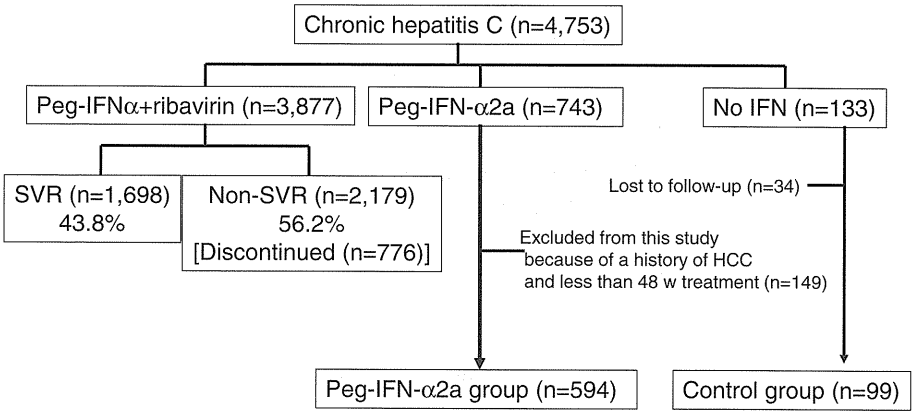

Fig. 1 Flow diagram of the patients' enrollment in the study. *Peg-IFNα* pegylated interferon α, *SVR* sustained viral response, *HCC* hepatocellular carcinoma, *w* week

study, 4,753 patients with chronic hepatitis C had been treated; Peg-IFN and ribavirin combination treatment had been administered to 3,877 patients, 743 patients had received Peg-IFN alone, and 133 patients had not agreed to receive IFN (a flow diagram of the enrollment of patients in this study is shown in Fig. 1). In the patients with Peg-IFN and ribavirin combination treatment, the SVR rate was 43.8 %; SVR was not achieved in 2,179 patients, and in 776 of these patients, the combination therapy was discontinued owing to adverse events or the patient's choice. Patients who failed to achieve an SVR were not included in this study, because the incidence of HCC is known to be reduced even in non-responders to IFN [17].

The backgrounds of the 594 patients studied are shown in Table 1. Findings from the liver biopsies of the patients were classified according to international standards [18]. Long-term PegIFNα-2a treatment is approved by the Japanese Medical Insurance system. Written informed consent was obtained from all patients prior to participation in this study. The study design was approved by the regional ethics committees of the 21 centers involved in this study, including the Musashino Red Cross Hospital, in accordance with the Helsinki Declaration. The 743 patients treated with PegIFNα-2a alone were not indicated for Peg-IFNα and ribavirin combination therapy because of anemia or heart disease. The 133 patients who did not agree to receive IFN served as the control group (see Fig. 1). A large proportion of the 594 study patients had advanced fibrosis of the liver and active inflammation. A dose of 90 μg PegIFNα-2a was administered to 512 and 82 patients weekly and biweekly, respectively, according to the patients' wishes. There were no significant differences between the weekly and biweekly groups in the patients' background data (data not shown).

The median duration of follow up in the PegIFNα-2a group was 1,273 days (range 228–2,768 days) and HCC was observed in 49 of the 594 patients (Table 1). Pre-treatment and on-treatment factors associated with the development of HCC were analyzed by Student's *t*-test, the Mann–Whitney *U*-test, and the χ^2 test (Table 2). Independent factors for the development of HCC were assessed by multivariate analysis using logistic regression. The

Table 1 Background data of patients treated with PegIFNα-2a ($n = 594$)

	$n = 594$
Age (years)	61.7 ± 11.7
Sex (male/female)	258/336
BMI	23.2 ± 3.3
Genotype (1/2)	443/151
Diagnosis (ASC/CH/LC)	4/460/130
History of excess alcohol consumption (≥60 g/day; yes/no)	118/376
Fibrosis (F0, 1, 2/F3, 4)	443/151
Inflammatory activity (A0, 1/A2, 3)	469/125
Diabetes mellitus (no/yes)	499/95
LDL cholesterol (mg/dL)	94.2 ± 31.1
Fasting blood sugar (mg/dL)	106.3 ± 28.5
White blood cell count (/mm^3)	4,360 ± 1,470
Red blood cell count (×10^6/μL)	423.8 ± 56.4
Hemoglobin (g/dL)	13.3 ± 1.8
Platelet count (×10^3/μL)	137 ± 56
Albumin (g/dL)	4.0 ± 0.5
Total bilirubin (mg/dL)	0.8 ± 0.6
AST (IU/L)	65.8 ± 47.8
ALT (IU/L)	72.1 ± 68.0
Gamma-GTP (IU/L)	55.2 ± 51.3
Esophageal varices (no/yes)	344/31
Alpha fetoprotein (ng/L)	6.9 (4.2–13.8)
Once weekly or biweekly PegIFNα-2a	512:82
Baseline HCV RNA (KIU/mL)	1,024 (73–2,130)
Development of HCC (no/yes)	545/49

PegIFN pegylated interferon, *BMI* body mass index, *ASC* asymtomatic carrier, *CH* chronic hepatitis, *LC* liver cirrhosis, *LDL* low-density lipoprotein, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GTP* guanosine triphosphate, *HCV* hepatitis C virus, *HCC* hepatocellular carcinoma

Values are means ± SD, with ranges in parentheses

incidence of HCC was analyzed according to the ALT, AFP, and hepatitis C virus (HCV) RNA levels 24 weeks after the start of PegIFNα-2a administration by using the Kaplan–Meier method. The risk of HCC was analyzed, using the Kaplan–Meier method, only in the non-responders with detectable HCV RNA during PegIFNα-2a administration by dividing them according to the ALT and AFP levels 24 weeks after the start of therapy. The incidence of HCC was compared between the patients with ALT levels of <41 IU/L and those with levels of ≥41 IU/L, and between patients with serum AFP levels of <10 ng/L and those with levels of ≥10 ng/mL at 24 weeks after starting treatment, because at most of the centers participating in the this study, the upper normal range of serum ALT is set at 40 IU/L, and the most significant difference in the incidence of HCC was observed between the PegIFNα-2a and control group with the cut-off serum ALT set at 41 IU/L and cutoff serum AFP set at 10 ng/mL, 24 weeks after starting treatment. The HCV RNA level was measured using the Amplicor Monitor method with a lower detection limit of 50 IU/L (Roche Diagnostics, Tokyo, Japan). A history of excess alcohol consumption was determined as >60 g alcohol per day in order to exclude alcoholic liver disease.

An asymptomatic carrier was defined as a patient with a serum ALT level within the normal range and minimal inflammation or fibrosis in the biopsied tissues of the liver. Chronic hepatitis was defined as mild-to-severe fibrosis of the liver according to liver biopsy [18]. The diagnosis of liver cirrhosis was based on the results of histological examination of the biopsied liver tissues.

Study 2: incidence of HCC in the PegIFNα-2a therapy and non-administration (control) groups in comparison with propensity-matched controls

Ninety-nine of the 133 chronic hepatitis C patients who had not received IFN were examined as controls; patients in this group received liver-protective agents such as glycyrrhizin or were untreated, and the group was observed for more than 1 year. None of the individuals in the control groups had received IFN alone or PegIFNα and ribavirin combination treatment. They were treated for a median of 1,395 days (range 75–6,556 days). Fifty-nine of these patients underwent liver biopsy before the treatment and were considered the control group for the propensity-matched study. For the propensity-matched study, 59 patients were selected from the PegIFNα-2a group according to their age, sex, platelet count, and total bilirubin levels, which had been identified as independent pretreatment risk factors for the development of HCC in Study 1. The rates of HCC were analyzed using the Kaplan–Meier method, and the risk of HCC was analyzed particularly in patients with advanced fibrosis of the liver (F3 and F4).

Table 2 Comparison of HCC and non-HCC patients with long-term PegIFNα-2a administration ($n = 594$)

	Patients with or without development of HCC		p value
	With HCC ($n = 49$)	Without HCC ($n = 545$)	
Pretreatment parameters			
Age (years)	63.8 ± 1.7	61.3 ± 0.5	<0.05
Sex (male/female)	32/17	226/319	<0.01
BMI	24.0 ± 0.5	23.1 ± 0.2	n.s.
Genotype (1/2)	47/6	397/148	n.s.
History of excess alcohol consumption (≥60 g/day; yes/no)	11/38	107/338	n.s.
Fibrosis (F0, 1, 2/F3, 4)	25/24	418/127	<0.001
Inflammatory activity (A0, 1/A2, 3)	7/42	462/83	<0.001
Diabetes mellitus (no/yes)	38/11	461/84	n.s.
LDL cholesterol (mg/dL)	88.2 ± 9.0	94.7 ± 2.6	n.s.
White blood cell count (/mm^3)	4,355 ± 210	4,360 ± 64	n.s.
Red blood cell count (×10^6/μL)	420.8 ± 8.1	424.1 ± 2.6	n.s.
Hemoglobin (g/dL)	13.6 ± 0.3	13.3 ± 0.1	n.s.
Platelet count (×10^3/μL)	106 ± 8	140 ± 2	<0.001
Albumin (g/dL)	3.8 ± 0.1	4.0 ± 0.1	<0.001
Total bilirubin (mg/dL)	1.2 ± 0.1	0.8 ± 0.1	<0.001
AST (IU/L)	78.1 ± 6.8	64.6 ± 2.1	n.s.
ALT (IU/L)	72.8 ± 9.7	72.0 ± 2.9	n.s.
Gamma-GTP (IU/L)	68.7 ± 7.5	53.9 ± 2.3	n.s.
Alpha fetoprotein (ng/L)	17.1 (4.4–36.8)	16.7 (4.1–23.1)	n.s.
Esophageal varices	29.0 % (9/31)	6.4 % (22/344)	<0.01
On-treatment parameters			
ALT (IU/L)	59.4 ± 5.7	44.6 ± 1.8	<0.05
Alpha fetoprotein (ng/L)	9.8 (4.6–17.4)	5.5 (3.7–11.1)	<0.01
HCV RNA level (KIU/mL)	236 (<0.5–2,210)	21 (<0.5–1,780)	<0.05

n.s. not significant

Statistical analysis

Categorical data were compared using the χ^2 test or Fisher's exact test. The distributions of continuous variables were analyzed using Student's t-test and the Mann–Whitney U-test for two groups. Multivariate analysis was

conducted using logistic regression. The cumulative incidence curve was determined using the Kaplan–Meier method and differences between groups were assessed by the log-rank test. For all methods, the level of significance was set at $p < 0.05$. Multivariate analysis of the risk of HCC was carried out using the Cox proportional hazard model. Statistical analyses were performed using the Statistical Package for the Social Sciences software version 11.0 (SPSS, Chicago, IL, USA). In Study 1, age, sex, platelet count, and total bilirubin levels were identified as independent factors for the development of HCC; therefore, these factors were selected for the propensity-matched control study (Study 2) in which 59 patients from the PegIFNα-2a group were included.

Results

Study 1

We analyzed the factors involved in the development of HCC in patients who received 90 μg PegIFNα-2a weekly or biweekly for more than a year. The incidence of HCC did not differ significantly between the groups treated with PegIFNα-2a weekly and biweekly (34 of 512 vs. 15 of 82, respectively). As shown in Table 2, univariate analysis revealed statistically significant differences in the pretreatment parameters including age, sex, fibrosis of the liver, platelet count, albumin level, and total bilirubin, between patients who developed HCC and those who did not. Endoscopy was carried out in 375 patients, and esophageal varices were noted in 31 of them. The incidence of HCC was higher in patients with esophageal varices than in those without varices [29.0 % (9 of 31) vs. 6.4 % (22 of 344)]. Assessment of on-treatment factors by univariate analysis revealed statistically significant differences in serum ALT, AFP, and HCV RNA levels 24 weeks after the start of PegIFNα-2a maintenance treatment (Table 2).

Multivariate analysis including pretreatment parameters revealed that age, sex, fibrosis of the liver, platelet count, and total bilirubin were independent risk factors for HCC development (Table 3). Multivariate analysis including on-treatment parameters identified ALT levels of ≥41 IU/L and AFP levels of ≥10 ng/L 24 weeks after the start of the PegIFNα-2a therapy as independent risk factors for HCC development (Table 3).

The incidence of HCC was significantly lower in patients with ALT levels of ≤40 IU/L than in those with ALT levels of ≥41 IU/L 24 weeks after the start of observation (Fig. 2). The incidence of HCC was also significantly lower in patients with AFP concentrations of <10 ng/mL at 24 weeks after the start of observation than in those with AFP concentrations of ≥10 ng/mL (Fig. 3). The dose of PegIFNα-2a was reduced to 45 μg in 16 patients because of neutropenia and thrombocytopenia. In addition, PegIFNα-2a was discontinued in 18 patients because of adverse events, including depression (7 patients), interstitial pneumonitis (3 patients), thrombocytopenia (3 patients), neutropenia (1 patient), itching (1 patient), and ascites (3 patients). No statistically significant differences were found between the patients with reduced dosage or treatment interruption and those without treatment modifications with respect to overall survival, HCC incidence, ascites formation, variceal bleeding, hepatic encephalopathy, and 2-point increases in the Child-Pugh score. No patients underwent liver transplantation.

Table 3 Independent risk factors for HCC development in patients treated with 90 μg PegIFNα-2a weekly or bi-weekly, evaluated by multivariate analysis (logistic regression analysis)

	Multivariate analysis		
	Odds ratio	95 % Confidence interval (CI)	*p*
Age (years) (every 5 years)	2.24	1.76–9.33	<0.005
Sex (male/female)	3.16	1.56–10.7	<0.005
Fibrosis (F3, 4/F0, 1, 2)	1.69	1.18–5.2	<0.01
Platelet count (<120 × 10^3/μL vs. ≥120 × 10^3/μL)	3.24	1.44–27.6	<0.01
Total bilirubin (mg/dL)	1.59	1.09–2.58	<0.05
ALT (at 24 weeks) (≥41 vs. <40 IU/L)	2.49	1.51–8.28	<0.05
AFP (at 24 weeks) (≥10 vs. <10 ng/L)	3.78	1.92–11.8	<0.01

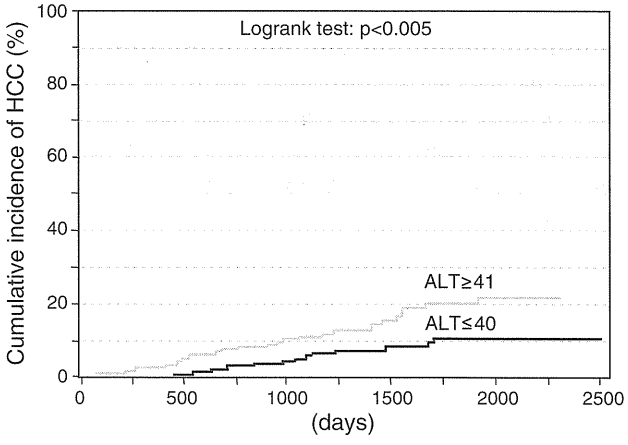

Fig. 2 Comparison of HCC rates in patients administered with PegIFNα-2a ($n = 594$) with respect to alanine aminotransferase (*ALT*) levels 24 weeks after the start of therapy. *Black line* patients with ALT ≥41 IU/L in the first 24 weeks, *gray line* patients with ALT ≤40 IU/L in the first 24 weeks

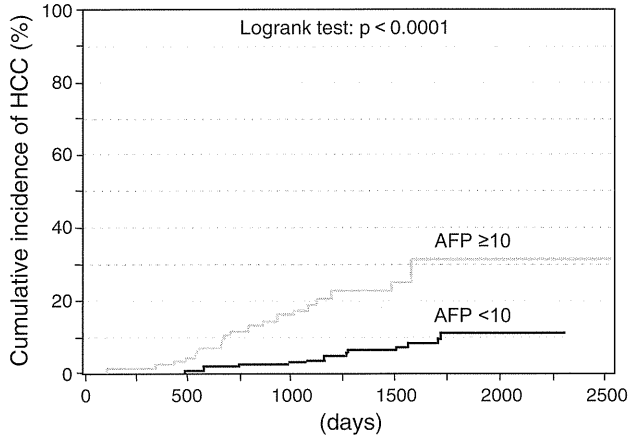

Fig. 3 Comparison of HCC rates in patients administered PegIFNα-2a ($n = 594$) with respect to alpha-fetoprotein (*AFP*) levels in the first 24 weeks after the start of therapy. *Black line* patients with AFP ≥10 ng/mL at 24 weeks, *gray line* patients with AFP <10 ng/mL at 24 weeks

Study 2

We compared the incidence of HCC between 59 patients in the control group and the same number of patients in the PegIFNα-2a group using the matched-pair test. The backgrounds of the patients are shown in Table 4. The PegIFNα-2a group had higher rates of advanced fibrosis (F3 and F4) and active inflammation (A2 and A3). No other differences were found between the two groups, except for the white blood cell count (Table 4).

Development of HCC was observed in 2 patients in the PegIFNα-2a group and 8 in the control group. The incidence of HCC was compared between the two groups, using the Kaplan–Meier method. The incidence of HCC in the PegIFNα-2a group was significantly lower than that in the control group (log-rank test, $p = 0.0187$; Fig. 4). Among the patients with advanced fibrosis of the liver (F3 and F4), those in the PegIFNα-2a group had a lower incidence of HCC than those in the control group. The independent risk factors for the development of HCC were analyzed using the stepwise Cox proportional hazard model. Only PegIFNα-2a administration and age were identified as independent risk factors for the development of HCC (Table 5).

Discussion

The number of HCC cases resulting from HCV infection continues to increase worldwide [19]. To date, IFN therapy is the most effective preventive measure against HCC in patients with chronic hepatitis C; furthermore, the incidence of HCC is reduced in patients who achieve an SVR to IFN [6–9] Therefore, achieving an SVR is the most effective approach for reducing the risk of developing HCC. In Japan, the incidence of HCC is elevated in older patients with hepatitis C. Corroborating this finding, the results of a Japanese study show a higher risk of HCC in patients aged 65 years and more [10]. Therefore, prevention of HCC in aged patients is an important challenge.

In the present multicenter, cooperative, retrospective study conducted in Japan, the incidence of HCC was reduced in patients who received 90 μg PegIFNα-2a weekly or biweekly and had AFP values of <10 ng/mL and ALT values of ≤40 IU/L 24 weeks after the start of the treatment. The results of the matched case–control study of the PegIFNα-2a group and the non-IFN control group show that the incidence of HCC was significantly lower in the PegIFNα-2a group than in the control group, especially in patients with advanced fibrosis of the liver (F3 and F4). However, there could have been a selection bias between

Table 4 Backgrounds of the patients in the propensity-matched control study (PegIFNα-2a group, $n = 59$; control group, $n = 59$)

	PegIFNα-2a group ($n = 59$)	Control group ($n = 59$)	p value
Age (years)	60.5 ± 13.0	63.3 ± 10.5	n.s.
Gender (male/female)	24/35	25/34	n.s.
BMI	22.9 ± 3.6	22.9 ± 3.4	n.s.
Genotype (1/2)	49/10	46/13	n.s.
History of excess alcohol consumption (60 g/day; yes/no)	10/49	4/55	n.s.
Fibrosis (F0, 1, 2/F3, 4)	37/22	43/16	<0.05
Development of HCC (F0–2/F3, 4)	1/1	1/7	n.s.
Inflammatory activity (A0,1/A2, 3)	19/40	30/29	<0.05
Diabetes mellitus (no/yes)	57/2	56/3	n.s.
LDL cholesterol (mg/dL)	95.3 ± 23.8	117.0 ± 4.2	n.s.
White blood cell count (/mm^3)	4,260 ± 1,239	5,193 ± 2,078	<0.05
Red blood cell count ($\times 10^{-4}$/μL)	430 ± 57.8	441 ± 44.9	n.s.
Hemoglobin (g/dL)	13.6 ± 1.5	13.6 ± 1.9	n.s.
Platelet count ($\times 10^{-3}$/μL)	14.5 ± 5.7	15.8 ± 5.7	n.s.
Albumin (g/dL)	4.1 ± 0.5	4.1 ± 0.4	n.s.
Total bilirubin (mg/dL)	0.7 ± 0.5	0.9 ± 0.7	n.s.
AST (IU/L)	58.3 ± 47.7	49.7 ± 26.6	n.s.
ALT (IU/L)	63.6 ± 68.7	58.0 ± 39.2	n.s.
Gamma-GTP (IU/L)	78.3 ± 81.3	55.3 ± 75.1	n.s.
Baseline alpha-fetoprotein (AFP) (ng/L)	7.2 (4.3–14.2)	7.7 (3.9–13.8)	n.s.
Baseline HCV RNA level (KIU/mL)	1,230 (24–3,870)	1,024 (38–3,110)	n.s.

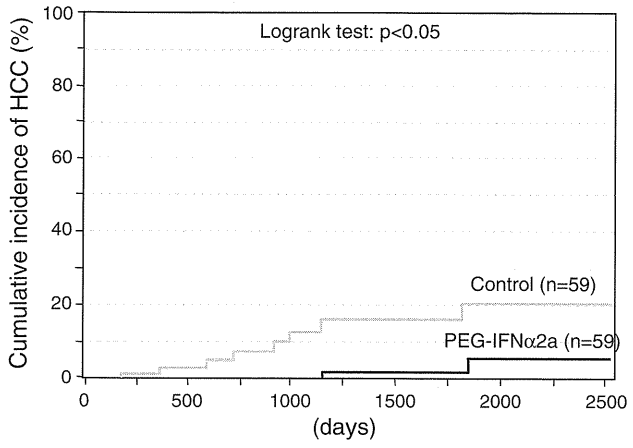

Fig. 4 Comparison of HCC rates between the long-term PegIFNα-2a administration group ($n = 59$) and non-administration group ($n = 59$) in the propensity-matched control study (Kaplan–Meier log-rank test, $p = 0.019$)

Table 5 Risk factors for HCC in the propensity-matched control study (Cox proportional hazard model)

Variables	Risk ratio	95 % CI	p value
PegIFN versus control	0.17	0.03–0.75	<0.05
Age (every 1 year)	1.12	1.02–1.25	<0.05
Fibrosis (F3, 4 vs. F0, 1, 2)	1.70	0.75–4.16	n.s.
Platelet count (every 10 × 10^3/μL)	0.89	0.73–1.09	n.s.
Albumin (every 1.0 g/dL)	0.80	0.10–6.68	n.s.
On-treatment AFP (<10 vs. ≥10 ng/L)	4.07	0.59–40.12	n.s.

the PegIFNα-2a group and the control group (patients who did not agree to receive IFN treatment), because this was a retrospective and non-randomized study. However, concordant with the findings of the HALT-C study [14], the present results show that PegIFNα-2a inhibits the development of HCC in patients with advanced fibrosis of the liver.

Recent studies show that polymorphisms in the host *IL28B* gene are important factors in the response to PegIFNα and ribavirin combination therapy [20, 21]. However, the mechanism of IL28B involvement in the response to PegIFNα and ribavirin has not been elucidated completely. A recent report has shown that IL28B is a significant factor in the development of HCC as well as in the response to IFN therapy [22]. Further studies are warranted to analyze the relationship between IL28B and inhibition of the development of HCC by PegIFNα in chronic hepatitis C.

Risk factors for the development of HCC have been discussed previously. Increased intrahepatic fat is involved in the development of HCC in chronic hepatitis C patients [23, 24]. In addition, diabetes-associated fat disorder [25, 26], hepatic iron overload [27], advanced fibrosis, older age, and fatty deposits in the liver are risk factors for HCC development [4]. Therefore, it is important to establish strategies to mitigate these risk factors to prevent the development of HCC and thus improve the outcomes of hepatitis C patients.

IFN therapy after HCC treatment is reported to inhibit the recurrence of tumors [28, 29], and a meta-analysis has revealed a trend toward inhibition of the recurrence of HCC [30, 31]. The prevention of HCC is an important issue that needs to be addressed to improve the survival of chronic hepatitis C patients. The findings of the present study and the HALT-C trial [14] indicate the effectiveness of long-term administration of maintenance IFN for preventing the development of HCC in chronic hepatitis C patients without an SVR. Improvement in ALT levels is also known to be an important predictor for the prevention of HCC [32]. A low AFP value during IFN administration is also recognized as a significant indicator of a lower risk of HCC [33, 34]. Recently, Osaki et al. [35] reported that a decrease of serum AFP during treatment with IFN was associated with a reduced incidence of HCC. Taking these findings and our own together, we conclude that maintenance administration of low-dose PegIFNα-2a weekly or biweekly to non-SVR patients with chronic hepatitis C decreases the incidence of HCC, especially in patients whose serum ALT and AFP levels are within the normal range 24 weeks after the start of treatment. The preventive effects of IFN against the development of HCC without elimination of the virus may be associated with its anticarcinogenic effects [16, 35]; however, the precise mechanism should be investigated.

The limitations of the present study are that it is retrospective and multicentric; therefore, potentially there may have been a selection bias. However, the reduction of the rate of development of HCC by maintenance administration of PegIFNα-2a in the patients in whom serum ALT and AFP levels were within the normal ranges 24 weeks after the start of treatment may be attributable to the anticarcinogenic effects of IFN without elimination of the virus.

Conclusion

The incidence of HCC was lower in non-SVR patients with chronic hepatitis C who were administered with maintenance low-dose PegIFNα-2a; especially in those whose serum ALT and AFP levels were within the normal ranges 24 weeks after the start of treatment.

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Conflict of interest Namiki Izumi received lecture fees from Chugai Co. and MSD Co. in 2011.

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Genetics of *IL28B* and HCV—response to infection and treatment

C. Nelson Hayes, Michio Imamura, Hiroshi Aikata and Kazuaki Chayama

Abstract | The *IL28B* locus attracted the attention of HCV researchers after a series of genome-wide association studies independently identified a strong association between common *IL28B* polymorphisms and the outcome of PEG-IFN-α plus ribavirin combination therapy in patients chronically infected with HCV genotype 1. This association was subsequently replicated for other HCV genotypes and has been linked to spontaneous eradication of HCV, development of steatosis and biochemical changes (such as altered levels of γ-glutamyl transpeptidase and LDL). Despite the introduction of direct-acting antiviral drugs, *IL28B* genetics are likely to play a part in patient selection and treatment decisions—moving towards a personalized approach to therapy. In HCV-infected patients with the so-called favourable *IL28B* genotype (rs12979860 CC; associated with better treatment response), hepatic expression levels of *IL28B* and interferon-stimulated genes seem to be reduced at baseline, but are induced more strongly after IFN-α administration, perhaps resulting in more effective elimination of the virus. Clarification of the mechanisms underlying these biological phenomena will lead to improved understanding of the antiviral effects of IFN-λ and, ideally, to the development of better therapies against HCV infection. This Review summarizes current understanding of the role of *IL28B* in HCV infection and response to therapy.

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Introduction

Shortly after the identification of HCV in 1989,[1] the development of HCV antibody tests—and thus improved screening and diagnosis—began to dramatically reduce the number of new cases of HCV infection by increasing awareness of this condition and by preventing transmission of HCV by blood transfusion or transplantation.[2] However, in 2011, an estimated 130–170 million people were chronically infected with HCV, putting them at an increased risk of cirrhosis, hepatocellular carcinoma and liver failure.[3] Despite the high costs and substantial toxicity of PEG-IFN-α plus ribavirin combination therapy, fewer than half of patients infected with the most common HCV genotype are able to achieve a sustained virological response (SVR),[4] which is defined as undetectable HCV RNA 6 months after the end of treatment. Although telaprevir and boceprevir are expected to greatly improve the rate of SVR, these direct-acting antiviral agents must currently be administered in combination with interferon (IFN)-α and ribavirin to help suppress viral breakthrough.[5] Identification of factors affecting response to IFN-α therapy, therefore, remains an important goal.

In 2009, a series of independent studies reported that patients infected with HCV genotype 1b who had a common variant in the *IL28B* locus (rs12979860 CC or rs8099917 TT) were significantly ($P<7.1\times10^{-08}$) more likely to respond to PEG-IFN-α plus ribavirin combination therapy than patients with other *IL28B* variants.[6–8] Such patients were also more likely to spontaneously resolve acute HCV infection without treatment.[9] These results have added a new dimension to HCV research and offer the potential for more personalized and effective therapy. In the 2 years since the publication of these landmark papers, hundreds of studies have examined the role of *IL28B* polymorphisms in HCV infection and treatment. This Review summarizes some of the major findings of the role of the *IL28B* locus in HCV infection, describing background information on *IL28B* and the part IL-28B (also known as IFN-λ3) plays in the elimination of HCV and response to therapy.

IL28 gene family and innate immunity

The type III IFN-λ family consists of three members: the cytokines IL-29, IL-28A and IL-28B (also known as IFN-λ1, IFN-λ2 and IFN-λ3, respectively). Although functionally an IFN, these cytokines are structurally related to the IL-10 family of cytokines.[10] Discovered in 2003 by computational prediction, the *IL28A*, *IL28B* and *IL29* genes are located in a cluster on chromosome 19 (Figure 1).[11,12] Amino acid sequences of the two isoforms of IL-28 (IL-28A and IL-28B) have 96% homology, both being 81% identical to the amino acid sequence of IL29.[12] As with IFN-α, the three IFN-λs can be triggered by viral infection and induce antiviral and antitumour activity through both innate and adaptive immune system pathways.[13,14] As shown in Figure 2a, although both IFN-α and IFN-λ family cytokines signal through

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the Jak–STAT (Janus kinase–signal transducer and activator of transcription) pathway[11,15] and activate an overlapping set of IFN-stimulated genes (ISGs),[16] fundamental differences exist in gene expression between IFN-α and IFN-λ.[17] Whereas IFN-α binds to the constitutively expressed type I IFN receptor, IFN-λ cytokines bind to a heterodimer of the IL-10 and IL-28 receptors (IL10R and IL28R, respectively),[11] the latter of which is only expressed in restricted cell types, including hepatocytes, epithelial cells and plasmacytoid dendritic cells.[18] Although both types of IFN induce expression of many of the same genes, the change in ISG expression in response to IFN-λ tends to be weaker overall but increases steadily over time,[16] whereas IFN-α triggers an early peak in ISG expression followed by a rapid decline.[19] This phenomenon seems to result from the distinct kinetics of IFN-λ-mediated activation of STAT,[19] involving differences in transcription factor remodelling efficiency[16] and greater dependence on the NF-κB pathway.[20] Therefore, differences in ISG expression through type I and III IFNs are possible under specific conditions.[20]

Key points

- The 130–170 million people chronically infected with HCV have an increased risk of cirrhosis, hepatocellular carcinoma and liver failure
- Several single nucleotide polymorphisms upstream of the *IL28B* gene are associated with spontaneous clearance of HCV and improved response to PEG-IFN-α plus ribavirin combination therapy
- In patients with the so-called favourable *IL28B* allele (rs12979860 CC), associated with better response to therapy, HCV RNA levels decline rapidly with treatment and IFN-α therapy induces strong interferon-stimulated gene (ISG) expression
- In patients with unfavourable *IL28B* genotypes (rs12979860 CC/TT), ISG expression tends to be refractory to further IFN stimulation, resulting in poor response to IFN therapy
- *IL28B* genotype might also predict response to telaprevir triple therapy, although it might not be as effective at predicting the treatment response in this scenario as with PEG-IFN-α plus ribavirin combination therapy

Effects of *IL28B* polymorphisms

An inherent limitation of genome-wide association studies (GWAS; Box 1) is the difficulty in tracing the link between changes at a single base and differences in the resulting phenotype. Understanding the genetic basis of differences in resolution of HCV infection is yet more complex because it involves coordinated activity between innate and adaptive immune effectors and is affected by multiple host and viral factors. The fairly strong independent effect of *IL28B* polymorphisms therefore suggest a role in the high-level regulation of antiviral defence against HCV. The effects of *IL28B* polymorphisms have been investigated for a number of aspects of response to HCV infection and treatment, including response to therapy, natural elimination of the virus, and changes in gene expression and lipid metabolism.

PEG-IFN-α plus ribavirin combination therapy

Striking differences in HCV clearance and response to treatment among ethnic groups,[21] as well as among patients infected with the same HCV inoculum,[22] have long suggested a role for host genetic factors. Early candidate gene studies identified single nucleotide polymorphisms (SNPs) in genes encoding proteins involved in response to HCV infection—including osteopontin, MxA (also known as MX1), OAS1, EIF2AK2 (also known as PKR), IFN-α receptor 1, and MAPKAPK3[23–26]—but practical insights gained from these studies have been limited. SNPs are defined as base pair variants at a specific genomic position that have a frequency greater than 1%.[27] As the number of SNPs examined increases, the probability of detecting spurious associations also increases sharply and a drawback of candidate gene studies, in particular, is the need to select appropriate target genes prior to analysis, limiting the ability to detect novel associations. Advances in high-throughput screening, however, have made it possible to screen representative SNPs across the entire genome using GWAS. This 'hypothesis-free' approach can detect SNPs associated with disease phenotype or response to treatment without requiring *a priori* candidate gene selection. Nonetheless, most SNPs are thought to have small, cumulative effects that require large sample sizes to be detected, and results of GWAS often fail to be replicated in other populations. Although the importance of ethnicity in treatment outcome suggests a genetic basis, the simultaneous independent discovery of a common variant upstream of the *IL28B* gene and the magnitude of its effect on treatment outcome was unusual (Table 1).[6–8]

Ge *et al.*[6] published the first report of an association between a common polymorphism on chromosome

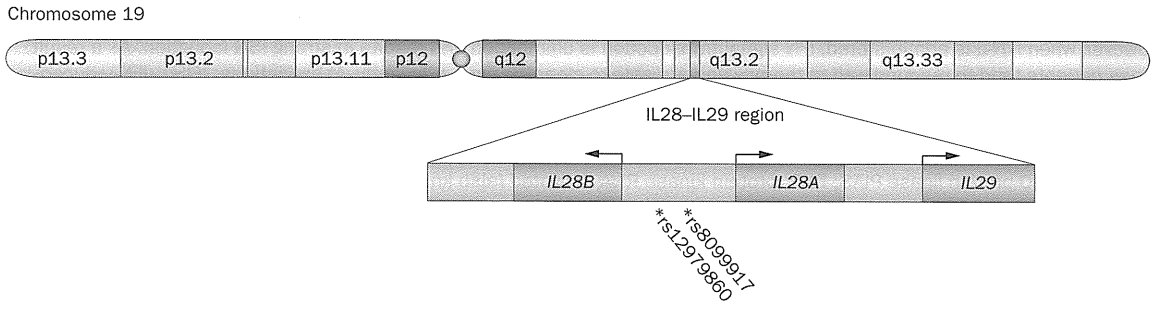

Figure 1 | The *IL28–IL29* locus on chromosome 19. The genes encoding the three members of the IFN-λ family, IL28A (IFN-λ2), IL28B (IFN-λ3) and IL29, (IFN-λ1) are clustered together on chromosome 19. The most important and validated SNPs with respect to response to IFN-α therapy, rs12979860 and rs8099917, are upstream of both *IL28B* and *IL28A* (owing to their antiparallel orientation), but are physically closer to *IL28B*.

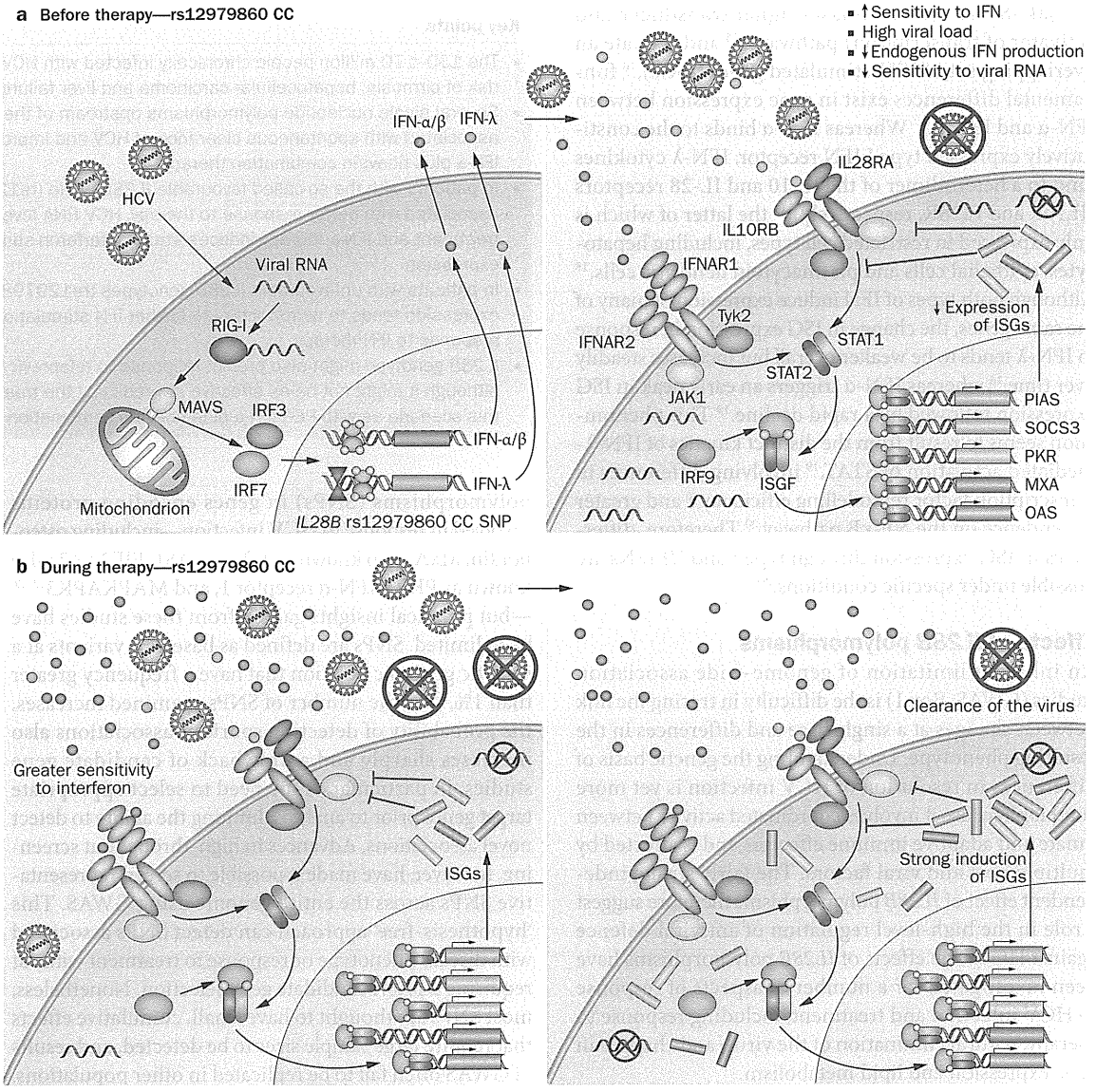

Figure 2 | Potential role of the favourable *IL28B* genotype in the response to interferon therapy. **a** | Intracellular HCV RNA is detected by surveillance molecules of the innate immune system, including the RIG-I–IFIH1 pathway, which causes the adaptor protein MAVS to induce expression and secretion of IFN-α, IFN-β and IFN-λ via IRFs. IFNs are recognized by receptors on the cell surface and initiate a signal cascade that results in induction of a large number of ISGs that collectively establish an antiviral state hostile to viral replication. IFN-α and IFN-β are recognized by IFNAR, whereas IFN-λ is recognized by the IL10R–IL28R receptor complex. Both receptors activate the Jak-STAT pathway, which induces the translocation of an ISGF complex to the nucleus, where it binds to the interferon-stimulated response elements of multiple ISGs. In individuals with the favourable allele (rs12979860 CC), the presence of viral RNA seems to induce only weak expression of IFN-λ, which in turn induces weak ISG expression. Although the response might be sufficient to clear the virus at low viral loads, the cell seems fairly tolerant of the virus and high viral loads can accumulate. **b** | During treatment, however, when IFN-α is administered, IFN signal transduction is unimpeded and results in strong ISG induction. As a result, these patients tend to respond well to therapy and are able to clear the virus efficiently. Abbreviations: IFIH1, IFN-induced helicase C domain-containing protein 1; IFNAR, IFN-α/β receptor 1; IL10R, IL-10 receptor; IL28R, IL-28 receptor; IRF, IFN regulatory factor; ISG, IFN-stimulated gene; ISGF, IFN-stimulated gene factor; Jak, Janus kinase; MAVS, mitochondrial antiviral-signalling protein; MXA, myxovirus resistance protein 1 (also known as MX1); OAS, 2'5'-oligoadenylate synthase; PIAS, E3 SUMO-protein ligase PIAS; PKR, protein kinase RNA-activated (also known as EIF2AK2); RIG-I, retinoic-acid inducible protein I; SOCS3, suppressor of cytokine signaling 3; STAT, signal transducer and activator of transcription.

19 and SVR on the basis of an analysis of a large cohort of white, African American and Hispanic patients with chronic HCV genotype 1 infection who were treated with 48 weeks of combination therapy with PEG-IFN-α plus ribavirin.[6] The rs12979860 SNP identified in this study is located in a noncoding region 3 kb upstream of *IL28B*. Patients homozygous for the major allele with the so-called favourable genotype (rs12979860 CC) were twice as likely to achieve an SVR as patients with the minor risk allele (rs12979860 TT or CT) following combination therapy.

The frequency of the major allele was proportional to the SVR rate across ethnic groups and explained half of the difference in SVR rates between African-American patients and American patients of European descent.

This report was followed by publication of independent studies by Tanaka *et al.*[7] and Suppiah *et al.*[8] on the role of rs8099917 (TT versus GT/GG genotypes), a neighbouring SNP in strong linkage disequilibrium with rs12979860, in 314 Japanese and 848 Australian patients, respectively. A fourth GWAS by Rauch *et al.*[28] confirmed the association between rs8099917 genotype and progression to chronic HCV infection and response to treatment in 465 white patients infected with HCV genotypes 1, 2, 3 or 4.

Using a candidate gene approach, McCarthy *et al.*[29] replicated the findings of Ge and colleagues[6] (that is, the association of rs12979860 with SVR) in a diverse cohort of 231 white and African-American patients infected with HCV genotypes 1, 2, or 3. Collectively, these studies suggest that either the rs12979860 CC or the rs8099917 TT genotype confers a 2–3-fold improved likelihood of response to dual combination therapy for chronic HCV and remains the strongest independent predictor of response to therapy even when other predictive factors are taken into account.[6,28]

Box 1 | Glossary terms for genome-wide studies

Candidate gene approach
Prior to the use of GWAS, genetic association studies were often performed by sequencing candidate genes known or suspected to be involved in with a disease or condition.

SNP
SNPs are base pair variants at a specific genomic location. Typically, only common SNPs with a population frequency greater than 1% or 5% are considered, due to the large number of samples required to detect associations involving rare SNPs.

GWAS
GWAS typically examine associations between common single nucleotide differences between patients in a disease group (cases) and a healthy group (controls) or other dichotomous classifications. Using high-throughput array-based methods, a large number of SNPs (for example, 500,000 or 1 million) can be assayed simultaneously for each patient.

Linkage disequilibrium
Linkage disequilibrium refers to combinations of alleles at different loci that occur together more often than expected by chance.

Causative SNP versus tagging SNPs
Due to linkage disequilibrium, GWAS can detect associations between a response variable and multiple SNPs in a region of DNA. In many cases, only one or a small number of SNPs directly affect the phenotype (for example, by changing an amino acid or altering transcription factor binding affinity). SNP arrays are based on a representative set of tagging SNPs that are expected to fall within linkage blocks that collectively cover most of the genome but may not include the causative SNPs.

Abbreviations: GWAS, genome-wide association studies; SNP, single nucleotide polymorphism.

Natural elimination of the virus

Only 20–30% of patients are able to resolve acute HCV infection without treatment, with the majority of those who remain infected progressing to chronic HCV infection.[9] However, individuals who have been infected with the same HCV inoculum (in this case, tainted product) have been noted to differ in their ability to spontaneously resolve the virus, suggesting that host genetics play a part in natural clearance of the virus (Table 2).[22]

In a GWAS of 1,362 patients infected with HCV, Rauch *et al.*[28] found that the rs8099917 TT genotype (patients homozygous for the major allele) strongly predicted spontaneous clearance of HCV infection. Thomas and colleagues[9] examined whether the SNP identified in the Ge *et al.*[6] study (rs12978860) was also associated with spontaneous clearance. They genotyped 1,008 patients with acute HCV infection and found that those with the rs12979860 CC genotype were more likely to spontaneously clear the virus than those with rs12979860 CT or TT genotype.[9] Tillmann *et al.*[30] also confirmed these results in a study of 136 German women, in whom spontaneous clearance occurred more often in patients with the CC genotype than either non-CC (CT or TT) genotype. Patients with a non-CC genotype were more likely to spontaneously clear the virus when they developed jaundice, but jaundice was not associated with viral clearance in patients with the CC genotype.[30]

Ruiz-Extremera and colleagues[31] examined the role of *IL28B* polymorphisms on vertical transmission of HCV between mother and child and found that whereas *IL28B* genotype in the mother and child are unrelated to vertical transmission, children with the rs12979860 CC genotype were more likely to spontaneously clear HCV genotype 1 infection. In a study of 138 Brazilian patients co-infected with HCV and HIV, patients with acute HCV who had the unfavourable rs12979860 CT/TT genotype were three times more likely to progress to chronic HCV infection than patients with the rs12979860 CC genotype.[32] Knapp *et al.*[33] noted that the frequency of the protective rs12979860 CC genotype was higher in patients who spontaneously resolved HCV infection than in individuals who were exposed to HCV but did not become infected, suggesting that the rs12979860 CC genotype is not associated with protection against acute infection. Interestingly, HCV-exposed uninfected individuals had a high frequency of killer cell immunoglobulin-like receptor 2DL3:group 1 HLA-C (KIR2DL3:HLA-C1),[33] which highlights the importance of the innate immune system in antiviral defence and suggests other heritable factors might be involved.

Even though high viral load is associated with poor response to treatment,[34] multiple studies have reported an association between high viral load and the favourable *IL28B* genotype (rs12979860 CC).[6,29,35–37] One potential explanation for this phenomenon is that patients with the favourable allele might be able to spontaneously clear the virus when the viral load is below a certain threshold. As a result, patients with the favourable *IL28B* genotype presenting with low viral loads might be relatively uncommon. Understanding how an individual SNP enables some patients to efficiently clear the virus in the absence of therapy should yield insight into the regulation of antiviral defences.

Change in viral load

Although the *IL28B* SNP remains the strongest pretreatment predictor of SVR,[38] on-treatment predictors such

Table 1 | Associations between *IL28B* homozygous major allele and response to HCV therapy

Study	SNP	Total number of patients	Odds ratio	*P* value	Population	HCV genotype
SVR with PEG-IFN-α plus ribavirin combination therapy						
Ge *et al.* (2009)[6]	rs12979860 CC	1,137	3.10	1.21×10^{-28}	White, African American, Hispanic	1
Suppiah *et al.* (2009)[8]	rs8099917	293	1.98	7.06×10^{-8}	White	1
Tanaka *et al.* (2009)[7]	rs8099917	142	12.10	3.11×10^{-15}	Japanese	1
Rauch *et al.* (2010)[28]	rs8099917	465	5.20	5.47×10^{-8}	Swiss	1–4
McCarthy *et al.* (2010)[29]	rs12979860 CC	231	5.80	9.00×10^{-6}	White, African American	1–3
Thompson *et al.* (2010)[38]	rs12979860 CC	1,671	5.20	$<1.00\times10^{-4}$	White, African American, Hispanic	1
Ochi *et al.* (2011)[37]	rs8099917	594	2.46	6.52×10^{-8}	Japanese, Taiwanese	1, 2
SVR with telaprevir triple therapy						
Akuta *et al.* (2010)[96]	rs8099917	66	10.60	$<1.00\times10^{-3}$	Japanese	1
Chayama *et al.* (2011)[70]	rs8099917	94	8.33	1.40×10^{-2}	Japanese	1
SVR with combination therapy for non-1b HCV genotypes*						
Mangia *et al.* (2010)[63]	rs12979860 CC	268	1.76	1.13×10^{-6}	White	2, 3
Asselah *et al.* (2011)[64]	rs12979860 CC	164	3.32	8.00×10^{-4}	Egyptian, European, sub-Saharan African	4
Kawaoka *et al.* (2011)[65]	rs8099917	83	4.35	2.00×10^{-2}	Japanese	2
Lindh *et al.* 2011[66]	rs12979860 CC	341	NA	2.00×10^{-2}	White	2, 3
Sakamoto *et al.* (2011)[67]	rs8099917	129	3.96	1.04×10^{-1}	Japanese	2
Sarrazin *et al.* (2011)[68]	rs12979860 CC	267	2.80	9.00×10^{-3}	German population	2, 3

Representative studies are shown, along with results based on the most significant SNP (rs12979860 or rs8099917), sample size, *P* value, odds ratio with respect to the favourable allele (if reported), study population, and viral genotype. *Initial *IL28B* studies focused mainly on the difficult-to-treat genotype 1 with 48 weeks of PEG-IFN-α plus ribavirin combination therapy. Other genotypes may respond better to therapy, and treatment guidelines may differ slightly with respect to stopping rules and overall duration of therapy.[97] Abbreviations: NA, not available; SVR, sustained virological response.

as rapid virological response (RVR; that is, undetectable HCV RNA by week 4 of therapy) are more directly linked to treatment outcome (Table 2).[39,40] When the effect of RVR is taken into account, *IL28B* genotype might no longer be an important predictor of treatment outcome,[41] especially during infection with HCV genotypes other than 1b.[42] However, *IL28B* genotype influences on-treatment predictors in several ways. On the one hand, initial baseline viral load tends to be higher in patients with the favourable *IL28B* genotype.[6,34–37] On the other hand, these patients seem to clear the virus more efficiently at each time point examined (for example, 48 h,[43] 4 weeks,[34] 12 weeks,[44] and so on). Consequently, patients with the favourable *IL28B* genotype are more likely to achieve RVR,[38,39,45] and even among patients who fail to achieve RVR, *IL28B* genotype remains the strongest predictor of SVR.[38,44] *IL28B* genotype might be linked to higher death rates of infected hepatocytes in patients with the favourable genotype[43] as well as lower daily viral production rates,[46] which could partially explain the improved efficiency of virological response in those with the *IL28B* CC genotype compared with non-CC *IL28B* genotypes.

ISG expression and viral replication

Both IFN-α and IFN-λ induce expression of hundreds of target ISGs and, subsequently, an antiviral state. Consequently, ISG expression might be the best predictor of treatment response regardless of *IL28B* genotype,[47] although genotyping one or a few *IL28B* SNPs is probably more practical than assaying ISG expression levels. Unexpectedly, however, hepatic ISG expression was found to be markedly higher in patients with the unfavourable *IL28B* allele,[48] and patients with high baseline ISG expression levels were found to respond poorly to IFN-α therapy.[49] Hepatic expression of several ISGs—including *MXA* (also known as *MX1*), *EIF2AK2*, *OAS1* and *ISG15*—was lower in patients with the favourable *IL28B* genotype than in those with the unfavourable *IL28B* genotype;[50] conversely, expression of genes that suppress the antiviral state were reduced.[51] However, Shebl *et al.*[52] found no evidence of an association between *IL28B* genotype and ISG expression in hepatocytes from uninfected individuals, implying that the association between *IL28B* genotype and ISG expression in the liver does not reflect normal expression levels in healthy individuals.[52] HCV infection in nonresponders might result in continual, but ineffectual, intrahepatic ISG expression, including expression of IFN-signalling inhibitors. Cells with such preactivated ISGs might not only fail to effectively clear the virus, but might have reduced sensitivity to therapeutic IFN-α (Figure 3).[49,53]

Biochemical changes and hepatic steatosis

During chronic HCV infection, differences in the cytokine profiles induced by the *IL28* polymorphisms

Table 2 | Associations between *IL28B* homozygous major allele and host response to HCV

Study	SNP	Total number of patients	Odds ratio	*P* value	Population	HCV genotype
Spontaneous clearance of HCV infection						
Thomas *et al.* (2009)[9]	rs12979860 CC	1,008	3.03	$<1.00\times10^{-12}$	European and African ancestry	1
Grebely *et al.* (2010)[71]	rs8099917 TT	163	3.78	4.40×10^{-2}	Australian	1–3
Montes-Cano *et al.* (2010)[72]	rs12979860 CC	731	3.13	6.20×10^{-5}	Spanish	NA
Tillmann *et al.* (2010)[30]	rs12979860 CC	136	NA	$<1.00\times10^{-3}$	German women	1b
Knapp *et al.* (2011)[33]	rs12979860 CC	397	2.97	1.00×10^{-4}	UK	NA
Rauch *et al.* (2010)[28]	rs8099917 TT	1,362	2.31	6.07×10^{-9}	Swiss	1–4
Improved rapid and early viral dynamics (RVR, EVR)						
Thompson *et al.* 2010[38]	rs12979860 CC	1,671	NA	$<1.00\times10^{-4}$	White, African American, Hispanic	1
Bochud *et al.* (2011)[41]	rs12979860 CC	242	NA	7.00×10^{-3}	White	1–3
Hayes *et al.* (2011)[35]	rs12979860 CC	817	1.37	1.40×10^{-8}	Japanese	1
Lin *et al.* (2011)[44]	rs12979860 CC	191	NA	$<1.00\times10^{-3}$	Taiwanese	1
Stattermayer *et al.* (2011)[45]	rs12979860 CC	682	NA	$<1.00\times10^{-3}$	Austrian	1–4
Ochi *et al.* (2011)[37]	rs8099917 TT	594	NA	6.70×10^{-5}	Japanese, Taiwanese	1, 2
Increased baseline viral load						
Ge *et al.* (2009)[6]	rs12979860 CC	1,475	NA	1.20×10^{-10}	White, African American, Hispanic	1
McCarthy *et al.* (2010)[29]	rs12979860 CC	231	2.13	6.10×10^{-3}	White, African American	1–3
Lindh *et al.* (2011)[66]	rs12979860 CC	341	NA	$<1.00\times10^{-3}$	White	2, 3
Ochi *et al.* (2011)[37]	rs8099917 TT	594	2.46	1.00×10^{-2}	Japanese, Taiwanese	1, 2
Increased viral clearance rate and death rates among infected hepatocytes						
Hsu *et al.* (2011)[46]	rs8099917 TT	145	NA	$<2.34\times10^{-2}$	Taiwanese	1, 2
Scott *et al.* (2011)[43]	rs12979860 CC	20	NA	4.00×10^{-2}	White, African American	1, 3

Representative studies are shown, along with results based on the most significant SNP (rs12979860 or rs8099917), sample size, *P* value, odds ratio with respect to the favorable allele (if reported), study population, and viral genotype. Abbreviations: EVR, early virological; NA, not available; RVR, rapid virological response; SVR, sustained virological response.

lead to altered biochemical and inflammatory states (Table 3).[54] Patients with an unfavorable *IL28B* genotype have been shown to have an increased risk of HCV-associated cirrhosis and more severe fibrosis.[55,56] However, Marabita *et al.*[57] reported that when the date of infection is taken into account, *IL28B* genotype is no longer associated with rate of fibrosis progression.

Lipid metabolism has an important role in HCV infection, and patients with high cholesterol levels tend to respond better to IFN therapy than those with lower cholesterol levels.[58] However, HCV infection and IFN administration both tend to depress cholesterol levels. Patients with the favourable *IL28B* genotype tend to have higher levels of total cholesterol, apolipoprotein B and LDL-cholesterol,[58] as well as a lower frequency of hepatic steatosis, than patients with the unfavourable *IL28B* genotype.[59]

Identification of the causative SNP

Many treatment-associated SNPs in the *IL28B* locus have been reported, although most studies have examined either rs12979860 or rs8099917.[28] Although rs12979860 seems to have better predictive value in some populations,[60] haplotypes that include both SNPs might be more accurate than either SNP individually.[61] This finding suggests that neither SNP is directly causative, but that both are linked to one, or more, as-yet-unidentified causative SNPs. Linkage disequilibrium (Box 1) is lowest in the African-American population, suggesting that examination of this group offers the best chance to identify the causative SNP.[6,62] Massively parallel sequencing has been used to identify new *IL28B* variants and Smith and colleagues[62] reported two SNPs—rs4803221 and rs7248668—that are more strongly associated with treatment failure than rs8099917. The authors do, however, point out that the high degree of homology among *IL28B* and *IL28A* complicates the task of unambiguous read mapping and increases the risk of detecting spurious associations.[62]

Although SNPs typically have little individual effect on treatment outcome, the minor allele (rs12979860 CT/TT) in the *IL28B* locus increases the risk of treatment failure 2–3-fold (observation based on references in Table 1), suggesting a direct effect on gene expression

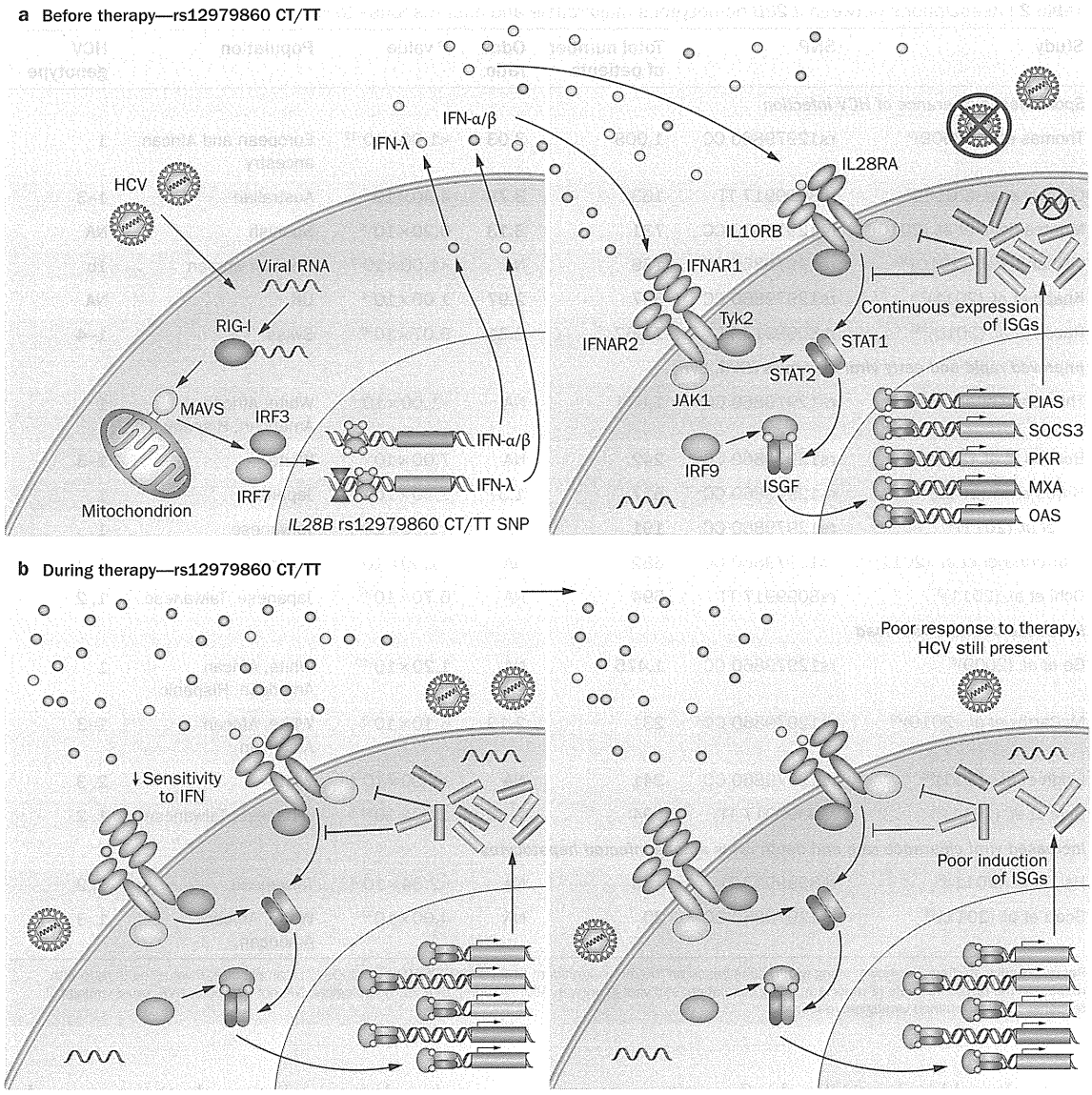

Figure 3 | Potential role of the unfavourable *IL28B* genotype in the response to interferon therapy. **a** | Intracellular HCV RNA is detected by surveillance molecules of the innate immune system, including the RIG-I–IFIH1 pathway, which causes the adaptor protein MAVS to induce expression and secretion of IFN-α, IFN-β and IFN-λ via IRFs. IFNs are recognized by receptors on the cell surface and initiate a signal cascade that results in induction of a large number of ISGs that collectively establish an antiviral state hostile to viral replication. IFN-α and IFN-β are recognized by IFNAR, whereas IFN-λ is recognized by the IL10R–IL28R receptor complex. Both receptors activate the Jak-STAT pathway, which induces the translocation of an ISGF complex to the nucleus, where it binds to the interferon-stimulated response elements of multiple ISGs. Unlike patients with the favourable *IL28B* (rs1297860 CC) genotype, in which viral RNA induces only modest ISG expression, patients with the unfavourable genotype (rs12979860 CT/TT) tend to have higher baseline ISG expression levels, suggesting continuous stimulation of the IFN signaling pathway in the presence of the virus. Nonetheless, the level of ISG expression seems to be insufficient to clear the virus, at the same time triggering negative regulation of the IFN signaling pathway through IFN-inhibitory molecules such as SOCS3 and PIAS. These pathways negatively regulate Jak-STAT signaling and make the cell less responsive to IFN signaling. **b** | As a result, even when IFN-α is administered as part of therapy, the cell is unable to induce a strong ISG expression, and the patient responds poorly to therapy. Abbreviations: IFIH1, IFN-induced helicase C domain-containing protein 1; IFNAR, IFN-α/β receptor 1; IL10R, IL-10 receptor; IL28R, IL-28 receptor; IRF, IFN regulatory factor; ISG, IFN-stimulated gene; ISGF, IFN-stimulated gene factor; Jak, Janus kinase; MAVS, mitochondrial antiviral-signalling protein; MXA, myxovirus resistance protein 1 (also known as MX1); OAS, 2'5'-oligoadenylate synthase; PIAS, E3 SUMO-protein ligase PIAS; PKR, protein kinase RNA-activated (also known as EIF2AK2); RIG-I, retinoic-acid inducible protein I; SOCS3, suppressor of cytokine signaling 3; STAT, signal transducer and activator of transcription.

resulting from a single base change. A number of possible mechanisms exist, and so far little evidence is available to reject some of the possibilities. The causative *IL28B* SNP might affect the strength, timing or specificity of *IL28B* expression by altering binding of transcription factors, enhancers or other regulatory elements, or it

Table 3 | Associations between *IL28B* homozygous major allele and biochemical and hepatic changes

Study	SNP	Total number of patients	Odds ratio	*P* value	Population	HCV genotype
Increased levels of cholesterol, LDL and apolipoprotein B100						
Li *et al.* (2010)[58]	rs12979860 CC	746	NA	8.90×10^{-10}	White, African American	1
Aizawa *et al.* (2011)[73]	rs8099917 TT	148	NA	6.40×10^{-3}	Japanese	1
Reduced frequency of hepatic steatosis						
Tillmann *et al.* (2011)[59]	rs12979860 CC	325	3.45	1.20×10^{-2}	White, African American	1
Reduced levels of γ-glutamyl transpeptidase						
Abe *et al.* (2010)[54]	rs8099917 TT	364	NA	1.00×10^{-3}	Japanese	1
Inflammatory activity, fibrosis and cirrhosis risk						
Barreiro *et al.* (2011)[76]	rs12979860 CT/TT	304	2.32	1.00×10^{-2}	Spanish	1, 3, 4
Fabris *et al.* (2011)[55]	rs12979860 CT/TT	412	NA	5.00×10^{-4}	Italian (white)	1–4
Falleti *et al.* (2011)[56]	rs12979860 CT/TT	629	1.68	$<5.00\times10^{-2}$	Italian (white)	1–4

Representative studies are shown, along with results based on the most significant SNP (rs12979860 or rs8099917), sample size, *P* value, odds ratio with respect to the favorable allele (if reported), study population, and viral genotype. Abbreviations: EVR, early virological; NA, not available; RVR, rapid virological response; SVR, sustained virological response.

may influence translation efficiency by altering DNA accessibility or mRNA stability.

Smith *et al.*[62] noted that several of the SNPs with the strongest reported associations are located in CpG regulatory regions and suggest that single-base substitutions could act by disrupting DNA methylation patterns. Even when the identity of the causative SNP is determined, rs12979860 and rs8099917 are, however, likely to remain as useful predictors of treatment response, especially in retrospective analyses.

Extended effects of *IL28B*

The role of *IL28B* genotype has been examined for a number of phenotypes in addition to its effect on spontaneous clearance and outcome of combination therapy (Tables 1–3). The effect of *IL28B* genotype on SVR has been replicated in diverse populations with consistently high odds ratios for outcome of therapy and spontaneous clearance (OR; range 2.5–12).[6–8,28,36–38] The effect seems to be strongest for difficult-to-treat HCV genotypes 1 and 4, but has also been reported for HCV genotypes 2 and 3, with intermediate ORs (1.5–4).[63–68] Initial evidence suggests that *IL28B* genotype might also be predictive of outcome after telaprevir triple therapy.[69,70] Multiple studies have also documented the effect of *IL28B* genotype on spontaneous clearance of the virus, although most studies have focused on patients of European ancestry[9,29,32,71,72] and few studies have examined the role, if any, of *IL28B* in exposed, uninfected individuals.[32]

IL28B genotype seems to be associated with differences in viral load early in treatment. As already mentioned, patients with the favourable genotype tend to have fairly high viral loads prior to treatment[6,35–37] that then rapidly decrease in the early weeks of therapy (for example RVR or early virological response).[34,37,38,41,44,45] The favourable *IL28B* genotype is also associated with biochemical differences, including increased cholesterol and LDL levels and reduced γ-glutamyl transpeptidase levels.[54,58,73] Differences in ISG expression levels,[48,50,74,75] viral clearance rate[46] and hepatocyte death rate[43] suggest differences in the cellular and immune response to HCV infection, although the role of *IL28B* in inflammatory activity, fibrosis risk and cirrhosis is unclear.[54–57,76] Given the number and diversity of ISG targets, small changes affecting IFN signalling are likely to have complex downstream effects. Further research will probably reveal additional roles for the *IL28B* genotype in resolution of viral infection.

Proposed role of *IL28B* on response to IFN

When HCV RNA is detected through the RIG-I–IFIH1 (also known as MDA5) or Toll-like receptor 3 pathways, the adaptor protein MAVS (also known as VISA or IPS1) induces expression of IFN-α, IFN-β and IFN-λ, which induce an intracellular antiviral state that suppresses viral replication (Figure 2a).[2,77–79] IFN-α and IFN-β signal through the IFN receptor (IFNAR), whereas IFN-λ signals through the IL10R–IL28R receptor complex.[11] Both receptors activate the Jak–STAT pathway, which upregulates a large number of ISGs by binding to the IFN-stimulated response element (ISRE; Figure 2a).[2] The underlying mechanism by which the rs12979860 SNP in the *IL28B* gene exerts its effect is not clear, but the unfavourable allele seems to lead to continuous activation of a subset of ISGs in the presence of intracellular HCV RNA.[53] Although this level of expression is not sufficient to effectively eliminate the virus from the cell, it might nonetheless upregulate IFN-inhibitory molecules such as SOCS3 and PIAS that negatively regulate Jak–STAT signalling, thereby reducing sensitivity to IFN signalling.[80] Therefore, the hepatocyte is not only unable to clear the virus from the cell but is unable to induce stronger ISG expression when IFN is administered during therapy.[53]

Several scenarios might arise in response to IFN therapy depending on the *IL28B* genotype of the host (Figures 2 and 3). In patients with the unfavourable genotype (rs12979860 CT/TT), prior to treatment, the

presence of viral RNA induces continuous ISG expression in hepatocytes. Although moderate ISG expression can partially impair viral replication, it also stimulates negative regulatory pathways that ultimately reduce IFN sensitivity. IFN-α administered during therapy fails to induce ISG expression strongly enough to eradicate the virus. As a result, the patient responds poorly to therapy (Figure 3). We are unaware of studies showing the long-term changes in ISG expression in patients with chronic HCV infection after IFN therapy. However, we speculate that ISG expression fails to return to preinfection levels as long as the virus remains active in the liver, which could contribute to the pathology of the disease and influence treatment options.

In those with the favourable *IL28B* allele (rs12979860 CC; Figure 2), prior to IFN-α treatment, the presence of HCV RNA seems to result in minimal IFN-λ expression. As a result, hepatocyte ISG expression remains low even in the presence of HCV RNA. Although this phenomenon might result in a higher baseline viral load in these patients than in patients with the unfavourable *IL28B* genotype, cells remain more sensitive to IFN. Therefore, IFN-α administered during therapy can result in stronger induction of ISG expression and more effective clearance of the virus.

Future perspectives

Therapeutic role of IFN-λ

In patients with the unfavourable *IL28B* genotype (rs12979860 CT/TT), increased constitutive expression of some ISGs could prevent cells from responding to IFN-α administered during therapy. However, IFN-λ signalling does not seem to become desensitized after prolonged stimulation, suggesting a role for IFN-λ in patients who fail to respond to IFN-α through ISG preactivation.[81] In an open-label study of 56 patients, Muir *et al.*[82] found that a 4-week course of PEG-IFN-λ (with or without ribavirin) was well tolerated and has clear antiviral activity with few adverse events.[82] The potent antiviral activity of IFN-λ coupled with reduced adverse effects owing to the restricted distribution of IFN-λ receptors suggest that it might serve as a less toxic alternative to IFN-α in HCV therapy.

Improved pretreatment predictive models

Although the *IL28B* SNP is currently the best single pretreatment predictor of SVR, not all patients with the favourable genotype achieve SVR, and some patients without it are nonetheless able to achieve SVR.[83,84] Therefore, this SNP alone might not be sufficiently discriminative to advise a course of treatment. SVR rates based on clinical studies could also be inflated because of requirements for a homogenous patient population with strict adherence to the treatment regimen,[6,62] whereas, in clinical practice, dose reductions, co-infection with HBV or HIV, and other complications might compromise the predictive effect of the *IL28B* SNP.

Useful prediction models must also take into account other host and viral factors that might influence outcome of treatment. Most current models use multivariate logistic regression, in which a number of continuous or categorical factors are evaluated simultaneously to find a minimal set of independent factors that can predict treatment outcome. A positive or negative coefficient is calculated for each factor in the model based on its effect size, and the probability of treatment success is calculated by multiplying the measured value for a factor by its coefficient and summing the results for all factors. The resulting sum is converted to a probability that represents the likelihood that the patient will respond successfully to therapy. Models can be evaluated in a test set by comparing the true positive rate with the true negative rate by plotting a receiver operating characteristic (ROC) curve and calculating the area under the curve (AUC). A model that accurately predicts success or failure for each patient would have an AUC of 1, whereas a model that over predicts or under predicts success will have a value less than 1 and a random model might have an AUC of 0.5.

When other independent factors are included (for example, age, sex, BMI, viral load, fibrosis stage, ISG expression and amino acid substitutions in viral proteins), current prediction models are able to achieve an ROC AUC approaching 0.85.[83,85,86] Although such models are certainly useful as a guide, an AUC of 0.85 implies that some patients who might respond to therapy will be predicted to fail and some patients who are not expected to respond to therapy may, nonetheless, successfully clear the virus.

Studies have also reported associations of *IL28B* with KIR (Killer-cell immunoglobulin-like receptors) and HLA genotypes,[87,88] CXC-chemokine ligand 10 (also known as IP-10)[89] and vitamin D levels.[90] These associations could further improve prediction accuracy by accounting for other genetic and environmental factors that influence the immune response and ability to respond to treatment. Predictive models are likely to become increasingly important as the number of alternative therapies increases and trade-offs between cost, risk of adverse effects and chance of success require more complex treatment decisions.

New therapies

The predictive role of *IL28B* SNPs is based on PEG-IFN-α plus ribavirin combination therapy. However, the treatment regimen for chronic HCV is currently undergoing a major change with the introduction of direct-acting antiviral agents.[5] Triple therapy with telaprevir or boceprevir is expected to dramatically improve the rate of SVR achieved,[5] raising the question of whether *IL28B* genotype will remain a useful predictor of treatment outcome. Initial data suggest that the favourable *IL28B* allele also predicts response to triple therapy (Table 1), but its predictive effect might not be as strong as for PEG-IFN-α plus ribavirin combination therapy.[70,91]

As an extension of the current standard of care, triple therapy still relies on IFN-α and ribavirin to suppress antiviral resistance.[5] Patients who fail to respond to IFN-α (owing to, for example, ISG preactivation) could also be more susceptible to antiviral resistance, and *IL28B* genotyping could help identify those patients who

are poor candidates for triple therapy. Not only might triple therapy be ineffective in such patients, but the lack of effective IFN response could fail to suppress the emergence of resistant strains, thereby complicating future treatment efforts and increasing the risk of horizontal transmission of resistant strains (for example, among high-risk subpopulations).

IL28B genotyping could also be useful in helping to predict and control recurrent HCV infection after liver transplantation, as re-infection with HCV following liver transplantation is common and requires post-transplant antiviral therapy.[92–94] The unfavourable *IL28B* genotype in recipients is associated with more severe recurrence of HCV infection, and *IL28B* genotypes of donors and recipients are independently associated with post-transplant treatment response, suggesting that donors with the favourable *IL28B* genotype might be preferentially allocated to HCV-infected patients.[93]

Conclusions

The role of *IL28B* in chronic HCV infection has been studied extensively following the identification of common genetic variants strongly predictive of treatment outcome in 2009. Results have been replicated in other populations and HCV genotypes, and predictive models have been developed that attempt to incorporate *IL28B* genotype into clinical decision-making. Even with the introduction of a new class of highly effective direct-acting antiviral agents, the *IL28B* SNP continues to serve as a useful predictor of treatment outcome and could help to establish treatment expectations and guide decisions for retreatment of prior nonresponders. Investigation of the underlying mechanism by which the *IL28B* genotype exerts its effect has yielded new insights into the regulation of antiviral defences that could have applications beyond the field of HCV research. Moving forward, the major challenge will be to incorporate this new knowledge into practical improvements in the clinic.

Review criteria

A search for original articles published between 1989 and 2011 and focusing on *IL28B* polymorphisms was performed in MEDLINE and PubMed. The search terms used were "hepatitis C virus", "interleukin 28b", "interferon lambda", "genome-wide association study", "single nucleotide polymorphism", "interferon-stimulated gene", and "spontaneous clearance" alone and in combination. All articles identified were English-language, full-text papers. We also searched the reference lists of identified articles for further relevant papers.

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Efficacy and safety of telaprevir, a new protease inhibitor, for difficult-to-treat patients with genotype 1 chronic hepatitis C

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SUMMARY. The aims of this phase III study were to assess the efficacy and safety of telaprevir in combination with peginterferon alfa-2b (PEG-IFN) and ribavirin (RBV) for difficult-to-treat patients who had not achieved sustained virological response (SVR) to prior regimens in Japan. The subjects were 109 relapsers (median age of 57.0 years) and 32 nonresponders (median age of 57.5 years) with hepatitis C virus genotype 1. Patients received telaprevir (750 mg every 8 h) for 12 weeks and PEG-IFN/RBV for 24 weeks. The SVR rates for relapsers and nonresponders were 88.1% (96/109) and 34.4% (11/32), respectively. Specified dose modifications of RBV that differed from that for the standard of care were introduced to alleviate anaemia. RBV dose reductions were used for 139 of the 141 patients. The SVR rates for relapsers did not depend on RBV dose reduction for 20–100% of the planned dose (SVR rates 87.5–100%, $P < 0.05$). Skin disorders were observed in 82.3% (116/141). Most of the skin disorders were controllable by anti-histamine and/or steroid ointments. The ratios of discontinuation of telaprevir only or of all the study drugs because of adverse events were 21.3% (30/141) and 16.3% (23/141), respectively. A frequent adverse event leading to discontinuation was anaemia. Telaprevir in combination with PEG-IFN/RBV led to a high SVR rate for relapsers and may offer a potential new therapy for nonresponders even with a shorter treatment period.

Keywords: direct-acting antiviral, peginterferon, ribavirin, sustained virological response, treatment failure.

INTRODUCTION

Hepatitis C virus (HCV) affects approximately 170 million people worldwide [1]; patients with chronic hepatitis C (CHC) eventually develop cirrhosis and hepatocellular carcinoma (HCC) [2,3]. The standard of care (SOC) with peginterferon plus ribavirin (RBV) for 48 weeks is most effective for eradicating HCV genotype 1 [4], which is a dominant genotype for CHC [1]. However, the sustained virological response (SVR) rate of SOC for the treatment of naïve patients with genotype 1 is approximately <50% [5,6]. The retreatment regimen for patients who do not achieve SVR is limited to exposure to peginterferon plus RBV with modification of dose and treatment duration. Some studies have been conducted to estimate the effectiveness of peginterferon plus RBV for 48 weeks for nonresponders to prior interferon-based combination therapy, and the SVR rates in most studies did not exceed 20% [7–9]. A large randomized study of patients who had not responded to previous treatment with peginterferon alfa-2b (PEG-IFN) plus RBV gave SVR rates for peginterferon alfa-2a 180 μg/kg plus RBV for 72 weeks that were not as high as those for 48 weeks (14%, 9%) [10]. HCV patients who had failed to achieve SVR with the combination therapy displayed high risk rates of decompensated cirrhosis, HCC and liver-related mortality [11]. Therefore, it is very important to establish new regimens to increase the SVR rate and shorten the treatment period for patients who do not achieve SVR with prior treatments.

Telaprevir, classified as a direct-acting antiviral agent, is a reversible, selective, orally bioavailable inhibitor of the nonstructural NS3/4A HCV serine protease [12]. Two phase II studies (PROVE 1 and PROVE 2) on the treatment of naïve patients with genotype 1 were conducted to assess the

Abbreviations: CHC, chronic hepatitis C; ETR, end of treatment response; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; PEG-IFN, peginterferon alfa-2b; RBV, ribavirin; RVR, rapid viral response; SOC, standard of care; SVR, sustained virological response.

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