Although PEG-IFN and RBV for patients with compensated cirrhosis has not been approved yet in Japan, the following recommendation is reasonable.

Recommendation 15: Patients with HCV-related compensated cirrhosis can be treated successfully with PEG-IFN and RBV but careful observation is needed because of an anticipated higher rate of adverse effects. (Level 3, Grade B.)

Retreatment with PEG-IFN and RBV combination therapy for patients who failed to respond to previous IFN treatment

Seven randomized controlled trials have been reported so far that examine the efficacy of PEG-IFN and RBV

combination therapy in patients who failed to respond to previous standard IFN therapy with or without RBV.^{105–111} The SVR rate varies among these trials ranging 6–45%, and was lower among non-responders to previous IFN therapy compared with relapsers. In a study using PEG-IFN α -2b and RBV at two different doses (1.5 μ g/kg per week of PEG-IFN α -2b together with 800 mg/day of RBV or 1.0 μ g/kg per week of PEG-IFN together with 1000–1200 mg/day of RBV), the SVR rate was low at 10% and 6% in non-responders to previous treatment, but was high at 50% and 32% in relapsers, respectively.¹⁰⁹ In a phase III clinical trial in Japan, the SVR rate was also low in non-responders but sufficiently high in relapsers.⁷⁷ Accordingly, PEG-IFN and RBV combination therapy is well indicated for patients who relapse after standard IFN therapy with or without RBV.

Data on retreatment of patients who failed to respond to previous PEG-IFN plus RBV therapy have been evaluated in two trials.^{112,113} In a randomized controlled trial that used two different doses of PEG-IFN- α -2a (360 or 180 μ g/week) with two different durations of therapy (72- or 48-week),¹¹² an SVR was achieved in 7–14% of patients. It should be noted, however, that the SVR was favorable at 52% in patients who achieved HCV RNA clearance from serum by week 12 in the 72-week treatment arm.¹¹² In the other trial that used PEG-IFN- α -2b and RBV in 2333 patients who failed to respond to previous PEG or standard IFN together with RBV, an SVR was achieved in 56% of patients whose HCV RNA was cleared from serum by week 12 and in 48% of those with genotype 1.¹¹³ Accordingly, it is reasonable to propose that SVR could be obtained by retreatment with PEG-IFN and RBV in patients who achieve HCV RNA clearance by week 12 of retreatment, even if they failed to respond to previous PEG-IFN and RBV combination therapy.^{112,113} In contrast, in the AASLD practice guideline, retreatment with PEG-IFN and RBV is not recommended for patients who did not achieve an SVR after a prior full course of PEG-IFN and RBV. Because it is still unclear who is more likely to respond to retreatment with PEG-IFN and RBV, and new drugs such as protease inhibitors may be indicated in the near future for patients who failed to respond to previous PEG-IFN and RBV therapy, data with retreatment of PEG-IFN and RBV should be accumulated to enable a conclusive recommendation.

Recommendation 16: Retreatment with PEG-IFN and RBV can be considered for non-responders and relapsers who were treated previously with IFN-based therapy with or without RBV. An SVR could be obtained in these

patients whose HCV RNA is cleared from serum by week 12 of retreatment with PEG-IFN and RBV. (Level 2b, Grade B.)

MONOTHERAPY WITH IFN OR PEG-IFN

IN JAPAN, IFN monotherapy has been used to treat HCV infection since 1992. Today, IFN monotherapy is used only in patients with specific characteristics because combination therapy with PEG-IFN and RBV has achieved a high rate of SVR. Recently, a large randomized control trial (RCT) of maintenance therapy with a low dose of PEG-IFN was reported.¹¹⁴ There were no differences in progression of liver disease between a PEG-IFN group and a control group. However, Japanese studies of elderly patients or patients who received maintenance therapy for longer periods showed that IFN can improve outcomes in advanced hepatic fibrosis.

Naïve patients with low viral loads

Previous studies showed that 3 MIU of IFN monotherapy achieved SVR rates of 15–45% in patients with fewer than 2×10^6 copies of HCV.^{115–118} Monotherapy with 180 μ g/week of PEG-IFN- α -2a or 1.5 μ g/kg per week of PEG-IFN- α -2b produced SVR rates of 16–46% in patients with fewer than 2×10^6 copies.^{119–121} In Japanese patients with fewer than 1×10^5 copies of HCV, 6 MIU of IFN treatment for 24 weeks achieved an SVR rate of 86% (127/148).¹²² PEG-IFN monotherapy for 48 weeks similarly achieved an SVR rate of 86% (106/123). A recent RCT showed that PEG-IFN monotherapy for 24 weeks produced the same SVR rate as similar treatment for 48 weeks in patients with fewer than 1×10^5 copies of HCV. On the basis of these results, monotherapy with IFN or PEG-IFN is considered to be an effective treatment for naïve patients with fewer than 5.0 log copies/mL of HCV.¹²³

Recommendation 17: Monotherapy with IFN or PEG-IFN can be considered for naïve patients with low viral loads (<5.0 log copies/mL). (Level 2a, Grade B.)

Patients with chronic kidney disease

Patients with chronic kidney disease (CKD) who undergo hemodialysis have a high prevalence of HCV infection. In Japan, one study reported that HCV RNA was detected in 117 (22%) of 543 patients who underwent maintenance hemodialysis.¹²⁴ Hemodialysis patients infected with HCV have a higher mortality rate than uninfected hemodialysis patients.¹²⁵ This higher

mortality is attributed to the frequent progression to cirrhosis and/or HCC in HCV-infected patients who receive hemodialysis.

Because RBV is excreted renally, it is currently contraindicated in patients with CKD who have a creatinine clearance of less than 50 mL/min. In addition, pharmacokinetic studies have shown that the clearance of IFN is lower in patients who undergo hemodialysis than in patients who have normal renal function.¹²⁶

Studies of antiviral therapy in patients who undergo hemodialysis suggest that IFN monotherapy is generally well tolerated and that SVR rates are higher than those in patients with normal renal function.¹²⁷ The overall SVR rate was reported to be 33–37% in hemodialysis patients.¹²⁸ However, the number of subjects in these trials was too low to support confident conclusions. Adverse events are common in this population, and many patients discontinue therapy prematurely because of such events. A recent RCT showed in EASL 2008 that 135 µg/week of PEG-IFN- α -2a for 48 weeks achieved an SVR rate of 39% (23/38), whereas a dose of 90 µg/week produced an SVR rate of 35% (16/43). In 74% of the patients, treatment was completed as scheduled.

Another important point is when to initiate antiviral therapy in hemodialysis patients. IFN might induce allograft rejection and renal failure.¹²⁹ Therefore, IFN therapy should be considered before renal transplantation. The next issue to be resolved is the efficacy and safety of low-dose RBV combination therapy in hemodialysis patients.

In 2008, KDIGO proposed guidelines for the treatment of patients with CKD.¹³⁰ In Japan, a committee including hepatologists and specialists for CKD is planning a clinical trial for HCV-infected patients with CKD.

Recommendation 18: 3 MIU of IFN thrice weekly or 90 or 135 µg of PEG-IFN- α -2a weekly is recommended for patients with CKD. (Level 2a, Grade B.)

Patients with acute HCV infection

Acute HCV infection progresses to chronic infection in approximately 70% of patients.¹³¹ Antiviral treatment should therefore be considered for this group of patients. On the other hand, it is difficult to identify patients with self-limited disease not requiring therapy. The results of previous studies indicate that anti-HCV treatment should be initiated if HCV RNA is detected continuously for more than 12–16 weeks. If treatment is initiated within this period, monotherapy with IFN or PEG-IFN achieves an SVR rate of more than 80% in patients with acute HCV infection.¹³² Reliable evidence

showing that additional treatment with RBV improves the SVR rate in such patients is not available.

Recommendation 19: Patients with acute HCV infection should be considered as candidates for antiviral therapy. If HCV RNA is detected continuously for 12 or 16 weeks from the onset, treatment with 6 MIU of IFN or 180 µg of PEG-IFN monotherapy should be initiated. (Level 2a, Grade B.)

Patients who receive curative treatment for HCC

Hepatocellular carcinoma frequently recurs in HCV-infected patients, even after curative therapy for HCC. Prevention of the recurrence of HCC is essential in such patients. Several RCT showed that the incidence of HCC was low in an IFN-treated group, compared to a control group (Table 4).^{133,134} For example, Kubo *et al.* reported that 3 MIU IFN monotherapy thrice weekly for 96 weeks inhibited the recurrence of HCC in patients who had undergone a curative resection.¹³⁴ Furthermore, Shiratori *et al.* performed an RCT in 74 patients who had received curative percutaneous ethanol injection therapy for HCC. They reported that second and third recurrences of HCC were less frequent in patients who received IFN.¹³⁵ In an Italian study of 150 patients who had undergone curative resection, the recurrence rate of HCC 2 years after operation was significantly lower among patients who received IFN.¹³⁶

Japanese studies showed that the survival rate was also improved by IFN treatment owing to the suppression of HCC and/or the progression of hepatic failure.^{137,138}

Recommendation 20: IFN therapy should be considered for patients after curative treatment for HCC. (Level 1, Grade A.)

Maintenance therapy for patients with advanced hepatic fibrosis

Previous studies of patients with advanced hepatic fibrosis, defined as a fibrosis score 3 or 4, showed that IFN monotherapy inhibited the occurrence of HCC, compared to patients who did not receive IFN.^{64,139,140} In Japanese studies, IFN was effective not only in SVR patients, but also in non-SVR patients.^{139,141} On the other hand, an Italian study showed that the incidence of HCC decreased only in cirrhotic patients in whom HCV was eradicated by IFN therapy.⁷⁵

Case-control studies in patients older than 60 years showed that a low dose of IFN reduced ALT and AFP levels and decreased the incidence of HCC, compared to a control group.^{142,143} RCT for IFN monotherapy non-

Table 4 Interferon monotherapy for patients after curative treatment for hepatocellular carcinoma

Author	Study design	No. of patients (IFN group vs non-IFN group)	Age (IFN group vs non-IFN group)	Interferon	Sustained virological response	Follow-up duration (months)	HCC recurrence (IFN group vs non-IFN group)	Survival (IFN group vs non-IFN group)
Ikeda <i>et al.</i>	RCT	10 vs 10	60 vs 65	beta	0	25	10% vs 70% $P = 0.0004$	
Kubo <i>et al.</i>	RCT	15 vs 15	62 vs 60	alpha	2 (13%)	54	60% vs 87% $P = 0.055$	80% vs 50% $P = 0.041$
Suou <i>et al.</i>	Pilot study	18 vs 22	61 vs 62	alpha	6 (33%)	60	28% vs 82% $P < 0.001$	100% vs 73% $P < 0.05$
Shiratori <i>et al.</i>	RCT	49 vs 25	61 vs 63	alpha	14 (29%)	60	80% vs 92%	68% vs 48%
Lin <i>et al.</i>	RCT	8 vs 6	61 vs 59	alpha	no data	27	63% vs 83% $P = 0.34$	
Jeong <i>et al.</i>	Prospective case-control study	16 vs 16	69 vs 68	alpha	2 (13%)	36	69% vs 80% $P = 0.157$	100% vs 88% $P = 0.45$
Sakaguchi <i>et al.</i>	Case-control study	24 vs 33	69 vs 67	alpha	1 (4%)	36	14% vs 73% $P = 0.011$	100% vs 94% $P = 0.25$
Mazzafarro <i>et al.</i>	RCT	76 vs 74	65 vs 67	alpha	2 (3%)	45	76% vs 94% $P = 0.49$	64% vs 52% $P = 0.47$
Akamatsu <i>et al.</i>	Retrospective study	53 vs 399	60 vs 68	no data	17 (32%)	72		88%, 71% vs 53.2% $P = 0.025$
Kudo <i>et al.</i>	Case-control study	43 vs 84	65 vs 66	alpha or pegylated IFN	2 (5%)	60	56% vs 71% $P = 0.04$	86% vs 56% $P = 0.004$

IFN, interferon; HCC, hepatocellular carcinoma; RCT, randomized control study.

responders showed that histological fibrosis and activity was improved in the assigned IFN-treated group. In contrast, in the untreated group, the fibrosis score did not decline.¹⁴⁴ In Japan, several studies support the effectiveness of low-dose IFN maintenance therapy.^{145–147} In the USA, an RCT of 53 patients in whom a histological response, but not a viral response was induced by 6 MIU of IFN showed that 3 MIU of IFN for 24 months improved the degree of hepatic fibrosis.

However, the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis (HALT-C) trial found no difference in the progression of liver disease between a low-dose PEG-IFN group and a control group.¹¹⁴ The large discrepancy in the effectiveness of IFN maintenance therapy between the HALT-C trial and Japanese trials might be attributed to several factors. First, the study designs differed. One of the most important differences was related to the patients' clinical characteristics. For example, patients enrolled in Japanese studies were older than those in the HALT-C trial. Elderly patients have a higher incidence of HCC than younger patients. It is suggested that the tumor-suppressive effect of IFN maintenance therapy might be more clearly demonstrated in a high-risk group, including elderly patients.¹³⁸

Until more data become available, the decision to perform IFN maintenance therapy should be made on an individual basis.

Recommendation 21: IFN maintenance therapy is a treatment option that can inhibit the progression of liver disease in patients with advanced hepatic fibrosis, especially in those who are elderly. However, the effect of monotherapy with IFN or PEG-IFN remains uncertain in non-responders to combination therapy with PEG-IFN plus RBV. (Level 2a, Grade C.)

CONSENSUS ON THERAPEUTIC STRATEGY FOR CH-C

Indication of antiviral therapy

IKEDA *ET AL.* elucidated the necessities of antiviral therapy for elderly patients with chronic HCV infection.¹³² At 5 and 10 years, hepatocarcinogenesis rates in the intermediate ($100\text{--}140 \times 10^9/\text{L}$) and low platelet ($<100 \times 10^9/\text{L}$) groups were 10.9% and 21.6% in the IFN group ($n = 217$) and 19.5% and 43.0% in the untreated group ($n = 459$), respectively ($P = 0.0005$). IFN independently decreased the risk of carcinogenesis risk with a hazard ratio of 0.56 ($P = 0.035$). On the other hand, in the high platelet ($\geq 150 \times 10^9/\text{L}$) group,

no significant difference was found in 5- and 10-year carcinogenesis rates between the IFN-treated group ($n = 228$) and the untreated group ($n = 585$) ($P = 0.69$). Furthermore, IFN treatment significantly increased cumulative survival in the lower platelet subgroup ($P = 0.0001$) but did not affect the higher platelet subgroup ($P = 0.08$). Thus, the necessities of antiviral therapy are shown to be greater in elderly patients with advanced fibrosis, although adverse effects of IFN are reported to be more frequent and the efficacy of IFN to be lower in such patients.^{148–150}

Therefore, the indication of antiviral therapy should be considered in the following order: the necessity of treatment, first; safety of treatment, second; and efficacy of treatment for a patient, last. Antiviral therapy should not be given up because the expected SVR rate is low.

Recommendation 22: Antiviral therapy should be offered even to CH-C patients whose SVR rates are expected to be low if type C chronic liver disease is the prognostic determinant (prognosis is improved by HCV elimination) for the individual patient, and the expected adverse effects are tolerable to the patients. (Level 6, Grade B/C.)

Effect of drug adherence of PEG-IFN and RBV on virological response

The relationship between drug exposure and antiviral effect of PEG-IFN plus RBV combination therapy has been reported in several papers.^{101,151–155} McHutchison *et al.* revealed that the SVR rate in patients who received 80% or more of their total planned doses of PEG-IFN- α -2b and RBV for 80% or more of the scheduled duration of therapy was significantly higher than that of patients who received less than 80% of one or both drugs (51% vs 34%) and also suggested that the impact of dose reduction was greatest in patients for whom the dose had to be decreased within the first 12 weeks of treatment.¹⁵²

Recently, Oze *et al.* evaluated how reducing drug doses affects complete early virological response (c-EVR) defined as HCV RNA negativity at week 12, using 984 patients with CH-C genotype 1.¹⁵⁶ As a result, the mean dose of PEG-IFN- α -2b, and not RBV, during the first 12 weeks was the independent factor for c-EVR ($P = 0.02$), not RBV.

Hiramatsu *et al.* reported on whether dose reduction of RBV (or PEG-IFN) has an effect on virological relapse in PEG-IFN plus RBV treatment for patients with CH-C genotype 1.¹⁵⁷ In the analysis of 472 patients responding to PEG-IFN- α -2b plus RBV, stepwise reduction of the

RBV dose was associated with a stepwise increase in relapse rate from 11% to 60% (Fig. 3):

Improving the treatment tolerability for genotype 2 or 3 patients has focused on dose reduction of treatment drugs. Weiland *et al.* examined low-dose PEG-IFN- α -2a (135 μ g/week) with a weight-based standard dose of RBV (11 mg/kg daily) for genotype 2 and 3 patients.¹⁵⁸ Recently, Inoue *et al.* reported neither PEG-IFN nor RBV drug exposure were critical in reaching rapid virological response and SVR.¹⁵⁹

Recommendation 23: In genotype 1 patients, PEG-IFN is dose-dependently correlated with c-EVR, independent of RBV dose. The administration over 80% of the scheduled dose of PEG-IFN- α -2a or over 1.2 μ g/kg per week of PEG-IFN- α -2b should be chosen as a starting dose: a marked dose reduction of PEG-IFN should not be risked at the start even for patients with disadvantage (e.g. aged patients). (Level 2b/3, Grade B.)

Recommendation 24: In genotype 1 patients, RBV shows a dose-dependent correlation with the relapse after treatment. Maintaining the RBV dose over 80% of the scheduled dose or over 10 mg/kg per day (12 mg/kg per day, if possible) during the complete treatment period can lead to suppression of the relapse in HCV genotype 1 patients responding to PEG-IFN- α -2b plus RBV, especially in c-EVR patients. (Level 2b/3, Grade B.)

Recommendation 25: In genotype 2/3 patients, reducing drug doses of PEG-IFN and RBV (down to 400 mg/day) has no significant effect on virological responses. (Level 2a, Grade B.)

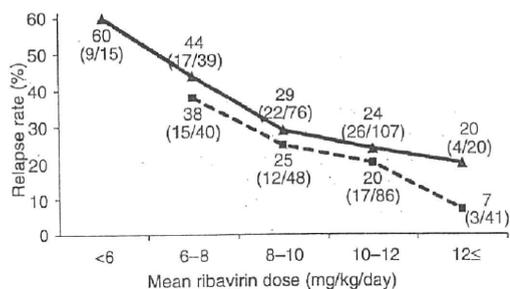


Figure 3 Relapse rate according to pegylated interferon (PEG-IFN)- α -2b and ribavirin doses during treatment of patients who completed treatment, which was stratified with the mean ribavirin doses (\blacktriangle). Group with the mean PEG-IFN dose < 1.4 μ g/kg/week (\blacktriangle). Group with the mean PEG-IFN dose \geq 1.4 μ g/kg/week (\blacksquare). There was no significant difference between the two PEG-IFN- α -2b-dose groups ($P = 0.17$).

Treatment for patients without elimination of HCV

Tarao *et al.* showed the rate of HCC appearance was significantly higher in HCV-related cirrhotic patients with a high ALT value (≥ 80 IU/mL) than in those with a lower ALT value (< 80 IU/mL).⁷⁰ This suggested that suppression of inflammation in the liver with HCV infection is very important to prevent the hepatocarcinogenesis in patients with HCV-related cirrhosis.

Omata *et al.* assessed the effects of oral ursodeoxycholic acid (UDCA) on serum biomarkers. CH-C patients with elevated ALT were assigned randomly to 150 ($n = 199$), 600 ($n = 200$) or 900 mg/day ($n = 197$) UDCA intake for 24 weeks. As a result, the median changes in serum ALT at the end of treatment were shown to be -15.3, -29.2 and -36.2%, respectively, although serum HCV RNA did not change in any group.¹⁶⁰

A glycyrrhizin product, Stronger Neo-Minophagen C (SNMC; Minophagen Pharmaceutical, Tokyo, Japan), is used widely in Japan and has been reported to improve ALT levels and liver inflammation.^{161,162} Furthermore, Ikeda *et al.* reported liver carcinogenesis was suppressed by long-term administration of glycyrrhizin, using a cohort of 1249 patients, and its favorable effect on hepatocellular carcinogenesis in those patients with IFN-resistant CH-C.^{163,164}

Repeated phlebotomy has been shown to be effective for the improvement of serum ALT as well as progression of fibrosis,³² however, it remains controversial whether the effects of IFN improve with extensive phlebotomy.¹⁶⁵⁻¹⁶⁹

In Japan, Yano *et al.* showed the iron removal by repeated phlebotomy improved serum ALT levels in patients with CH-C.¹⁷⁰

Recommendation 26: Patients whose HCV RNA was not eradicated by PEG-IFN plus RBV and whose ALT and/or AFP levels were not improved by IFN monotherapy or those without indication for IFN therapy should be treated with the liver-supporting therapy (SNMC, UDCA), and if the effect of this medication is inadequate, phlebotomy can be used in combination. (Level 3/6, Grade B/C.)

Treatment of patients with decompensated cirrhosis

The compensated patients who failed to eradicate HCV by antiviral therapy and decompensated patients should be referred for consideration of liver transplantation and liver supporting therapy should be performed. Long-

term nutritional supplementation with oral branched-chain amino acid (BCAA) has been shown to be useful to prevent progressive hepatic failure and to improve surrogate markers.^{171,172} Early interventional with oral BCAA was shown to prolong the liver transplant waiting period by preserving hepatic reserve in cirrhosis.

Recommendation 27: Patients with compensated cirrhosis for the prevention of hepatocellular carcinogenesis, should be treated by not only IFN but also with liver supporting therapy (SNMC, UDCA) and/or phlebotomy and/or BCAA in order to improve the liver inflammation, and AFP levels. (Level 3, Grade C.)

Novel antiviral drugs

Telaprevir, a protease inhibitor specific to the HCV nonstructural 3/4A serine protease, reduced HCV RNA levels rapidly in early studies. McHuthison *et al.* reported the improved SVR rate with triple therapy for 12 weeks followed by PEG-IFN- α -2a and RBV for 12 weeks.

Thus, the treatment for CH-C is progressing. Therefore, as a treatment strategy, PEG-IFN plus RBV combination therapy should be performed early for aged patients and the patients with the advanced fibrosis. However, the novel antiviral drugs, such as protease inhibitors and polymerase inhibitors, should be taken into account as a candidate of treatment for the patients who can wait for the oncoming drugs.

Recommendation 28: Novel antiviral drugs, such as a protease inhibitor or a polymerase inhibitor, in combination with PEG-IFN plus RBV, can improve the SVR rates in genotype 1 CH-C patients. (Level 2a, Grade A.)

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Original Article

Phase I/II study of a fine-powder formulation of cisplatin for transcatheter arterial chemoembolization in hepatocellular carcinoma

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Aim: The clinical feasibility of transcatheter arterial chemoembolization (TACE) with fine-powder cisplatin (CDDP) in patients with hepatocellular carcinoma (HCC) has not been investigated. A phase I/II study was conducted to investigate the safety and tolerability of fine-powder CDDP when it was used with lipiodol and gelatin sponge particles for TACE.

Methods: Fine-powder CDDP emulsified in lipiodol was injected into tumor arteries. Embolization was subsequently performed with gelatin sponge particles. The CDDP dose was started at 45 mg/m² (level 1) and increased to 65 mg/m² in 10 mg/m² increments.

Results: Thirteen patients were enrolled in phase I study since no dose limiting toxicity was observed in any patients, even in seven patients at level 3 (65 mg/m²), the recommended dose was 65 mg/m². The major adverse event was grade 3 thrombocytopenia, which occurred in 8% of

patients. The incidence of hematological toxicities was 15% for leukocytopenia, 84% for thrombocytopenia, and 84% for anemia. Increased serum total bilirubin was observed in 54% and increased aspartate aminotransferase or alanine aminotransferase in all patients. All digestive tract symptoms (nausea 77%, anorexia 84%, vomiting 31%) were grade 2 or lower. Total adverse events were grade 3 or higher in 44%. The response rate in 19 patients who received the recommended dose was 21%.

Conclusions: TACE with a fine-powder formulation of CDDP at a dose of 65 mg/m² is well tolerated in patients with unresectable HCC.

Key words: cisplatin, hepatocellular carcinoma, transcatheter arterial chemoembolization

INTRODUCTION

TRANSCATHETER ARTERIAL CHEMOEMBOLIZATION (TACE) is a widely used treatment for unresectable hepatocellular carcinoma (HCC).¹ Gelatin sponge particles are mainly used for embolization, and almost always in conjunction with lipiodol.² Doxorubicin (DXR), epirubicin (EPI), mitomycin C, cisplatin (CDDP), and others, have been used as anticancer agents, and response rates range from 15% to 73%.^{3–8}

The response rate to TACE with CDDP varies (15–73%).^{3,4,7} The standard concentration of CDDP

solution in Japan (0.5 mg/mL) cannot be mixed with an appropriate dose of lipiodol. A fine-powder formulation of CDDP (IA-call[®], Nippon Kayaku, Tokyo) has recently been developed, making possible the preparation of high-concentration aqueous solutions. The response rate was 33.8% against HCC by hepatic arterial infusion with high-concentration CDDP solutions (1.43 mg/mL). Moreover, unlike conventional preparations, fine-powder CDDP is readily miscible when mixed with lipiodol and thereby easy to use for TACE. However, the safety and tolerability of using fine-powder CDDP for TACE is unknown in patients with HCC.

We conducted a phase I/II study of fine-powder CDDP for TACE using lipiodol and gelatin sponge particles in HCC patients. The primary objectives of this study were to evaluate the safety and tolerability of fine-powder CDDP, and to determine the recommended dose (RD). The secondary objective was to evaluate the tumor response.

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Pre-treatment prediction of response to pegylated-interferon plus ribavirin for chronic hepatitis C using genetic polymorphism in *IL28B* and viral factors

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Background & Aims: Pegylated interferon and ribavirin (PEG-IFN/RBV) therapy for chronic hepatitis C virus (HCV) genotype 1 infection is effective in 50% of patients. Recent studies revealed an association between the *IL28B* genotype and treatment response. We aimed to develop a model for the pre-treatment prediction of response using host and viral factors.

Methods: Data were collected from 496 patients with HCV genotype 1 treated with PEG-IFN/RBV at five hospitals and universities in Japan. *IL28B* genotype and mutations in the core and IFN sensitivity determining region (ISDR) of HCV were analyzed to predict response to therapy. The decision model was generated by data mining analysis.

Results: The *IL28B* polymorphism correlated with early virological response and predicted null virological response (NVR) (odds ratio = 20.83, $p < 0.0001$) and sustained virological response (SVR) (odds ratio = 7.41, $p < 0.0001$) independent of other covariates. Mutations in the ISDR predicted relapse and SVR independent of *IL28B*. The decision model revealed that patients with the minor *IL28B* allele and low platelet counts had the highest NVR (84%) and lowest SVR (7%), whereas those with the major *IL28B* allele and mutations in the ISDR or high platelet counts had the lowest NVR (0–17%) and highest SVR (61–90%). The model had high reproducibility and predicted SVR with 78% specificity and 70% sensitivity.

Conclusions: The *IL28B* polymorphism and mutations in the ISDR of HCV were significant pre-treatment predictors of response to PEG-IFN/RBV. The decision model, including these host and viral factors may support selection of optimum treatment strategy for individual patients.

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Introduction

Hepatitis C virus (HCV) infection is the leading cause of cirrhosis and hepatocellular carcinoma worldwide [1]. The successful eradication of HCV, defined as a sustained virological response (SVR), is associated with a reduced risk of developing hepatocellular carcinoma. Currently, pegylated interferon (PEG-IFN) plus ribavirin (RBV) is the most effective standard of care for chronic hepatitis C but the rate of SVR is around 50% in patients with HCV genotype 1 [2,3], the most common genotype in Japan, Europe, the United States, and many other countries. Moreover, 20–30% of patients with HCV genotype 1 have a null virological response (NVR) to PEG-IFN/RBV therapy [4]. The most reliable method for predicting the response is to monitor the early decline of serum HCV-RNA levels during treatment [5] but there is no established method for prediction before treatment. Because PEG-IFN/RBV therapy is costly and often accompanied by adverse effects such as flu-like symptoms, depression and hematological abnormalities, pre-treatment predictions of those patients who are unlikely to benefit from this regimen enables ineffective treatment to be avoided.

Recently, it has been reported through a genome-wide association study (GWAS) of patients with genotype 1 HCV that single nucleotide polymorphisms (SNPs) located near the *IL28B* gene are strongly associated with a response to PEG-IFN/RBV therapy in

Keywords: *IL28B*; ISDR; Peg-interferon; Ribavirin; Data mining; Decision tree.
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Table 1. Baseline characteristics of all patients, and patients assigned to the model building or validation groups.

	All patients n = 496	Model group n = 331	Validation group n = 165
Gender: male	250 (50%)	170 (51%)	80 (48%)
Age (years)	57.1 ± 9.9	56.8 ± 9.7	57.5 ± 10.2
ALT (IU/L)	78.6 ± 60.8	78.1 ± 61.4	79.7 ± 59.8
GGT (IU/L)	59.3 ± 63.6	58.9 ± 62.0	60.2 ± 66.9
Platelets (10 ⁹ /L)	154 ± 53	153 ± 52	154 ± 56
Fibrosis: F3-4	121 (24%)	80 (24%)	41 (25%)
HCV-RNA: >600,000 IU/ml	408 (82%)	273 (82%)	136 (82%)
ISDR mutation: ≤1	220 (88%)	290 (88%)	145 (88%)
Core 70 (Arg/Gln or His)	293 (59%)/203 (41%)	197 (60%)/134 (40%)	96 (58%)/69 (42%)
Core 91 (Leu/Met)	299 (60%)/197 (40%)	200 (60%)/131 (40%)	99 (60%)/66 (40%)
<i>IL28B</i> : Minor allele	151 (30%)	101 (31%)	50 (30%)
SVR	194 (39%)	129 (39%)	65 (39%)
Relapse	152 (31%)	103 (31%)	49 (30%)
NVR	150 (30%)	99 (30%)	51 (31%)

ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; ISDR, interferon sensitivity determining region; Arg, arginine; Gln, glutamine; His, histidine; Leu, leucine; Met, methionine; Minor, heterozygote or homozygote of minor allele; SVR, sustained virological response; NVR, null virological response.

Japanese [6], European [7], and a multi-ethnic population [8,9]. The last three studies focused on the association of SNPs in the *IL28B* region with SVR [7–9] but we found a stronger association with NVR [6]. In addition to these host genetic factors, we have reported that mutations within a stretch of 40 amino acids in the NSSA region of HCV, designated as the IFN sensitivity determining region (ISDR), are closely associated with the virological response to IFN therapy: a lower number of mutations is associated with treatment failure [10–13]. Amino acid substitutions at positions 70 and 91 of the HCV core region (Core70, Core91) also have been reported to be associated with response to PEG-IFN/RBV therapy: glutamine (Gln) or histidine (His) at Core70 and methionine (Met) at Core91 are associated with treatment resistance [4,14]. The importance of substitutions in the HCV core and ISDR was confirmed recently by a Japanese multicenter study [15]. How these viral factors contribute to response to therapy is yet to be determined. For general application in clinical practice, host genetic factors and viral factors should be considered together.

Data mining analysis is a family of non-parametric regression methods for predictive modeling. Software is used to automatically explore the data to search for optimal split variables and to build a decision tree structure [16]. The major advantage of decision tree analysis over logistic regression analysis is that the results of the analysis are presented in the form of flow chart, which can be interpreted intuitively and readily made available for use in clinical practice [17]. The decision tree analysis has been utilized to define prognostic factors in various diseases [18–25]. We have reported recently its usefulness for the prediction of an early virological response (undetectable HCV-RNA within 12 weeks of therapy) to PEG-IFN/RBV therapy in chronic hepatitis C [26].

This study aimed to define the pre-treatment prediction of response to PEG-IFN/RBV therapy through the integrated analysis of host factors, such as the *IL28B* genetic polymorphism and various clinical covariates, as well as viral factors, such as mutations in the HCV core and ISDR and serum HCV-RNA load. In addition,

for the general application of these results in clinical practice, decision models for the pre-treatment prediction of response were determined by data mining analysis.

Materials and methods

Patients

This was a multicentre retrospective study supported by the Japanese Ministry of Health, Labor and Welfare. Data were collected from a total of 496 chronic hepatitis C patients who were treated with PEG-IFN alpha and RBV at five hospitals and universities throughout Japan. Of these, 98 patients also were included in the original GWAS analysis [6]. The inclusion criteria in this study were as follows (1) infection by genotype 1b, (2) lack of co-infection with hepatitis B virus or human immunodeficiency virus, (3) lack of other causes of liver disease, such as autoimmune hepatitis, and primary biliary cirrhosis, (4) completion of at least 24 weeks of therapy, (5) adherence of more than 80% to the planned dose of PEG-IFN and RBV for the NVR patients, (6) availability of DNA for the analysis of the genetic polymorphism of *IL28B*, and (7) availability of serum for the determination of mutations in the ISDR and substitutions of Core70 and Core91 of HCV. Patients received PEG-IFN alpha-2a (180 µg) or 2b (1.5 µg/kg) subcutaneously every week and were administered a weight adjusted dose of RBV (600 mg for <60 kg, 800 mg for 60–80 kg, and 1000 mg for >80 kg daily) which is the recommended dosage in Japan. Written informed consent was obtained from each patient and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional ethics review committee. The baseline characteristics are listed in Table 1. For the data mining analysis, 67% of the patients (331 patients) were assigned randomly to the model building group and 33% (165 patients) to the validation group. There were no significant differences in the clinical backgrounds between these two groups.

Laboratory and histological tests

Blood samples were obtained before therapy and were analyzed for hematologic tests and for blood chemistry and HCV-RNA. Sequences of ISDR and the core region of HCV were determined by direct sequencing after amplification by reverse-transcription and polymerase chain reaction as reported previously [4,11]. Genetic polymorphism in one tagging SNP located near the *IL28B* gene (rs8099917) was determined by the GWAS or DigiTag2 assay [27]. Homozygosity (GG) or heterozygosity (TG) of the minor sequence was defined as having the *IL28B* minor allele, whereas homozygosity for the major sequence (TT) was

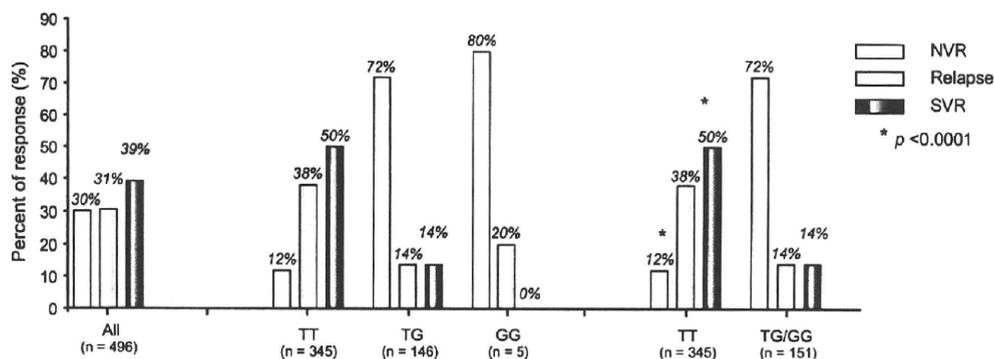


Fig. 1. Association between the *IL28B* genotype (rs8099917) and treatment response. The rates of response to treatment are shown for each rs8099917 genotype. The rate of null virological response (NVR), relapse, and sustained virological response (SVR) is shown. The *p* values are from Fisher's exact test. The rate of NVR was significantly higher ($p < 0.0001$) and the rate of SVR was significantly lower ($p < 0.0001$) in patients with the *IL28B* minor allele compared to those with the major allele. [This figure appears in colour on the web.]

defined as having the *IL28B* major allele. In this study, NVR was defined as a less than 2 log reduction of HCV-RNA at week 12 and detectable HCV-RNA by qualitative PCR with a lower detection limit of 50 IU/ml (Amplicor, Roche Diagnostic systems, CA) at week 24 during therapy. RVR (rapid virological response) and complete early virological response (cEVR) were defined as undetectable HCV-RNA at 4 weeks and 12 weeks during therapy and SVR was defined as undetectable HCV-RNA 24 weeks after the completion of therapy. Relapse was defined as reappearance of HCV-RNA after the completion of therapy. The stage of liver fibrosis was scored according to the METAVIR scoring system: F0 (no fibrosis), F1 (mild fibrosis: portal fibrosis without septa), F2 (moderate fibrosis: few septa), F3 (severe fibrosis: numerous septa without cirrhosis) and F4 (cirrhosis). Percentage of steatosis was quantified in 111 patients by determining the average proportion of hepatocytes affected by steatosis.

Statistical analysis

Associations between pre-treatment variables and treatment response were analyzed by univariate and multivariate logistic regression analysis. Associations between the *IL28B* polymorphism and sequences of HCV were analyzed by Fisher's exact test. SPSS software v.15.0 (SPSS Inc., Chicago, IL) was used for these analyses. For the data mining analysis, IBM-SPSS Modeler version 13.0 (IBM-SPSS Inc., Chicago, IL) software was utilized as reported previously [26]. The patients used for model building were divided into two groups at each step of the analysis based on split variables. Each value of each variable was considered as a potential split. The optimum variables and cut-off values were determined by a statistical search algorithm to generate the most significant division into two prognostic subgroups that were as homogeneous as possible for the probability of SVR. Thereafter, each subgroup was evaluated again and divided further into subgroups. This procedure was repeated until no additional significant variable was detected or the sample size was below 15. To avoid over-fitting, 10-fold cross validation was used in the tree building process. The reproducibility of the resulting model was tested with the data from the validation patients.

Results

Association between the *IL28B* (rs8099917) genotype and the PEG-IFN/RBV response

The rs8099917 allele frequency was 70% for TT ($n = 345$), 29% for TG ($n = 146$), and 1% for GG ($n = 5$). We defined the *IL28B* major allele as homozygous for the major sequence (TT) and the *IL28B* minor allele as homozygous (GG) or heterozygous (TG) for the minor sequence. The rate of NVR was significantly higher (72% vs. 12%, $p < 0.0001$) and the rate of SVR was significantly lower (14% vs. 50%, $p < 0.0001$) in patients with the *IL28B* minor allele compared to those with the major allele (Fig. 1).

Effect of the *IL28B* polymorphism, substitutions in the ISDR, Core70, and Core91 of HCV on time-dependent clearance of HCV

Patients were stratified according to their *IL28B* allele type, the number of mutations in the ISDR, the amino acid substitutions in Core70 and Core91, and the rate of undetectable HCV-RNA at 4, 8, 12, 24, and 48 weeks after the start of therapy was analyzed (Fig. 2A–D). The rate of undetectable HCV-RNA was significantly higher in patients with the *IL28B* major allele than the minor allele, in patients with two or more mutations in the ISDR compared to none or only one mutation, in patients with arginine (Arg) at Core70 rather than Gln/His, and in patients with leucine (Leu) at Core91 rather than Met. The difference was most significant when stratified by the *IL28B* allele type. The rate of RVR and cEVR was significantly more frequent in patients with the *IL28B* major allele compared with those with the *IL28B* minor allele: 9% vs. 3% for RVR ($p < 0.005$) and 57% vs. 11% for cEVR ($p < 0.0001$). These findings suggest that *IL28B* has the greatest impact on early virological response to therapy.

Association between substitutions in the ISDR and relapse after the completion of therapy

Patients were stratified according to the *IL28B* allele, number of mutations in the ISDR, and amino acid substitutions of Core70 and Core91, and the rate of relapse was analyzed (Fig. 3A and B). Among patients who achieved cEVR, the rate of relapse was significantly lower in patients with two or more mutations in the ISDR compared to those with only one or no mutations (15% vs. 31%, $p < 0.005$) (Fig. 3B). On the other hand, the relapse rate was not different between the *IL28B* major and minor alleles within patients who achieved RVR (3% vs. 0%) or cEVR (28% vs. 29%) (Fig. 3A). Amino acid substitutions of Core70 and Core91 were not associated with the rate of relapse (data not shown).

Factors associated with response by multivariate logistic regression analysis

By univariate analysis, the minor allele of *IL28B* ($p < 0.0001$), one or no mutations in the ISDR ($p = 0.03$), high serum level of

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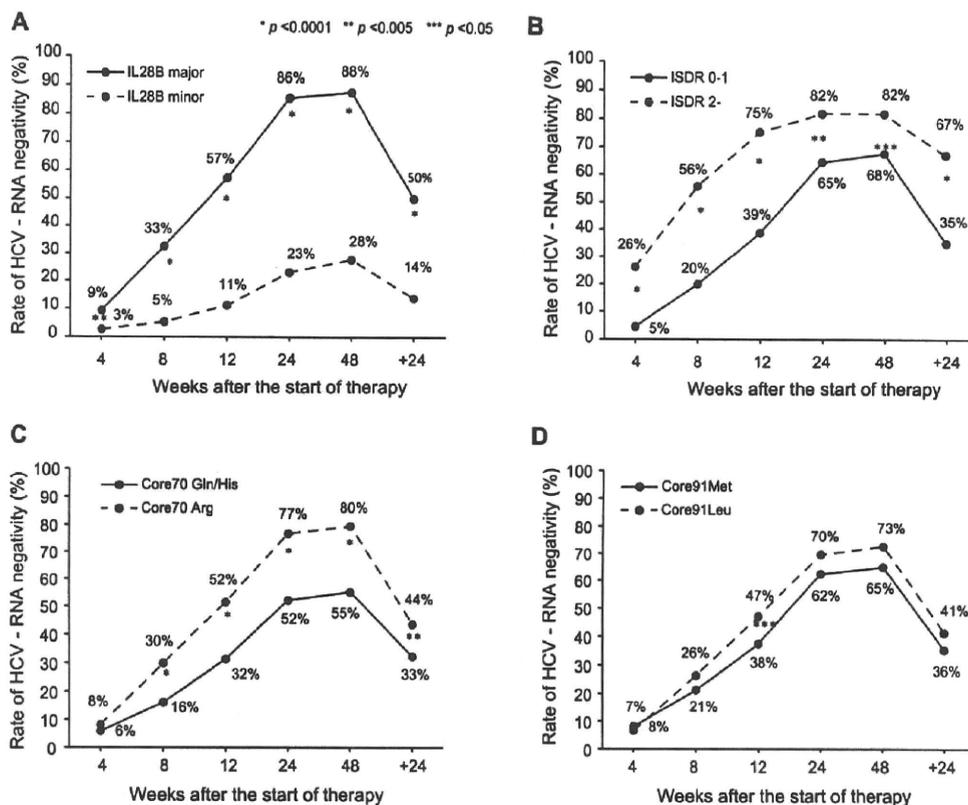


Fig. 2. Effect of *IL28B* mutations in the ISDR, Core70 and Core91 of HCV on time-dependent clearance of HCV. The rate of undetectable HCV-RNA was plotted for serial time points after the start of therapy (4, 8, 12, 24, and 48 weeks) and for 24 weeks after the completion of therapy. Patients were stratified according to (A) the *IL28B* allele (minor allele vs. major allele), (B) the number of mutations in the ISDR (0-1 mutation vs. 2 or more mutations), amino acid substitutions of (C) Core70 (Gln/His vs. Arg), and (D) Core91 (Met vs. Leu). The *p* values are from Fisher's exact test.

HCV-RNA ($p = 0.035$), Gln or His at Core70 ($p < 0.0001$), low platelet counts ($p = 0.009$), and advanced fibrosis ($p = 0.0002$) were associated with NVR. By multivariate analysis, the minor allele of *IL28B* (OR = 20.83, 95%CI = 11.63–37.04, $p < 0.0001$) was associated with NVR independent of other covariates (Table 2). Notably, mutations in the ISDR ($p = 0.707$) and at amino acid Core70 ($p = 0.207$) were not significant in multivariate analysis due to the positive correlation with the *IL28B* polymorphism ($p = 0.004$ for ISDR and $p < 0.0001$ for Core70, Fig. 4).

Genetic polymorphism of *IL28B* also was associated with SVR (OR = 7.41, 95% CI = 4.05–13.57, $p < 0.0001$) independent of other covariates, such as platelet counts, fibrosis, and serum levels of HCV-RNA. Mutation in the ISDR was an independent predictor of SVR (OR = 2.11, 95% CI = 1.06–4.18, $p = 0.033$) but the amino acid at Core70 was not (Table 3).

Factors associated with the *IL28B* polymorphism

Patients with the *IL28B* minor allele had significantly higher serum level of gamma-glutamyltransferase (GGT) and a higher

frequency of hepatic steatosis (Table 4). When the association between the *IL28B* polymorphism and HCV sequences was analyzed, Gln or His at Core70, that is linked to resistance to PEG-IFN and RBV therapy [4,14,15], was significantly more frequent in patients with the minor *IL28B* allele than in those with the major allele (67% vs. 30%, $p < 0.0001$) (Fig. 4). Other HCV sequences with an IFN resistant phenotype also were more prevalent in patients with the minor *IL28B* allele than those with the major allele: Met at Core91 (46% vs. 37%, $p = 0.047$) and one or no mutations in the ISDR (94% vs. 85%, $p = 0.004$) (Fig. 4).

Data mining analysis

Data mining analysis was performed to build a model for the prediction of SVR and the result is shown in Fig. 5. The analysis selected four predictive variables, resulting in six subgroups of patients. Genetic polymorphism of *IL28B* was selected as the best predictor of SVR. Patients with the minor *IL28B* allele had a lower probability of SVR and a higher probability of NVR than those with the major *IL28B* allele (SVR: 14% vs. 50%, NVR: 72% vs.

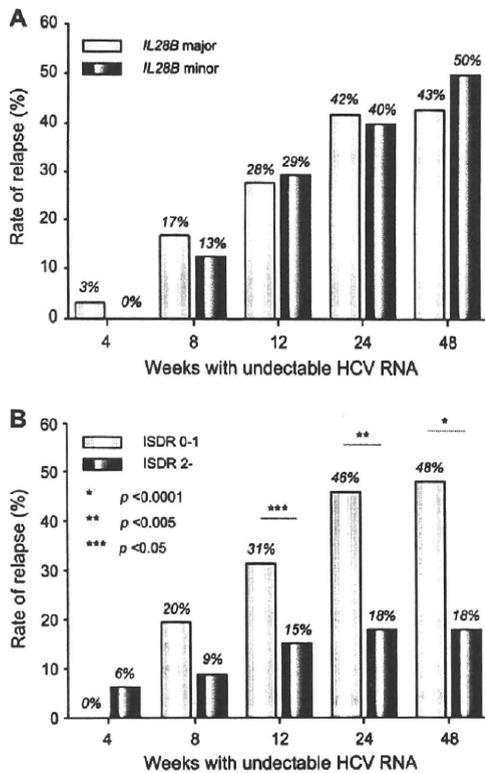


Fig. 3. Association between relapse and the *IL28B* allele or mutations in the ISDR. The rate of relapse was calculated for patients who had undetectable HCV-RNA at serial time points after the start of therapy (4, 8, 12, 24, and 48 weeks). Patients were stratified according to (A) the *IL28B* allele (minor allele vs. major allele) and (B) the number of mutations in the ISDR (0-1 mutation vs. 2 or more mutations). The *p* values are from Fisher's exact test. [This figure appears in colour on the web.]

12%). After stratification by the *IL28B* allele, patients with low platelet counts ($<140 \times 10^9/L$) had a lower probability of SVR and higher probability of NVR than those with high platelet counts ($\geq 140 \times 10^9/L$): for the minor *IL28B* allele, SVR was 7% vs. 19%, and NVR was 84% vs. 62%, and for the major *IL28B* allele, SVR was 32% vs. 66% and NVR was 16% vs. 8%. Among patients with the major *IL28B* allele and low platelet counts, those with two or more mutations in the ISDR had a higher probability of SVR and lower probability of relapse than those with one or no mutations in the ISDR (SVR: 75% vs. 27%, and relapse: 8% vs. 57%). Among patients with the major *IL28B* allele and high platelet counts, those with a low HCV-RNA titer ($<600,000$ IU/ml) had a higher probability of SVR and lower probability of NVR and relapse than those with a high HCV-RNA titer (SVR: 90% vs. 61%, NVR: 0% vs. 10%, and relapse: 10% vs. 29%). The sensitivity and specificity of the decision tree were 78% and 70%, respectively. The area under the receiver operating characteristic (ROC) curve of the model was 0.782 (data not shown). The pro-

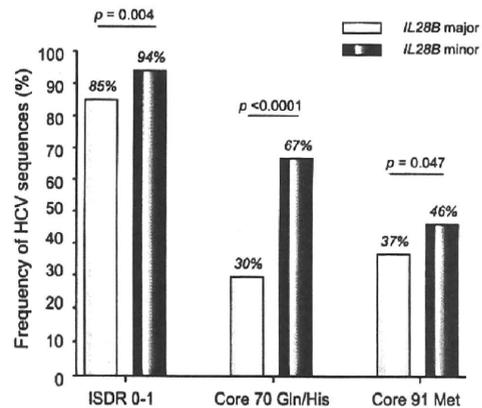


Fig. 4. Associations between the *IL28B* allele and HCV sequences. The prevalence of HCV sequences predicting a resistant phenotype to IFN was higher in patients with the minor *IL28B* allele than those with major allele. (A) 0 or 1 mutation in the ISDR of NS5A, (B) Gln or His at Core70, and (C) Met at Core91. *p* values are from Fisher's exact test. [This figure appears in colour on the web.]

portion of patients with advanced fibrosis (F3-4) was 39% (84/217) in patients with low platelet counts ($<140 \times 10^9/L$) compared to 13% (37/279) in those with high platelet counts ($\geq 140 \times 10^9/L$).

Validation of the data mining analysis

The results of the data mining analysis were validated with 165 patients who differed from those used for model building. Each patient was allocated to one of the six subgroups for the validation using the flow-chart form of the decision tree. The rate of SVR and NVR in each subgroup was calculated. The rates of SVR and NVR for each subgroup of patients were closely correlated between the model building and the validation patients ($r^2 = 0.99$ and 0.98) (Fig. 6).

Discussion

The rate of NVR after 48 weeks of PEG-IFN/RBV therapy among patients infected with HCV of genotype 1 is around 20-30%. Previously, there have been no reliable baseline predictors of NVR or SVR. Because more potent therapies, such as protease and polymerase inhibitor of HCV [28,29] and nitazoxanide [30], are in clinical trials and may become available in the near future, a pre-treatment prediction of the likelihood of response may be helpful for patients and physicians, to support clinical decisions about whether to begin the current standard of care or whether to wait for emerging therapies. This study revealed that the *IL28B* polymorphism was the overwhelming predictor of NVR and is independent of host factors and viral sequences reported previously. The *IL28B* encodes a protein also known as IFN-lambda 3, which is thought to suppress the replication of various viruses including HCV [31,32]. The results of the current study and the findings of the GWAS studies [6-9] may provide the rationale for developing diagnostic testing or an IFN-lambda based therapy for chronic hepatitis C in the future.