Table 3 Baseline characteristics in untreated and treated patients

Features at the baseline	Untreated (n = 1,130)	Treated (n = 982)	Differences p value
Age (years)	31 (1–81)	36 (6–75)	<0.001
Men	705 (62.4 %)	726 (71.9 %)	<0.001
Chronic hepatitis	1,094 (96.8 %)	937 (96.4 %)	0.079
Cirrhosis	36 (3.2 %)	45 (3.6 %)	
AST (IU/L)	27 (3–1,776)	56 (6–2,192)	<0.001
ALT (IU/L)	28 (2–3,020)	96 (8–2,740)	<0.001
γ-GTP (IU/L)	20 (4–1,494)	45 (4–1,278)	<0.001
Total bilirubin (mg/dL)	0.5 (0.1–20.1)	0.7 (0.2–21.2)	<0.001
Albumin (g/dL)	4.4 (2.2–5.8)	4.3 (1.1–5.4)	<0.001
Platelets ($\times 10^3$/mm^3)	202 (40–443)	181 (40–483)	<0.001
α-Fetoprotein (μg/L)	4 (1–2,060)	4 (1–1,610)	<0.001
HBeAg-negative status	857 (75.8 %)	312 (31.8 %)	<0.001
HBsAg (IU/mL)	2,240 (0.06–141,000)	5,270 (0.09–277,000)	<0.001
HBcrAg (log U/mL)	3.6 (<3.0 to >6.8)	> 6.8 (<3.0 to >6.8)	<0.001
Genotypes [A/B/C/others (%)]	5.7/20.0/72.6/1.7	3.4/11.1/84.9/0.5	<0.001
HBV DNA (log copies/mL)	4.7 (<2.1 to >9.1)	8.0 (<2.1 to >9.1)	<0.001

Median values with the range in parentheses or numbers with the percentage in parentheses are given

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

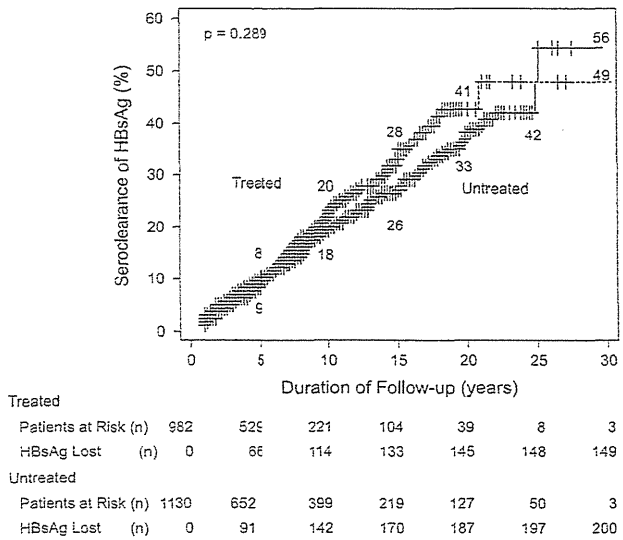


Fig. 2 Comparison of HBsAg seroclearance rates between 982 treated and 1,130 untreated patients. Numbers of patients at risk and those of patients who lost HBsAg are indicated below each time point

($p < 0.001$), HBV DNA ($p = 0.002$), HBsAg ($p < 0.001$), HBcrAg ($p = 0.003$), and precore wild-type ($p = 0.013$) (Table 6).

Factors contributing to HBsAg seroclearance in treated patients

In the 982 treated patients, factors influencing HBsAg seroclearance in univariate analysis by the Cox regression analyses were age ≥50 [RR 1.91 ($p = 0.001$)]; male gender [RR 2.14 ($p = 0.001$)], no family history in third-degree or closer relatives [RR 1.58 ($p = 0.005$)]; previous treatment with interferon [RR 2.13 ($p < 0.001$)]; chronic hepatitis [RR 3.12 ($p < 0.001$)]; AST ≥50 IU/L [RR 1.47 ($p = 0.031$)]; γ-GTP ≥20 IU/L [RR 1.87 ($p = 0.001$)]; platelets ≤150 × 10^3/mm^3 [RR 2.10 ($p < 0.001$)]; HBeAg-negative status [RR 2.53 ($p < 0.00$)]; HBV DNA ≤5 log copies/mL [RR 2.07 ($p = 0.001$)]; HBsAg ≤2,000 IU/mL [RR 2.29 ($p < 0.001$)]; HBcrAg ≤4 log U/mL [RR 2.28 ($p = 0.003$)]; and the wild-type precore sequence [RR 2.04 ($p = 0.011$)].

In multivariate analysis, only 3 factors contributed to HBsAg seroclearance: no family history in third-degree or closer relatives [RR 2.22 ($p = 0.006$)]; previous treatments with interferon [RR 3.15 ($p < 0.001$)]; and HBeAg-negative status [RR 3.75 ($p < 0.001$)] (Table 7).

Discussion

In Japan, perinatal materno-fetal transmission was the main route of HBV infection, but this transmission has been prevented since 1986 by the national campaign to prevent it by immunoprophylaxis with combined passive-active immunization of babies born to HBeAg-positive carrier mothers. However, HCC develops in about 10 % of the patients who have established chronic HBV infection by materno-fetal infection or through child-to-child transmission. Hence, HBsAg seroclearance is crucially required for preventing the development of cirrhosis followed by HCC.

In the present study, we analyzed 2,112 patients with persistent HBV infection to establish the factors

Table 4 Differences between the baseline characteristics of 917 untreated patients in whom HBsAg persisted and 213 those who lost HBsAg

Features at the baseline	HBsAg persisted (n = 917)	HBsAg lost (n = 213)	Differences p value
Age (years)	37 (1–81)	44 (0–80)	<0.001
Men	553 (60.3 %)	152 (71.4 %)	0.003
HBV in family members	349 (38.1 %)	76 (35.7 %)	0.509
Chronic hepatitis	893 (97.4 %)	201 (94.4 %)	0.020
AST (IU/L)	27 (3–1,144)	25 (6–1,776)	0.283
ALT (IU/L)	28 (6–1,960)	27 (6–3,020)	0.389
γ-GTP (IU/L)	22 (1–1,494)	29 (4–1,092)	<0.001
Total bilirubin (mg/dL)	0.6 (0.2–20.1)	0.7 (0.1–4.0)	0.257
Albumin (g/dL)	4.3 (2.0–5.3)	4.4 (1.6–5.7)	0.004
Platelets ($\times 10^3$/mm^3)	203 (40–443)	203 (33–417)	0.473
α-Fetoprotein (μg/L)	3 (1–2,060)	1 (1–478)	0.373
Genotypes [A/B/C/others (%)]	5.7/19.0/73.3/1.9	5.5/24.7/69.2/0.7	<0.001
HBeAg-negative status	663 (72.3 %)	194 (91.1 %)	<0.001
HBV DNA (log copies/mL)	4.9 (<2.1 to >9.1)	3.8 (<2.1 to >9.1)	<0.001
HBsAg (IU/mL)	3,100 (1.94–141,000)	149 (0.06–88,800)	<0.001
HBcrAg (log U/mL)	3.9 (<3.0 to >6.8)	2.9 (<3.0 to >6.8)	<0.001
Wild-type precore sequence	441 (48.1 %)	160 (75.0 %)	<0.001
Wild-type core promoter sequence	320 (34.9 %)	47 (22.0 %)	0.001

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

Table 5 Factors influencing the seroclearance of HBsAg in untreated patients evaluated by time-dependent uni- and multivariate analyses

Factors	Univariate analysis HBsAg clearance Relative risk (95 % CI)	p value	Multivariate analysis HBsAg clearance Relative risk (95 % CI)	p value
Age ≥50 years	1.63 (1.19–2.23)	0.002	1.61 (1.09–2.37)	0.018
Male gender	1.08 (0.79–1.48)	0.618		
No HBV infection in family	1.38 (1.02–1.86)	0.037		
Cirrhosis	1.19 (0.73–1.93)	0.484		
AST ≥50 IU/L	1.01 (0.70–1.45)	0.979		
ALT ≥50 IU/L	0.93 (0.68–1.27)	0.633		
γ-GTP ≥20 IU/L	1.17 (0.85–1.61)	0.330		
Total bilirubin ≥1 mg/dL	1.41 (0.80–2.49)	0.239		
Albumin ≥4 g/dL	0.78 (0.51–1.18)	0.239		
Platelets >150 × 10^3/mm^3	0.99 (0.67–1.46)	0.946		
α-Fetoprotein ≤10 μg/L	0.84 (0.48–1.47)	0.543		
Genotype A or B	1.17 (0.81–1.69)	0.410		
HBeAg-negative status	0.78 (0.79–2.07)	0.314		
HBV DNA ≥5 log copies/mL	0.84 (0.58–1.24)	0.383		
HBsAg ≤2,000 IU/mL	1.87 (1.19–2.91)	0.006	1.77 (1.12–2.77)	0.014
HBcrAg ≥4 log U/mL	0.85 (0.50–1.45)	0.555		
Wild-type precore sequence	0.99 (0.60–1.52)	0.967		
Wild-type core promoter sequence	0.78 (0.35–1.73)	0.538		

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

contributing to HBsAg seroclearance. The overall rate of HBsAg seroclearance was 1.75 % annually. The annual seroclearance rates of HBsAg are reported to be 1.7 % in Korea [14] and 1.6 % in Taiwan [15–17], as well as 2.5 % in Goto Islands of Japan, where HBV infections are very prevalent [18]. In 1,271 natives in Alaska, the rate of HBsAg seroclearance was 0.7 % annually [19]. These differences could be ascribed, in part, to HBV genotypes distinct among Asian countries and Alaska. Since treatment with IFN and/or nucleot(s)ide analogues has suppressive effects on the development of HCC [6, 20], they may influence HBsAg seroclearance.

Table 6 Differences in baseline characteristics between the 833 treated patients in whom HBsAg persisted and 149 those who lost HBsAg

Features at the baseline	HBsAg persisted (n = 833)	HBsAg lost (n = 149)	Differences p value
Age (years)	41 (13–88)	43 (17–71)	0.285
Men	601 (72.2 %)	124 (83.2 %)	0.004
HBV in family members	496 (59.6 %)	72 (48.3 %)	0.010
Chronic hepatitis	802 (96.3 %)	134 (89.9 %)	0.001
AST (IU/L)	54 (6–2,192)	78 (7–888)	0.010
ALT (IU/L)	93 (8–2,740)	118 (8–1,700)	0.117
γ-GTP (IU/L)	44 (4–1,278)	46 (4–1,278)	0.023
Total bilirubin (mg/dL)	0.7 (0.2–21.2)	0.7 (0.3–8.4)	0.273
Albumin (g/dL)	4.3 (1.1–5.4)	4.5 (1.4–5.3)	0.281
Platelets ($\times 10^3$/mm^3)	182 (40–483)	171 (50–391)	<0.001
α-Fetoprotein (μg/L)	4 (1–1,610)	4 (1–765)	0.682
Genotypes [A/B/C/others (%)]	3.2/10.7/85.1/1.0	5.1/12.4/81.6/0.9	0.565
HBeAg-negative status	230 (27.6 %)	79 (53.0 %)	<0.001
HBV DNA (log copies/mL)	7.8 (<2.1 to >9.1)	8.3 (<2.1 to >9.1)	0.002
HBsAg (IU/mL)	7,880 (0.04–277,000)	1,380 (0.04–188,000)	<0.001
HBcrAg (log U/mL)	6.9 (<3.0 to >6.8)	5.9 (<3.0 to >6.8)	0.003
Wild-type precore sequence	554 (66.6 %)	61 (41.2 %)	0.013
Wild-type core promoter sequence	274 (32.9 %)	67 (45.0 %)	0.836

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

Table 7 Factors influencing the seroclearance of HBsAg in treated patients evaluated by time-dependent uni- and multivariate analyses

Factors	Univariate analysis HBsAg clearance Relative risk (95 % CI)	p value	Multivariate analysis HBsAg clearance Relative risk (95 % CI)	p value
Age ≥50 years	1.91 (1.32–2.77)	0.001		
Male gender	2.14 (1.37–3.33)	0.001		
No HBV infection in family	1.58 (1.15–2.19)	0.005	2.22 (2.32–3.94)	0.006
Treatments (interferon vs. others)	2.13 (1.53–2.98)	<0.001	3.15 (1.69–5.87)	<0.001
Chronic hepatitis	3.12 (2.05–4.74)	<0.001		
AST ≥50 IU/L	1.47 (1.04–2.09)	0.031		
ALT ≥50 IU/L	1.29 (0.82–1.92)	0.201		
γ-GTP ≥20 IU/L	1.87 (1.30–2.70)	0.001		
Total bilirubin ≥1 mg/dL	1.35 (0.87–2.08)	0.179		
Albumin ≥4 g/dL	1.11 (0.66–1.86)	0.688		
Platelets ≤150 × 10^3/mm^3	2.10 (1.49–2.96)	<0.001		
α-Fetoprotein ≤10 μg/L	1.33 (0.92–1.92)	0.136		
Genotype A or B vs. others	1.16 (0.74–1.82)	0.529		
HBeAg-negative status	2.53 (1.83–3.50)	<0.001	3.75 (2.09–6.74)	<0.001
HBV DNA ≤5 log copies/mL	2.07 (1.37–3.13)	0.001		
HBsAg ≤2,000 IU/mL	2.29 (1.52–3.47)	<0.001		
HBcrAg ≤4 log U/mL	2.28 (1.31–3.97)	0.003		
Wild-type precore sequence	2.04 (1.18–3.55)	0.011		
Wild-type core promoter sequence	1.18 (0.63–2.21)	0.608		

Wild-type precore sequence, G1896; wild-type core promoter sequence, A176.2/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

Therefore, we went on to extend our analysis to untreated patients and those treated with IFN or nucleotide analogues separately. Criteria for upper or lower levels of each parameter were set, taking into consideration the median value or a cutoff value with the lowest p value of the entire 2,112-patient cohort (Table 1), and unified for untreated and treated patients (Tables 5, 7).

Firstly, in the univariate analysis, age, no family history of HBV infection in third-degree or closer relatives, and decreased HBsAg levels lowered the annual rate of HBsAg seroclearance significantly. In multivariate analysis, age ≥50 years (RR 1.61, $p = 0.018$) and HBsAg ≤2,000 IU/mL (RR 1.77, $p = 0.014$) decreased the annual rate of HBsAg seroclearance significantly. Kato et al. [18] reported high HBsAg seroclearance rates in patients over 40 or over 50 years; in our patients, also, age ≥50 years increased RR to 1.61 ($p = 0.018$). As for HBsAg and HBV DNA, low HBsAg and HBV DNA levels increased the HBsAg seroclearance rate to 37.7 %, and therefore, low HBsAg levels are an important factor. In actuality, HBsAg levels ≤2,000 IU/mL increased the rate of HBsAg seroclearance with RR 1.77 ($p = 0.014$).

In treated patients, by contrast, age, the male gender, no HBV infections in third-degree or closer relatives, treatment with IFN, chronic hepatitis, high AST levels, high γ-GTP levels, low platelet counts, HBeAg-negative status, low HBsAg levels, low HBcrAg levels and the wild-type precore sequence were significant factors in univariate analysis. In multivariate analysis, no HBV infections in third-degree or closer relatives (RR 2.22, $p = 0.006$), interferon treatments (RR 3.15, $p < 0.001$), and HBeAg-negative status (RR 3.75, $p < 0.001$) were significant factors.

Thus, there were differences in factors predictive of the HBsAg loss between untreated and treated patients. Remarkably, age and HBsAg titer were independent factors in untreated patients, whereas family history and negative HBeAg were independent factors in treated patients. Since this work studied patients who were followed for a long time (>15 years), age and HBsAg titer were factors for clearance of HBsAg in untreated patients. Treated patients, in contrast, would have included more patients with HBeAg, with a good response to antiviral treatment, as well as those without family history who would have been infected with HBV with a sorter duration than those with family history. In other words, most untreated patients were those with favorable clinical course, in whom HBsAg titer gradually decreased and eventually lost it with time. In fact, there would be many such patients, the majority of whom do not visit hospitals and are unaware of HBV infection, who may have unapparent liver disease. Treated patients, on the other hand, would have had higher risks for cirrhosis and HCC, owing to elevated ALT/AST levels; this risk is especially high for patients with a family history of HBV [21]. Therefore, patients with family history would not be able to easily lose HBsAg.

In treated patients, IFN led to HBsAg loss more effectively than other treatments [RR 2.13, $p < 0.001$ (Table 7)]. The immunomodulatory activity of IFN, which is not shared by nucleot(s)ide analogues, would have accelerated the immune response to HBV required for the seroclearance of HBsAg. Of the 333 patients who received IFN, 190 (57 %) were treated with IFN multiply. In them, seroclearance of HBsAg was achieved in 49 of the 190 (26 %) patients with multiple IFN treatments in comparison with 41 of the 143 (29 %) with single IFN treatment. Owing to indications for IFN, patients who received IFN tended to be younger, without previous treatments and higher HBV DNA as well as ALT levels. They might have increased the rate of HBsAg loss that was higher with IFN than other treatments.

Since this is a retrospective cohort study of patients visiting our hospital for more than 15 years, and there has been so much innovation in the treatment of chronic hepatitis B during that period, treated and untreated patients have different backgrounds at the baseline. Hence, treated patients had higher ALT and HBV DNA levels with severer liver disease than untreated patients (Table 3). This might have been responsible, at least in part, for the failure in finding differences in the rate of HBsAg loss between untreated and treated patients (Fig. 2). Future studies will be aimed at analyzing contributing factors in treated and matched controls. This will allow us to analyze factors contributing to HBsAg seroclearance in the treatment of patients with chronic hepatitis B.

Acknowledgments This work was supported in part by grants from the Ministry of Health, Labour and Welfare in Japan.

Conflict of interest These authors disclose the following: Dr. Kumada reports having received investigator, lecture, and consulting fees from Dainippon Sumitomo Pharma Co., MSD KK, Bristol-Myers Squibb, Pharma International, Dentsu Sudler, and Hennessey Inc. Dr. Ikeda reports having received investigator, lecture, and consulting fees from Dainippon Sumitomo Pharma Co. No other potential conflicts of interest relevant to this article were reported.

References

1. Ganem D, Prince AM. Hepatitis B virus infection—natural history and clinical consequences. N Engl J Med. 2004;350:1118–29.
2. Lee WM. Hepatitis B virus infection. N Engl J Med. 1997;337:1733–45.
3. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA. 2006;295:65–73.

4. Huo TI, Wu JC, Lee PC, Chau GY, Lui WY, Tsay SH, et al. Seroclearance of hepatitis B surface antigen in chronic carriers does not necessarily imply a good prognosis. Hepatology. 1998;28:231–6.
5. Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology. 2006;130:678–86.
6. Arase Y, Ikeda K, Suzuki F, Suzuki Y, Saitoh S, Kobayashi M, et al. Long-term outcome after hepatitis B surface antigen seroclearance in patients with chronic hepatitis B. Am J Med. 2006;119:71.e9–16.
7. Chen YC, Sheen IS, Chu CM, Liaw YF. Prognosis following spontaneous HBsAg seroclearance in chronic hepatitis B patients with or without concurrent infection. Gastroenterology. 2002;123:1084–9.
8. Chu CM, Liaw YF. HBsAg seroclearance in asymptomatic carriers of high endemic areas: appreciably high rates during a long-term follow-up. Hepatology. 2007;45:1187–92.
9. Yuen MF, Wong DK, Fung J, Ip P, But D, Hung I, et al. HBsAg seroclearance in chronic hepatitis B in Asian patients: replicative level and risk of hepatocellular carcinoma. Gastroenterology. 2008;135:1192–9.
10. Suzuki F, Arase Y, Suzuki Y, Akuta N, Sezaki H, Seko Y, et al. Long-term efficacy of interferon therapy in patients with chronic hepatitis B virus infection in Japan. J Gastroenterol. 2012;47:814–22.
11. Kobayashi M, Suzuki F, Akuta N, Hosaka T, Sezaki H, Yatsuji H, et al. Loss of hepatitis B surface antigen from the serum of patients with chronic hepatitis treated with lamivudine. J Med Virol. 2007;79:1472–7.
12. Akuta N, Suzuki F, Suzuki Y, Sezaki H, Hosaka T, Someya T, et al. Favorable efficacy of long-term lamivudine therapy in patients with chronic hepatitis B: an 8-year follow-up study. J Med Virol. 2005;75:491–8.
13. Hosaka T, Suzuki F, Kobayashi M, Seko Y, Kawamura Y, Sezaki H, et al. Clearance of hepatitis B surface antigen during long-term nucleot(s)ide analog treatment in chronic hepatitis B: results from a nine-year longitudinal study. J Gastroenterol. 2012. doi:10.1007/s00535-012-0688-7.
14. Ahn SH, Park YN, Park JY, Chang HY, Lee JM, Shin JE, et al. Long-term clinical and histological outcomes in patients with spontaneous hepatitis B surface antigen seroclearance. J Hepatol. 2005;42:188–94.
15. Kim JH, Lee JH, Park SJ, Bae MH, Kim JH, Kim do Y, et al. Factors associated with natural seroclearance of hepatitis B surface antigen and prognosis after seroclearance: a prospective follow-up study. Hepatogastroenterology. 2008;55:578–81.
16. Liaw YF, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. Hepatol Int. 2008;2:263–83.
17. Liu J, Yang HI, Lee MH, Lu SN, Jen CL, Wang LY, et al. Incidence and determinants of spontaneous hepatitis B surface antigen seroclearance: a community-based follow-up study. Gastroenterology. 2010;139:474–82.
18. Kato Y, Nakao K, Hamasaki K, Kato H, Nakata K, Kusumoto Y, et al. Spontaneous loss of hepatitis B surface antigen in chronic carriers, based on a long-term follow-up study in Goto Islands, Japan. J Gastroenterol. 2000;35:201–5.
19. McMahon BJ, Holck P, Bulkow L, Snowball M. Serologic and clinical outcomes of 1536 Alaska natives chronically infected with hepatitis B virus. Ann Intern Med. 2001;135:759–68.
20. Simonetti J, Bulkow L, McMahon BJ, Homan C, Snowball M, Negus S, et al. Clearance of hepatitis B surface antigen and risk of hepatocellular carcinoma in a cohort chronically infected with hepatitis B virus. Hepatology. 2010;51:1531–7.
21. Shiraki K, Yoshihara N, Sakurai M, Eto T, Kawana T. Acute hepatitis B in infants born to carrier mothers with the antibody to hepatitis B e antigen. J Pediatr. 1980;97:768–70.

Original article

Telaprevir is effective given every 12 h at 750 mg with pegylated interferon-α2b and ribavirin to Japanese patients with HCV-1b IL28B rs8099917 TT

*Yoshiiku Kawakami[1,2], Fumitaka Suzuki[3], Yoshiyasu Karino[4], Joji Toyota[4], Hiromitsu Kumada[3], Kazuaki Chayama[1,2,5]**

[1]Department of Gastroenterology and Metabolism, Applied Life Sciences, Institute of Biomedical & Health Sciences, Hiroshima University, Hiroshima, Japan
[2]Liver Research Project Center, Hiroshima University, Hiroshima, Japan
[3]Department of Hepatology, Toranomon Hospital, Tokyo, Japan
[4]Department of Gastroenterology, Sapporo Kosei General Hospital, Hokkaido, Japan
[5]Laboratory for Digestive Diseases, RIKEN Center for Integrative Medical Sciences, The Institute of Physical and Chemical Research (RIKEN), Hiroshima, Japan

*Corresponding author e-mail: chayama@hiroshima-u.ac.jp

Background: The aim of this study is to explore the efficacy, safety and pharmacokinetics of 750 mg telaprevir (TVR) given at 8 or 12 h intervals during triple therapy with pegylated interferon-α2b (PEG-IFN) and ribavirin (RBV) for patients with chronic HCV infection.
Methods: A total of 52 patients with high viral loads of HCV genotype 1b who were expected to respond well to therapy (rs8099917 TT genotype or relapse to previous therapy) were randomly assigned to two groups who were given 750 mg TVR at either 8 h (q8h) or 12 h (q12h) intervals in combination with PEG-IFN and RBV for 12 weeks, followed by 12 additional weeks of treatment with PEG-IFN and RBV alone. The primary end point of the study was undetectable HCV RNA at 12 weeks after the end of treatment (sustained virological response $[SVR]_{12}$).
Results: SVR_{12} rates were 92.3% (24/26) for both q8h and q12h. The changes in mean $\log_{10}$ HCV RNA levels and viral response were also similar in q8h compared to q12h, whereas pharmacokinetic properties such as maximum plasma concentration, area under the concentration-time curve at 24 h and trough plasma concentration of TVR were slightly higher in q8h than in q12h ($P>0.2$). The frequency of TVR discontinuation due to anaemia or renal damage was significantly higher in q12h than in q8h (6/26 [23%] versus 0/20 [0%], respectively; $P=0.02$).
Conclusions: TVR given at 12 h intervals should be considered for patients with lower body weight, especially patients with prior relapse and with IL28B polymorphisms at rs8099917 TT (interferon-λ 4 ss469415590 polymorphism TT/TT) genotype in patients with genotype 1b HCV infection.

Introduction

There are estimated to be 170 million HCV carriers worldwide [1,2]. Approximately 30% of carriers develop serious liver diseases, such as decompensated cirrhosis and hepatocellular carcinoma [3,4]. Eradication of the virus is necessary to prevent the development of severe liver damage in these patients.

Telaprevir (TVR), an HCV NS3/4A serine protease inhibitor, has recently been approved in the US, Canada, the European Union and Japan for treatment of patients with chronic HCV genotype 1 infection. In Phase III studies, sustained virological response (SVR) rates increased significantly in both treatment-naive as well as previously treated patients when TVR was administered in combination with pegylated interferon (PEG-IFN) and ribavirin (RBV) compared to PEG-IFN and RBV alone [5–7]. High SVR rates were also observed in Phase III studies in Japan [8,9]; however, side effects of triple therapy in the Japanese studies were so severe that many patients were forced to discontinue therapy due to adverse events, such as anaemia and fatigue [5–9]. Anaemia, in particular, is commonly associated with triple therapy. The frequency of anaemia ranged from 15% to 19% [5,6] in patients treated with PEG-IFN and RBV alone, whereas in patients treated with triple

therapy, the frequency of anaemia increased to between 30% and 37% [5,6]. In addition, RBV dose-reduction rates and discontinuation rates of TVR treatment due to severe adverse events are higher in Japan than in the US and European Union [5–9]. The higher discontinuation rate may result from taking the same standard prescription dosage of TVR despite the lighter body weight of Japanese patients compared with patients in other countries. Japanese patients also tend to be relatively older, and may therefore be at greater risk of severe side effects due to poorer drug metabolism rates. The aim of this study is thus to compare effects and safety of triple therapy with TVR administered at 12 h intervals compared with the standard 8 h interval regimen. We also studied pharmacokinetics of TVR in both groups of patients to see how the reduction of TVR affects the concentration of TVR.

Methods

Patients

We enrolled patients at Hiroshima University Hospital (Hiroshima, Japan), Toranomon Hospital (Tokyo, Japan) and Sapporo Kosei General Hospital (Hokkaido, Japan). Patients were enrolled from August 2012, and the last patient completed follow-up in May 2013. Criteria for inclusion were age between 20 and 70 years, chronic infection with HCV genotype 1b, and plasma HCV RNA level ≥100,000 IU/ml. We selected patients who were expected to respond well to triple therapy based on one of the following criteria: patients with the treatment-favourable rs8099917 TT genotype in the IFN-λ 3 (IL28B) locus or patients who experienced relapse during prior treatment with PEG-IFN and RBV combination therapy. In order to avoid poor response to reduction of TVR, we excluded patients who were expected to have poor response to the therapy, including prior non-responders to PEG-IFN and RBV therapy (that is, patients who failed to become negative for HCV RNA) and patients with rs8099917 T/G or G/G genotypes. Exclusion criteria also included liver disease due to other causes, decompensated cirrhosis, presence of liver cancer, HBV or HIV infection, renal insufficiency, history of heart disease or cerebral infarction, and pregnancy or current breastfeeding. IL28B rs8099917, IFN-λ 4 (IFNL4) ss469415590 and inosine triphosphate pyrophosphatase (ITPA) polymorphism (rs1127354) were genotyped using the Invader assay (Third Wave Technologies, Madison, WI, USA), TaqMan assay (Life Technologies, Carlsbad, CA, USA) or by direct sequencing, as described elsewhere [10–12]. Amino acid substitutions in the HCV core were determined using direct sequencing of PCR products after extraction and reverse transcription of HCV RNA. Core amino acid substitutions at positions 70 and 91 (core 70 and core 91, respectively) were determined as reported by Akuta *et al.* [13,14]. The demographic and baseline characteristics of patients are shown in Table 1. Median body weight was 62.3 kg and 25 (48%) patients had body weight <60 kg. IFNL4 ss469415590 and IL28B rs8099917 genotypes were completely linked, except in one patient (Additional file 1).

Study design and randomization

This was an exploratory prospective multicenter randomized study. Experimental procedures were approved by the institutional review boards at participating hospitals, and informed consent was obtained from all participants. Sample size was not based on hypothesis testing other than the precision estimate of SVR. If we assume that 80%, 85% and 90% of subjects will have undetectable HCV RNA 12 weeks after the end of therapy (SVR_{12}), then 25 subjects per arm would yield two-sided 95% confidence intervals of 64.3% to 95.7%, 71.0% to 99.0% and 78.0% to 100%, respectively. The study was conducted in accordance with the Declaration of Helsinki, and the trial was registered with UMIN Clinical Trials (UMIN000006758). Randomization was stratified according to the combination of prior treatment experience and amino acid substitution at HCV core amino acid 70 (treatment-naive and wild type, naive and mutant, transient response and wild type, transient response and mutant, non-response and wild type, or non-response and mutant), age (<60 or ≥60 years), gender (male or female) and baseline haemoglobin level (<13 or ≥13 g/dl). As shown in Table 1, the demographic and baseline characteristics were well balanced in the two groups of patients.

Mythos (Osaka, Japan), a third party institute that was not involved in the conduct of the study, randomly allocated the two groups of patients to different doses of TVR by means of computer-generated randomization codes.

Study procedures

TVR was administrated at a randomized dose of 750 mg after meals at 8 h (q8h) or 12 h (q12h) intervals. PEG-IFN-α2b (PegIntron; MSD, Tokyo, Japan) was administered subcutaneously at a dose of 1.5 μg/kg of body weight once weekly, and oral RBV (Rebetol; MSD) was administered at a total dose of 600 to 1,200 mg/day based on body weight. Patients received 12 weeks of treatment with TVR plus PEG-IFN/RBV followed by PEG-IFN/RBV alone for an additional 12 weeks. Follow-up observation was performed for 12 weeks (Additional file 2). RBV dosage was reduced or discontinued as required, based on reduction of haemoglobin levels or the development of adverse events. When haemoglobin decreased

Table 1. Baseline characteristics of patients

Characteristic	TVR 750 mg q8h (*n*=26)	TVR 750 mg q12h (*n*=26)	*P*-value
Gender	-	-	0.77
Male	17	18	-
Female	9	8	-
Age, years	61 (24-68)	61 (37-70)	0.99
Body weight, kg	61.4 (39-82)	63.3 (40-81)	0.76
Body mass index, kg/m^2	23.5 (16.8-32.0)	22.5 (17.8-27.7)	0.61
White blood cell count, cells/mm^3	4,890 (3,500-8,920)	4,995 (2,970-11,830)	0.67
Haemoglobin, g/dl	14.2 (12.2-16.5)	15.2 (11.4-17.4)	0.17
Platelet count, $\times 10^4$ cells/μl	15.9 (5.7-25.3)	16.9 (5.2-25.6)	0.74
ALT, IU/l	36 (16-292)	40 (14-117)	0.62
γ-GTP, IU/l	26 (13-125)	20 (10-192)	0.25
eGFR, ml/min	80 (62-105)	80 (60-120)	0.61
HCV RNA, log IU/ml	6.8 (5.3-7.4)	6.9 (5.2-7.8)	0.26
Previous IFN therapy	-	-	0.42
Treatment-naive	14	11	-
Relapse	9	11	-
Non-response	3	4	-
rs8099917	-	-	0.32
TT	25	26	-
TG	1	0	-
ss469415590	-	-	0.49
TT/TT	24	26	-
TT/ΔG	2	0	-
rs1127354	-	-	0.39
CC	18	20	-
Non-CC	8	5	-
ND	0	1	-
HCV core 70	-	-	0.67
Wild type	17	20	-
Mutant	6	4	-
ND	3	2	-

Data are median (range) or *n*. ALT, alanine aminotransferase; eGFR, estimated glomerular filtration rate; IFN, interferon; ND, not done; q8h, every 8 h; q12h, every 12 h; TVR, telaprevir; γ-GTP, γ-glutamy transpeptidase.

<10 g/dl, the daily dose of RBV was reduced from 600 to 400 mg, from 800 to 600 mg and from 1,000 to 600 mg, depending on the initial dose of each patient. RBV was withdrawn when haemoglobin decreased <8.5 g/dl. Decrease of TVR dose was not permitted, but administration was stopped if necessary due to the development of adverse events.

Efficacy assessments

Serum HCV RNA levels were measured using COBAS TaqMan HCV RNA 2.0 assay (Roche Diagnostics, Tokyo, Japan), with a lower limit of quantification of 25 IU/ml and a lower limit of detection of 10 IU/ml. The lower limit of detection was used in the determination of undetectable HCV RNA at week 4. HCV RNA levels were measured on day 1 and at the following times: weeks 2, 4, 8, 12, 16, 20, 24 and every 4 weeks until 12 weeks after the end of treatment.

End points

The primary end point was the proportion of patients who had undetectable plasma HCV RNA 12 weeks after the end of treatment (SVR_{12}). The secondary end point was the rate of discontinuation of the therapy due to adverse events.

Pharmacokinetic assessments

Blood samples were collected immediately prior to administering the first morning dose, and at week 2 at 1, 2.5, 4, 6, 8 and 12 h after the first dose to determine the concentration of TVR (750 mg q8h or 750 mg q12h) in the plasma. Plasma concentrations of TVR were determined using a HPLC apparatus fitted with a mass spectrometer. Area under the concentration-time curve (AUC) at 24 h ($AUC_{24\ h}$) was calculated by multiplying $AUC_{8\ h}$ by 3 or $AUC_{8\ h}$ by 2. The maximum plasma concentration (C_{max}) and trough plasma concentration (C_{trough}) were

directly determined from the observed values at week 2. RBV concentration was measured prior to the morning dose at week 2.

Safety assessments

Safety assessments including physical examinations, clinical laboratory tests and evaluation of adverse events were performed at each hospital visit during and after treatment at least every 4 weeks until 12 weeks after cessation of the therapy.

Statistical analyses

Analysis was performed on the intention-to-treat population, defined as all randomly assigned patients who received one dose of the study medication. Categorical variables between groups were compared using Fisher's exact test and continuous variables using the Mann-Whitney test. All analyses were performed using R version 2.15.3.

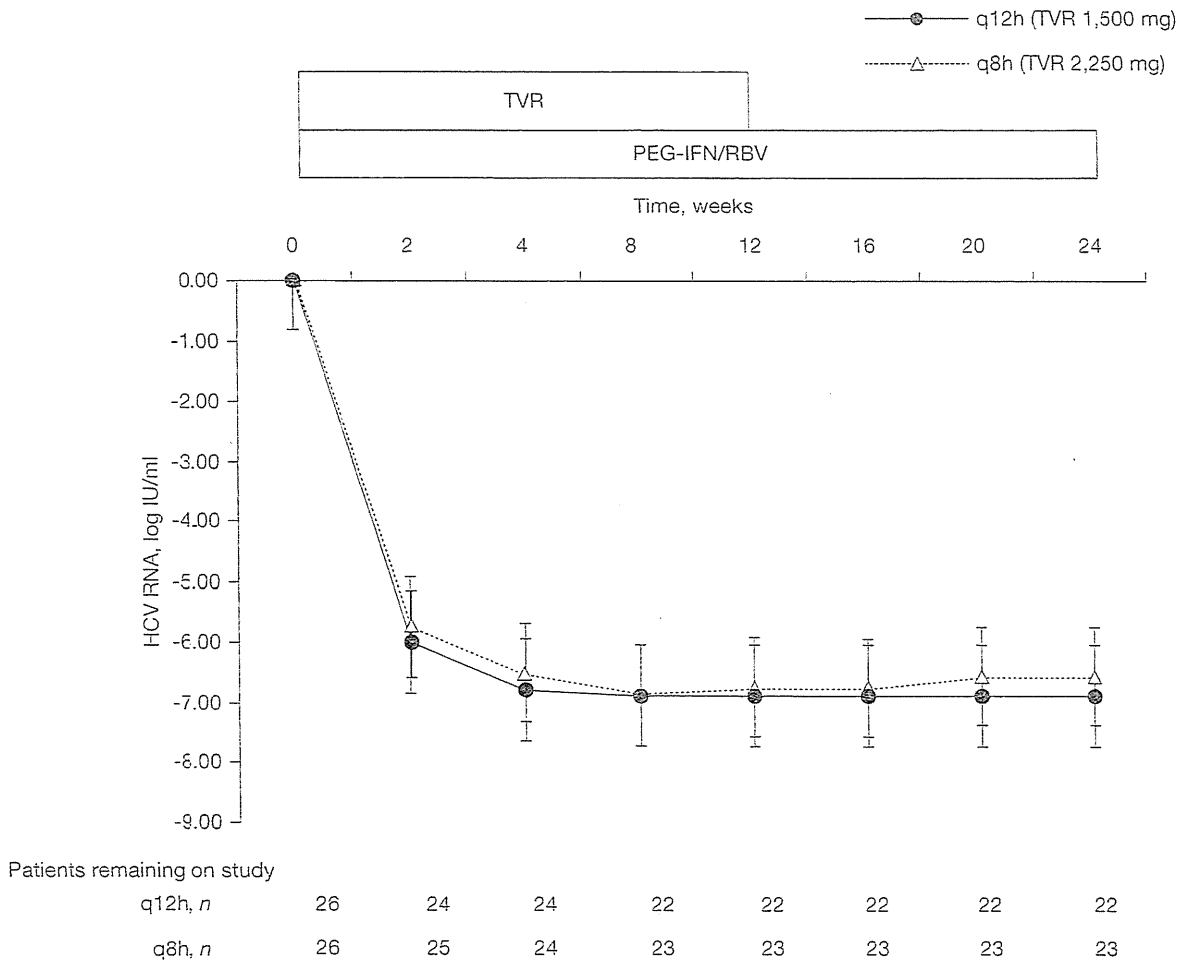
Results

Efficacy

SVR_{12} rates were 92.3% (24/26) for both q8h and q12h (Additonal file 2). The percentage of patients with undetectable HCV RNA at weeks 2, 4, 12, 24 and at 12 weeks after the end of treatment (SVR_{12}) was not statistically different between the two groups of patients (Additional file 2). Similar decreases in mean $\log_{10}$ HCV RNA levels were observed in both groups of patients (Figure 1). The SVR_{12} rate did not differ when the patients were divided by response to previous therapy, age, gender and platelet count (Additional file 1). These results show that the antiviral effect of triple therapy was nearly equivalent between the two patient groups.

Four patients did not achieve SVR_{12}. The characteristics of these four patients were as follows: median 64 years (range 62–65), male/female gender n=3/1, median viral

Figure 1. Decrease of HCV RNA during therapy



Data are shown as mean (sd). PEG-IFN, pegylated interferon; RBV, ribavirin; q8h, every 8 h; q12h, every 12 h; TVR, telaprevir.

load 6.9 log IU/ml (range 5.8–7.2) and median platelet count 17×10^4 cells/μl (range 12–22).

Pharmacokinetics

Mean pharmacokinetic parameters of TVR are shown in Table 2. C_{trough} was slightly lower in the q12h group than in the q8h group. $AUC_{24\ h}$ was also slightly higher in the q8h group than in the q12h group. However, these differences were not statistically significant. C_{max} was similar in both groups of patients.

The mean (SD) of RBV concentration (C_{trough}) at week 2 in the q8h and q12h groups was 1,706 (221) and 1,562 (222) ng/ml, respectively. Although the concentration was slightly higher in the q8h group than in the q12h group, the difference was not statistically significant (P=0.515).

Safety

There were no deaths or serious adverse effects. Adverse events with a frequency of >5% in total patients are listed in Table 3. The overall safety profile was similar in both groups of patients except for the frequency of renal damage. The ratios of discontinuation of all treatment due to adverse events were 12% (3/26) in the q8h group and 15% (4/26) in the q12h group (Additional file 1). Discontinuation of TVR occurred in 42.3% (11/26) of patients in the q8h group and 21.4% (6/28) of patients in the q12h group. Frequency of TVR discontinuation due to anaemia or renal damage was significantly higher in q12h than in q8h (6/26 [23%] versus 0/20 [0%], respectively; P=0.02; Additional file 1).

For anaemia, decreases of mean haemoglobin levels were similar during the initial 6 weeks. Although mean haemoglobin levels continued to decrease in the q8h group, haemoglobin levels stopped decreasing in the q12h group after week 6 (Figure 2). Low haemoglobin (<8.5 g/dl) occurred in 8 (30.8%) patients in the q8h group and 6 (23.1%) patients in the q12h group. The genotype of the ITPA single nucleotide polymorphism (SNP) had no significant effect on the frequency of anaemia. In terms of renal damage, during the 12 weeks of the triple therapy, estimated glomerular filtration rate decreased significantly more in the q8h group than in the q12h group (Figure 3).

Adherence to PEG-IFN and RBV treatment was higher in the q12h group, although the difference was not statistically significant (Additional file 1).

Table 2. Pharmacokinetic parameters of telaprevir at week 2

Pharmacokinetic parameter	TVR 750 mg q8h (n=10)	TVR 750 mg q12h (n=10)	P-value
C_{trough}, μg/ml	2.80 (1.33)	2.00 (0.59)	0.243
1 h, μg/ml	2.93 (1.35)	3.07 (0.81)	0.661
2.5 h, μg/ml	3.60 (1.66)	3.24 (1.22)	0.842
4 h, μg/ml	3.42 (1.40)	3.03 (1.02)	0.661
6 h, μg/ml	3.02 (1.41)	2.51 (0.97)	0.549
8 h, μg/ml	2.48 (1.37)	1.98 (0.77)	0.549
12 h, μg/ml	3.42 (1.47)	1.36 (0.70)	<0.001
C_{max}, μg/ml	3.90 (1.50)	3.74 (0.99)	0.720
$AUC_{24\ h}$, μg•h/ml[a]	74.91 (32.91)	57.16 (18.12)	0.243

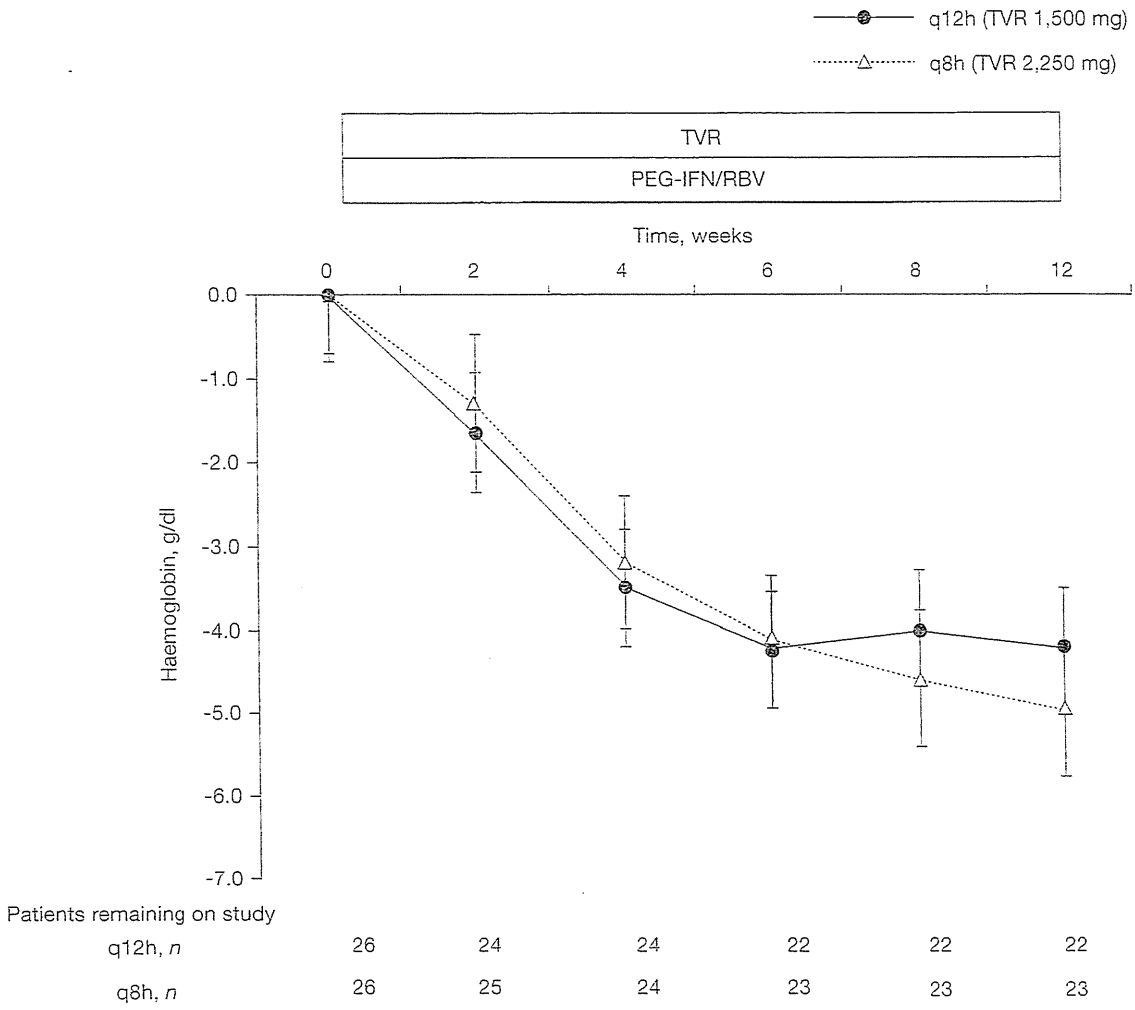
All values are expressed as mean (SD). [a]Area under the curve (AUC) at 24 h ($AUC_{24\ h}$) was calculated by multiplying $AUC_{8\ h}$ by 3 or $AUC_{12\ h}$ by 2. C_{max}, maximum plasma concentration; C_{trough}, trough plasma concentration; q8h, every 8 h; q12h, every 12 h; TVR, telaprevir.

Table 3. Adverse events occurring in >5% of participants

Adverse event	TVR 750 mg q8h (n=26)	TVR 750 mg q12h (n=26)	P-value	All (n=52)
White blood cell count decreased	26 (100)	26 (100)	1.00	52 (100)
Platelet count decreased	26 (100)	26 (100)	1.00	52 (100)
Anaemia	26 (100)	26 (100)	1.00	52 (100)
Blood creatinine increased (eGFR decreased)	21 (80.8)	12 (46.2)	0.02	33 (63.5)
Skin rash	11 (42.3)	13 (50)	0.59	24 (46.2)
Blood uric acid increased	10 (38.5)	6 (23.1)	0.37	16 (30.1)
Anorexia	4 (15.4)	2 (7.7)	0.67	6 (11.5)
General fatigue	3 (11.5)	1 (3.8)	0.61	4 (7.7)

Data are n (%). eGFR, estimated glomerular filtration rate; q8h, every 8 h; q12h, every 12 h; TVR, telaprevir.

Figure 2. Time course of haemoglobin levels during the triple therapy

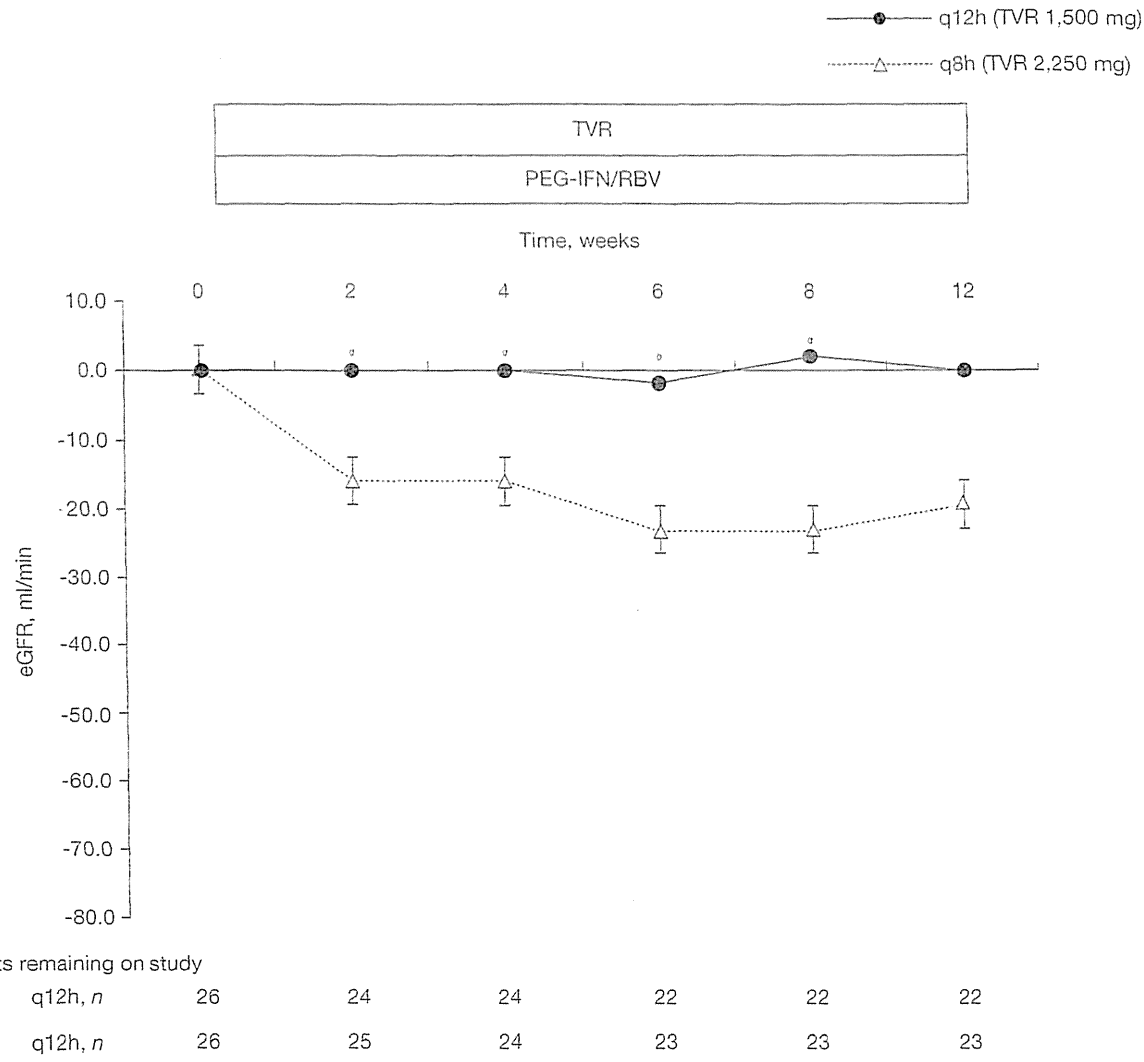


Change from baseline haemoglobin concentrations are noted as mean (sd). PEG-IFN, pegylated interferon; q8h, every 8 h; q12h, every 12 h; RBV, ribavirin; TVR, telaprevir.

Discussion

With the introduction of TVR, the eradication rate of HCV has improved significantly [5–7]. However, severe adverse effects associated with TVR have also been reported, some or which occur more frequently in Japanese patients [8,9]. The dose of TVR for use in triple therapy was determined based on a dose-finding study conducted in the US and Europe [15], which found that the q8h dosage regimen achieved the greatest reduction of HCV RNA. However, body weights of Japanese patients who were treated with TVR, PEG-IFN-α2b and RBV [9] were 61–63 kg compared to 79–91 kg among American and European patients who were treated with boceprevir, PEG-IFN-α2b and RBV combination therapy [16]. As the dose of TVR is the same among countries where triple therapy is approved, we considered the possibility that the dose of TVR might be too high for smaller Japanese patients and could be reduced. Suzuki *et al.* [17] previously reported that the antiviral effect of triple therapy was similar when patients were given TVR at 1,500 mg/day (q8h at 500 mg) compared with those given at 2,250 mg/day (q8h at 750 mg) in the Japanese patients, suggesting that reduction of TVR might be possible. However, the treatment period of their study was only 12 weeks, and the study was a non-randomized controlled study with a small number of patients. Therefore, we conducted a randomized controlled trial to confirm that the dose reduction is as effective as the approved regimen. Therefore, we also attempted to test if TVR is as effective when administered at 12 h intervals instead of 8 h intervals, based on a pharmacokinetics study in which Marcellin *et al.* [18] found no difference in viral response and safety profiles

Figure 3. Time course of eGFR levels from baseline during triple therapy



Statistically significant differences between patients treated with 1,500 mg versus 2,250 mg telprevir (TVR): $^{a}P<0.01$, $^{b}P<0.05$. eGFR, estimated glomerular filtration rate; PEG-IFN, pegylated interferon; q8h, every 8 h; q12h, every 12 h; RBV, ribavirin.

between patients treated with the triple therapy with TVR 2,250 mg (q12h) and TVR 2,250 mg (q8h). Furthermore, Buti *et al.* [19] reported that the effectiveness and safety were similar between patients treated with triple therapy with 2,250 mg TVR (q12h) and 2,250 mg TVR (q8h) in the OPTIMIZE trial (Phase IIIb).

We showed in this study that the effect of TVR given q12h at 750 mg with PEG-IFN-α2b and RBV is the same as TVR given q8h among Japanese chronic hepatitis C patients. However, four patients failed to achieve SVR_{12}, and all treatment was discontinued within 4 weeks in these patients. Safety profiles were similar except for differences in the frequency of anaemia and renal damage. Haemoglobin levels continued to decline only in patients who received the larger 2,250 mg dose, whereas haemoglobin levels plateaued by week 6 in patients who received the 1,500 mg dose. We also found that the 1,500 mg dosage was also accompanied with a lower frequency of renal damage (Figure 3). Incidence of TVR discontinuation was significantly less frequent in patients treated with the 1,500 mg regimen. These results suggest that reduction of TVR to 1,500 mg and administration of the drug q12h is as effective as the approved 2,250 mg dose and is less likely to result in premature termination of TVR therapy (Additional file 1).

We assessed the effect of reduced TVR only in patients who relapsed under previous PEG-IFN/RBV therapy or had the IL28B SNP rs8099917 TT genotype that is associated with a good response to IFN therapy. Patients who had relapsed during previous

PEG-IFN/RBV therapy have been reported to respond well to triple therapy [9]. The majority of patients with the rs8099917 TT genotype have also been reported to successfully eradicate the virus with triple therapy [20,21]. The effect of TVR reduction on patients who are expected to be difficult to treat should be further explored in a different trial.

Until recently it was unknown why SNPs near the IL28B locus, such as rs8099917 and rs12979860, are associated with the outcome of IFN therapy. However, the recent characterization of IFNL4 and its association with polymorphism ss469415590 (TT or ΔG) has shed light on this issue [22]. Genotype ss469415590 TT, which fails to express functional IFNL4, is associated with both eradication of HCV by PEG-IFN plus RBV combination therapy as well as spontaneous clearance of the virus [22]. As this polymorphism is in strong linkage disequilibrium with rs8099917 and rs12979860 in Asian populations [22], it is assumed that in the majority of patients the IL28B and IFNL4 ss469415590 genotypes are in complete linkage disequilibrium, and in fact, there was only one patient who had a discrepancy between ss469415590 and rs8099917 genotypes (Additional file 1). Taken together, patients with ss469415590 genotype TT/TT are expected to be successfully treated with the 1,500 mg regimen.

Our results were obtained from Japanese patients with body weights between 61 and 63 kg in each group of patients (Table 1). Results obtained here should be confirmed in patients with a larger body weight. Alternatively, administration of TVR based on body weight should be considered in order to maintain high eradication rates while reducing the risk of adverse effects. However, it should be noted that the limitations of the study are the relatively small patient numbers and enrolling two main groups including prior relapsers and treatment-naive patients with favourable INFL4 genotypes. A more comprehensive study is essential in the future.

Acknowledgements

We thank Hiroko Kawahara and Yuki Tanaka for excellent technical assistance, and Yuko Nagaoki, Michio Imamura, Mika Tsuzuno and Sanae Furuya for preparation of the paper. Part of this work was carried out at the Analysis Center of Life Science, Hiroshima University, Hiroshima, Japan.

This study was supported in part by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Labor and Health and Welfare.

Disclosure statement

The authors declare no competing interests.

Additional files

Additional file 1: Supplementary tables illustrating a comparison between IFNL3 (rs8099917) and IFNL4 (ss469415590) genotypes; SVR_{12} rates stratified by response to previous therapy, age, gender and platelet count; adverse events leading to discontinuation of all treatment or TVR only; and the rate of treatment completion without reduction or discontinuation can be found at http://www.intmedpress.com/uploads/documents/3050_Kawakami_Additional_File_1.pdf

Additional file 2: Supplementary figures displaying the study design; enrolment and outcomes; and the cumulative rate of undetectable HCV RNA in serum during treatment can be found at http://www.intmedpress.com/uploads/documents/3050_Kawakami_Additional_File_2.pdf

References

1. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* 2005; 5:558–567.
2. Perz JF, Armstrong GL, Farrington LA, *et al.* The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol* 2006; 45:529–538.
3. Hoofnagle JH. Course and outcome of hepatitis C. *Hepatology* 2002; 36 Suppl 1:S21–S29.
4. Seeff LB, Hoofnagle JH. National Institutes of Health consensus development conference: management of hepatitis C: 2002. *Clin Liver Dis* 2003; 7:261–287.
5. Jacobson IM, McHutchison JG, Dusheiko G, *et al.* TVR for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; 364:2405–2416.
6. Zeuzem S, Andreone P, Pol S, *et al.* TVR for retreatment of HCV infection. *N Engl J Med* 2011; 364:2417–2428.
7. Sherman KE, Flamm SL, Afdhal NH, *et al.* Response-guided TVR combination treatment for hepatitis C virus infection. *N Engl J Med* 2011; 365:1014–1024.
8. Kumada H, Toyota J, Okanoue T, *et al.* TVR with peginterferon and ribavirin for treatment-naive patients chronically infected with HCV of genotype 1 in Japan. *J Hepatol* 2012; 56:78–84.
9. Hayashi N, Okanoue T, Tsubouchi H, *et al.* Efficacy and safety of TVR, a new protease inhibitor, for difficult-to-treat patients with genotype 1 chronic hepatitis C. *J Viral Hepat* 2012; 19:e134–e142.
10. Ohnishi Y, Tanaka T, Ozaki K, *et al.* A high-throughput SNP typing system for genomewide association studies. *J Hum Genet* 2001; 46:471–477.
11. Suzuki A, Yamada R, Chang X, *et al.* Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylargininedeiminase 4, are associated with rheumatoid arthritis. *Nat Genet* 2003; 34:395–402.
12. Suzuki F, Suzuki Y, Akuta N, *et al.* Influence of ITPA polymorphism on decreases of hemoglobin during treatment with pegylated IFN, ribavirin and TVR. *Hepatology* 2011; 53:415–421.
13. Akuta N, Suzuki F, Sezaki H, *et al.* Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. *Intervirology* 2005; 48:372–380.

14. Akuta N, Suzuki F, Kawamura Y, *et al.* Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. *J Hepatol* 2007; 46:403–410.

15. Reesink HW, Zeuzem S, Weegink CJ, *et al.* Rapid decline of viral RNA in hepatitis C patients treated with VX-950: a phase Ib, placebo-controlled, randomized study. *Gastroenterology* 2006; 131:997–1002.

16. Poordad F, McCone J, Jr., Bacon BR, *et al.* Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011; 364:1195–1206.

17. Suzuki F, Suzuki Y, Sezaki H, *et al.* Exploratory study on TVR given every 8 h at 500 mg or 750 mg with peginterferon-alpha-2b and ribavirin in hepatitis C patients. *Hepatol Res* 2013; 43:691–701.

18. Marcellin P, Forns X, Goeser T, *et al.* TVR is effective given every 8 or 12 hours with ribavirin and peginterferon alfa-2a or 2b to patients with chronic hepatitis C. *Gastroenterology* 2011; 140:459–468.

19. Buti M, Agarwa K, Horsmans Y, *et al.* OPTIMIZE Trial: non-inferiority of twice daily telaprevir versus administration every 8 hours in treatment-naive, genotype 1 HCV-infected patients. *63rd American Association for the Study of Liver Diseases.* 9–13 November 2012, Boston, MA, USA. Abstract LB-8.

20. Chayama K, Hayes CN, Abe H, *et al.* IL28B but not ITPA polymorphism is predictive of response to pegylated interferon, ribavirin, and TVR triple therapy in patients with genotype 1 hepatitis C. *J Infect Dis* 2011; 204:84–93.

21. Akuta N, Suzuki F, Miharu M, *et al.* Amino acid substitution in hepatitis C virus core region and genetic variation near the interleukin 28B gene predict viral response to TVR with peginterferon and ribavirin. *Hepatology* 2010; 52:421–429.

22. Prokunina-Olsson L, Muchmore B, Tang W, *et al.* A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. *Nat Genet* 2013; 45:164–171.

Accepted 9 October 2013; published online 5 November 2013

Hepatology Research 2014; 44: 983–992 doi: 10.1111/hepr.12280

Original Article

Interleukin 28B polymorphism predicts interferon plus ribavirin treatment outcome in patients with hepatitis C virus-related liver cirrhosis: A multicenter retrospective study in Japan

Satoshi Shakado,[1,2] Shotaro Sakisaka,[1,2] Takeshi Okanoue,[3] Kazuaki Chayama,[4] Namiki Izumi,[5] Joji Toyoda,[6] Eiji Tanaka,[7] Akio Ido,[8] Tetsuo Takehara,[9] Kentaro Yoshioka,[10] Yoichi Hiasa,[11] Hideyuki Nomura,[12] Masataka Seike,[13] Yoshiyuki Ueno[14] and Hiromitsu Kumada[15]

[1]*Department of Gastrcenterology and Medicine,* [2]*Division of Advanced Clinical Research for Viral Hepatitis and Liver Cancer, Faculty of Medicine, Fukuoka University, Fukuoka,* [3]*Saiseikai Suita Hospital,* [9]*Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, Suita,* [4]*Department of Gastroenterology and Metabolism, Applied Life Sciences, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima,* [5]*Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Musashino,* [6]*Department of Gastroenterology and Hepatology, Sapporo Kousei Hospital, Sapporo,* [7]*Department of Medicine, Shinshu University School of Medicine, Matsumoto,* [8]*Department of Gastroenterology and Hepatology, Kagoshima University, Kagoshima,* [10]*Department of Liver, Biliary Tract and Pancreas Diseases, Fujita Health University, Toyoake,* [11]*Department of Gastroenterology and Metabology, Ehime University Graduate School of Medicine, Tcuon,* [12]*The Center for Liver Disease, Shin-Kokura Hospital, Kitakyushu,* [13]*Department of Gastroenterology, Faculty of Medicine, Oita University, Yufu,* [14]*Department of Gastroenterology, Yamagata University, Yamagata, and* [15]*Department of Hepatology, Toranomon Hospital, Kawasaki, Japan*

Aim: This study evaluated the efficacy of interferon plus ribavirin and examined whether interleukin 28B (IL28B) polymorphism influenced treatment outcome in Japanese patients with hepatitis C virus (HCV)-related liver cirrhosis (LC).

Methods: Fourteen collaborating centers provided details of 261 patients with HCV-related LC undergoing treatment with interferon plus ribavirin. Univariate and multivariate analyses were used to establish which factors predicted treatment outcome.

Results: Eighty-four patients (32.2%) achieved a sustained virological response (SVR). SVR rates were 21.6% (41/190) in patients with HCV genotype 1 with high viral load (G1H) and 60.6% (43/71) in patients with non-G1H. In patients with non-G1H, treatment outcome was effective irrespective of IL28B polymorphism. In those with G1H, SVR was achieved in 27.1% of patients with the IL28B rs8099917 TT allele compared with 8.8% of those with the TG/GG alleles ($P = 0.004$). In patients with G1H having TT allele, treatments longer than 48 weeks achieved significantly higher SVR rates than treatments less than 48 weeks (34.6% vs 16.4%, $P = 0.042$). In patients with G1H having TG/GG alleles, treatments longer than 72 weeks achieved significantly higher SVR rates than treatments less than 72 weeks (37.5% vs 4.1%, $P = 0.010$).

Conclusion: Interferon plus ribavirin treatment in Japanese patients with non-G1H HCV-related LC was more effective than those with G1H and not influenced by IL28B polymorphism. In those with G1H, IL28B polymorphism may predict SVR and guide treatment duration: SVR rates were higher in those with the TT allele treated for more than 48 weeks and those with the TG/GG alleles treated for more than 72 weeks.

Key words: cirrhosis, hepatitis C virus, interferon, interleukin 28B, ribavirin

Correspondence: Dr Satoshi Shakado, Department of Gastroenterology and Medicine, Faculty of Medicine, Fukuoka University, 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan. Email: shakado @cis.fukuoka-u.ac.jp
Received 29 August 2013; revision 15 November 2013; accepted 17 November 2013.

INTRODUCTION

CHRONIC HEPATITIS C virus (HCV) infection is a leading cause of liver cirrhosis worldwide.[1] Patients with HCV-related liver cirrhosis (LC) are at increased risk of hepatic decompensation and hepatocellular

carcinoma (HCC).[2–4] The therapeutic goal in these patients should be the prevention of liver-related mortality. A randomized trial conducted in Japan was the first to suggest that interferon (IFN) may reduce the risk of HCC in patients with HCV-related LC.[5] Recent studies have shown that patients with HCV-related LC who achieved a sustained virological response (SVR) with antiviral therapy had a significant reduction in liver-related mortality.[6,7] However, patients with HCV-related LC show a lower SVR rate than non-cirrhotic patients, as well as a reduced tolerance to the therapy.[8,9] A previous meta-analysis revealed that the overall SVR rate in patients with cirrhosis was 33.3%, and was significantly higher in patients with HCV genotypes 2 and 3 (55.4%) than in those with HCV genotypes 1 and 4 (21.7%).[10]

Genome-wide association studies have recently shown that single nucleotide polymorphisms (SNP) near the interleukin 28B (IL28B) region (rs8099917, rs12979860) are the most powerful predictors of SVR to pegylated (PEG) IFN plus ribavirin in patients with HCV genotype 1 infection.[11–13] However, it is not clear whether IL28B polymorphism can be used to predict the virological response to treatment of HCV-related LC. This study evaluated the efficacy of IFN plus ribavirin, and the association between IL28B polymorphism and the treatment efficacy in Japanese patients with HCV-related LC.

METHODS

THIS WAS A multicenter retrospective study of patients with HCV-related LC who had received treatment with IFN plus ribavirin in 14 hospitals in Japan.

Patient selection

Data were collected from 290 patients with HCV-related LC receiving treatment with IFN plus ribavirin in 14 academic and community hospitals. All patients had compensated HCV-related LC with clinical or histological data available. The diagnosis of cirrhosis met at least one of the following criteria: liver biopsy specimens with cirrhosis, diffuse formation of the nodules on the liver surface in peritoneoscopy, over 12.5 kPa in liver stiffness values on transient elastography, signs of portal hypertension on ultrasound scan (splenomegaly, portal vein enlargement, re-permeabilization of the umbilical vein, or presence of portal-systemic shunts), presence of esophageal varices on endoscopy or positive values using the following discriminant by Ikeda and colleagues: $z = 0.124 \times (\gamma\text{-globulin [\%]}) + 0.001 \times$ (hyaluronate) ($\mu g\ L^{-1}$) $- 0.075 \times$ (platelet count [$\times 10^4$ counts/mm^3]) $- 0.413 \times$ sex (male, 1; female, 2) $- 2.005$.[14–16] Principal investigators in 14 hospitals identified eligible patients and entered data in a predefined database.

Combination therapy

Of the 290 patients identified, 29 were not genotyped for IL28B SNP, thus the data of 261 patients were analyzed. A total of 190 patients were infected with HCV genotype 1 with high viral load (>100 KIU/mL) (G1H) (72.8%) and the remaining 71 (27.2%) were classified as non-G1H. Twenty-two patients were HCV genotype 1 with low viral load, 46 were genotype 2a or 2b, and three were of unknown genotype. Two hundred and twenty-four (85.8%) patients were treated with PEG IFN-α-2b (1.5–1.0 μg/kg bodyweight per week), 20 (7.7%) patients were treated with PEG IFN-α-2a (45–180 μg/week) and the remaining 17 (6.5%) patients were treated with IFN-α-2b or IFN-β. IFN-α-2b and IFN-β were administrated at a median dose of 6 million units each day (seven times per week for the initial 2 or 4 weeks, followed by three times per week thereafter). All patients also received oral ribavirin (600–1000 mg/day). Median treatment duration was 48 and 28 weeks in G1H and non-G1H, respectively. The individual attending physician determined the treatment regimes and their duration.

Virological response during therapy and definitions

The efficacy end-point was SVR, defined as undetectable serum HCV RNA 24 weeks after treatment. Relapse was defined as undetectable serum HCV RNA at the last treatment visit but detectable serum HCV RNA again at the last follow-up visit. Breakthrough was defined as reappearance of serum HCV RNA during treatment. A non-responder was defined as serum HCV RNA never undetectable during treatment. A rapid virological response (RVR) was defined as undetectable serum HCV RNA at treatment week 4, and a complete early virological response (cEVR) was defined as undetectable serum HCV RNA at treatment week 12. A late virological response (LVR) was defined as detectable serum HCV RNA at 12 weeks that became undetectable within 36 weeks of the start of treatment.

Determination of IL28B genotype

Interleukin 28B (rs8099917) was genotyped in each of the 14 hospitals by Invader assay, TaqMan assay or by direct sequencing, as previously described.[17,18]

Statistical analysis

Results were analyzed on the intention-to-treat principle. Mean differences were tested using Student's *t*-test. The difference in the frequency distribution was analyzed with Fisher's exact test. Univariate and multivariate logistic regression analyses were used to identify factors independently associated with SVR. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. The parameters that achieved statistical significance on univariate analysis were entered into multivariate logistic regression analysis to identify significant independent factors. Data were analyzed with JMP version 9.0 for Macintosh (SAS Institute, Cary, NC, USA). All statistical analyses were two sided, and $P < 0.05$ was considered significant.

RESULTS

OF THE 261 patients included in our analysis, 84 patients (32.2%) achieved SVR (Fig. 1). The rate of relapse and breakthrough was 24.9% and the non-responder rate was 33.3%. There were 25 patients (9.6%) who required early discontinuation of treatment because of adverse events. Baseline demographic and clinical features are summarized in Table 1. The age of the patients was 60.7 ± 8.9 years and 50.6% were male. Of the patients studied, 125 patients (47.9%) had been treated with IFN previously, and 75 (28.7%) had not responded to previous treatment. One hundred and six patients (40.6%) had been treated for HCC before. There were 85 patients with esophageal varices (32.6%). There were 190 patients with G1H and 133 (70%) of these had the TT allele at IL28B rs8099917. There were 71 patients in the non-G1H group, 51 (71.8%) of whom were found to have the TT allele at IL28B rs8099917.

Virological response rates in patients with G1H and non-G1H HCV-related LC

The SVR rates were 21.6% (41/190) in patients with G1H and 60.6% (43/71) in patients with non-G1H (Table 2). There were no statistically significant differences between the G1H and non-G1H groups with regard to dose reduction rates of IFN or ribavirin. Dose reduction of IFN was required in 51.3% of patients and dose reduction of ribavirin in 53.6% of patients. Treatment duration in patients in the G1H group was significantly longer than those in the non-G1H group ($P = 0.010$).

Association between IL28B rs8099917 genotype and treatment response

Sustained virological response was achieved in 37.0% of patients with the rs8099917 TT allele and 20.8% in those with the TG or GG allele. Virological responses, including SVR, relapse and breakthrough, in patients with the rs8099917 TT allele were significantly higher than in those with rs8099917 TG or GG allele ($P = 0.013$ and 0.012, respectively; Table 3). The proportion of non-responders among patients with the rs8099917 TG or GG allele was significantly higher than in those with the TT allele ($P = 0.002$). There was no

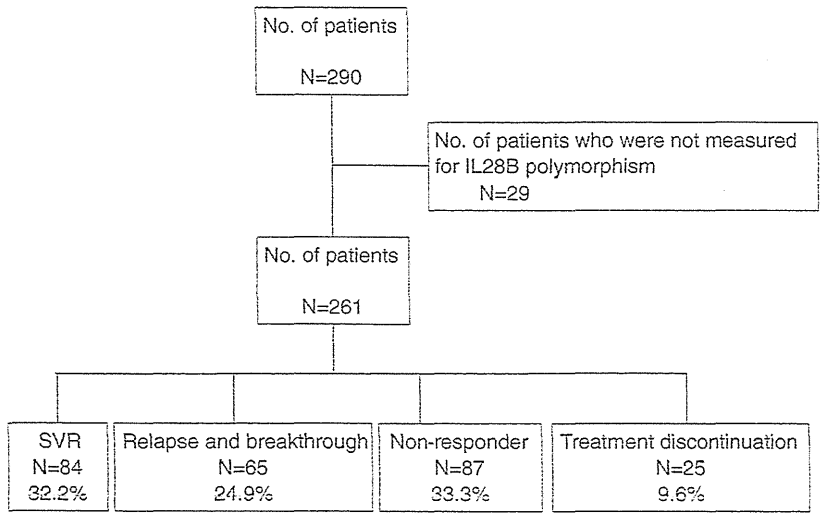


Figure 1 Flowchart showing the characteristics of the study cohort. IL28B, interleukin 28B; SVR, sustained virological response.

Table 1 Summary of demographic and baseline characteristics ($n = 261$)

	G1H, $n = 190$	Other than G1H, $n = 71$	All patients, $n = 261$
Sex (M : F)	95:95	37:34	132:129
Age (years)	60.5 ± 9.3	61.2 ± 7.8	60.7 ± 8.9
BMI (kg/m^2)	23.8 ± 3.5	23.4 ± 3.2	23.7 ± 3.4
IFN treatment history	91 (47.9%)	34 (47.9%)	125 (47.9%)
HCC treatment history	75 (39.5%)	31 (43.7%)	106 (40.6%)
Presence of EV	60 (31.6%)	25 (35.2%)	85 (32.6%)
Total bilirubin (mg/dl)	1.1 ± 0.9	1.1 ± 1.4	1.1 ± 1.2
AST (IU/L)	79.1 ± 44.2	75.8 ± 57.7	79.9 ± 52.7
ALT (IU/L)	82.4 ± 56.4	81.9 ± 75.4	83.3 ± 66.2
GGT (IU/L)	83.8 ± 107.8	87.0 ± 140.1	84.6 ± 115.8
Albumin (g/dL)	3.7 ± 0.5	3.8 ± 0.4	3.7 ± 0.5
Prothrombin (%)	86.2 ± 14.4	83.7 ± 16.7	85.5 ± 15.1
WBC (/μL)	4407 ± 1592	4190 ± 1930	4348 ± 1667
Hemoglobin (g/dL)	13.2 ± 1.8	13.1 ± 1.8	13.1 ± 1.8
Platelets (10^4/mm^3)	11.8 ± 6.7	11.8 ± 6.3	11.8 ± 6.6
AFP (ng/mL)	48.9 ± 224.7	24.0 ± 29.3	45.4 ± 193.9
DCP (mAU/mL)	66.8 ± 372.3	155.3 ± 620.4	92.4 ± 450.8
IL28B (TT : TG + GG)	133:57	51:20	184:77

All values are expressed as mean ± standard deviation.
AFP, α-fetoprotein; ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; DCP, des-γ-carboxy prothrombin; EV, esophageal varices; G1H, genotype 1 with high viral load; GGT, γ-glutamyltransferase; HCC, hepatocellular carcinoma; IFN, interferon; IL28B, interleukin 28B rs8099917 genotype; WBC, white blood cell.

significant association between the IL28B genotype and the incidence of adverse events.

Among patients in the G1H group, SVR was achieved in 27.1% (36/133) of those with the TT allele and 8.8% (5/57) of those with the TG or GG allele (Table 4). There was no statistically significant difference between IL28B genotype and viral response in patients with non-G1H.

Table 2 Summary of treatment and sustained virological response rates ($n = 261$)

	G1H, $n = 190$	Other than G1H, $n = 71$	All patients, $n = 261$
Dose reduction of IFN	$n = 98$ (51.6%)	$n = 36$ (50.7%)	$n = 134$ (51.3%)
Dose reduction of RBV	$n = 107$ (56.3%)	$n = 33$ (46.5%)	$n = 140$ (53.6%)
Treatment duration (weeks)			
Mean ± SD	45.3 ± 21.6	37.7 ± 19.6	43.2 ± 21.4
Median	48	28	48
SVR	$n = 41$ (21.6%)	$n = 43$ (60.6%)	$n = 84$ (32.2%)

G1H, genotype 1 with high viral load; IFN, interferon; RBV, ribavirin; SD, standard deviation; SVR, sustained virological response.

Table 3 Association between IL28B rs8099917 polymorphism and treatment response in 261 hepatitis C virus-related liver cirrhotic patients

IL28B	TT ($n = 184$)	TG + GG ($n = 77$)	*P*-value
SVR	68 (37.0%)	16 (20.8%)	0.013
Relapse and breakthrough	54 (29.3%)	11 (14.3%)	0.012
Non-responder	44 (23.9%)	43 (55.8%)	0.002
Discontinuation	18 (9.8%)	7 (9.1%)	1.000

IL28B, interleukin 28B rs8099917 genotype; SVR, sustained virological response.

Table 4 Sustained virological response associated between IL28B rs8099917 polymorphism and G1H in hepatitis C virus-related liver cirrhosis patients

IL28B	TT ($n = 184$)	TG + GG ($n = 77$)	*P*-value
G1H	36/133 (27.1%)	5/57 (8.8%)	0.004
Other than G1H	32/51 (62.7%)	11/20 (55.0%)	0.596

G1H, genotype 1 with high viral load; IL28B, interleukin 28B rs8099917 polymorphism.

Predictive factors associated with SVR

Differences in the characteristics of patients with SVR and those in whom SVR was not achieved are summarized in Table 5. Neither age, sex, alanine transaminase, aspartate aminotransferase, prothrombin activity, hemoglobin nor platelet counts appeared to significantly influence the chance of achieving SVR. The patients who achieved SVR had a lower body mass index, higher white blood cell count and higher serum albumin than those who did not, and were more likely to have non-G1H and the TT allele of IL28B rs8099917. Multivariate analysis identified that possession of the IL28B rs8099917 TT allele (OR = 2.85; 95% CI, 1.01–9.15; $P = 0.047$) and non-G1H (OR = 6.49; 95% CI, 1.77–26.43; $P = 0.005$) as significant determinants of SVR.

Treatment duration and efficacy in patients with G1H

Of the patients with G1H, 79 (41.6%) received less than 48 weeks of treatment. The number receiving 48–52 weeks, 53–72 weeks, over 72 weeks and unknown duration of treatment were 54 (28.4%), 41 (21.6%), 14 (7.4%) and two (1.1%), respectively. The median duration of treatment in patients who achieved RVR and cEVR was 48 weeks, but was significantly longer (66 weeks) in those with an LVR ($P < 0.001$). Table 6 shows the SVR rates of those with different IL28B genotypes and on-treatment viral response. The SVR rate in patients who achieved LVR was significantly lower than those who achieved RVR and cEVR ($P = 0.002$). Of the patients with G1H found to have the IL28B TG or GG genotype, none achieved RVR and only two achieved cEVR.

Predictors of SVR in patients with G1H and the TT allele

Patients with G1H and the TT allele who achieved SVR had higher platelet counts, higher serum albumin and had undergone over 48 weeks of treatment. Multivariate analysis identified platelet count (OR = 1.08; 95% CI, 1.01–1.18; $P = 0.047$), serum albumin (OR = 2.78; 95% CI, 1.14–7.42; $P = 0.031$) and over 48 weeks of treatment duration (OR = 2.53; 95% CI, 1.07–6.49; $P = 0.042$) as significant determinants of SVR (Table 7).

Predictors of SVR in patients with G1H and the TG or GG allele

Patients who had G1H and the TG or GG allele who achieved SVR had a higher total dose of ribavirin ($P = 0.011$) and more than 72 weeks of treatment duration ($P = 0.010$).

Treatment tolerability and adverse events

Table 8 illustrates details of the patients who experienced adverse events higher than grade 2. There were

Table 5 Factors associated with sustained virological response in hepatitis C virus-related liver cirrhosis patients

Factors	SVR (+), ($n = 84$)	SVR (−), ($n = 177$)	*P*-value	Multivariate analyses		
				Odds ratio	95% CI	*P*-value
BMI (kg/m^2)	22.9 ± 3.5	24.0 ± 3.3	0.019			
WBC (/μL)	4727 ± 2096	4168 ± 1376	0.013			
Albumin (g/dL)	3.83 ± 0.48	3.68 ± 0.46	0.018			
Other than G1H	$n = 43$ (51.2%)	$n = 28$ (15.8%)	<0.001	6.49	1.77–26.43	0.005
IL28B TT	$n = 68$ (81.0%)	$n = 116$ (65.5%)	0.012	2.85	1.01–9.15	0.047

P-values were obtained by logistic regression model.
BMI, body mass index; CI, confidence interval; G1H, genotype 1 with high viral load; IL28B, interleukin 28B rs8099917 polymorphism; SVR, sustained virological response; WBC, white blood cell.

Table 6 Sustained viral response rates between IL28B genotype and on-treatment viral response in the patients with G1H

	IL28B TT	IL28B TG/GG	All patients
RVR	7/7	0/0	7/7
	100%	0%	100%
cEVR	15/26	1/2	16/28
	57.7%	50%	57.1%
LVR	14/44	4/11	18/55
	31.8%	36.4%	32.7%

cEVR, complete early virclogical response (defined as serum HCV RNA negative at treatment week 12); G1H, genotype 1 with high viral load; HCV, hepatitis C virus; IL28B, interleukin 28B rs8099917; LVR, late virclogical response (defined as serum HCV RNA detectable at 12 weeks and undetectable at 36 weeks after the start of treatment); RVR, rapid virological response (defined as serum HCV RNA negative at treatment week 4).

two cases of liver decompensation, two cases of interstitial pneumonia, one case of cerebral hemorrhage and one case of cerebral infarction. The cause of death in two patients was decompensation of LC. In one patient, treatment was stopped after 4 weeks, and in another, treatment was stopped after 32 weeks because of hepatic failure. The IFN dose was reduced in 134 patients (51.3%), and the ribavirin dose was reduced in 140 patients (53.6%) and discontinued in 60 patients (23.0%). Among patients who had treatment discontinued, 27 patients (10.3%) had treatment withdrawn because of no virological response and 33 patients (12.6%) because of severe adverse events. In patients in whom treatment was discontinued, three patients had SVR and five had a relapse.

IL28B alleles predicting SVR in G1H group

The influence of IL28B rs8099917 genotype on SVR in G1H is shown in Figure 2. Overall, there were 84 patients (32.2%) who achieved SVR with IFN plus ribavirin in HCV-related LC. The SVR was 60.6% in those with non-G1H, and was not significantly influenced by IL28B rs8099917 genotype (the SVR in TT patients was 62.7% compared with 55.0% in TG or GG patients). In contrast, in patients with G1H, the SVR of patients with IL28B rs8099917 genotype TT was significantly higher than those with rs8099917 TG or GG (27.1% vs 8.8%, $P = 0.004$). In patients with G1H and IL28B TT, the SVR of those treated for over 48 weeks was significantly higher than those treated for less than 48 weeks (34.6% vs 16.4%, $P = 0.042$). In patients with G1H and IL28B TG/GG, the SVR of those treated for over 72 weeks was significantly higher than those treated for less than 72 weeks (37.5% vs 4.1%, $P = 0.010$).

Table 8 Adverse events higher than grade 2

	No. of patients (%)
Anemia	63 (24.1%)
Thrombocytopenia	31 (11.9%)
Leukopenia	19 (7.3%)
Rash and itching	17 (6.5%)
Fatigue and general malaise	15 (5.7%)
Gastrointestinal disorders	5 (1.9%)
Depression	5 (1.9%)
Development of hepatocellular carcinoma	3 (1.1%)
Respiratory disorders	3 (1.1%)
Liver decompensation	2 (0.8%)
Malignant neoplasm	2 (0.8%)
Interstitial pneumonia	2 (0.8%)
Cerebral hemorrhage	1 (0.4%)
Cerebral infarction	1 (0.4%)
Cholangitis	1 (0.4%)
Retinal hemorrhage	1 (0.4%)
Diabetes decompensation	1 (0.4%)
Palpitation	1 (0.4%)

DISCUSSION

WE FOUND THAT in Japanese patients with G1H HCV-related LC, the likelihood of achieving SVR with IFN plus ribavirin combination therapy was influ-

Table 7 Factors associated with sustained virological response in the patients with G1H and TT allele of IL28B rs8099917 ($n = 133$)

Factors	SVR (+) ($n = 36$)	SVR (−) ($n = 97$)	*P*-value	Multivariate analyses		
				Odds ratio	95% CI	*P*-value
Platelets (10^4/mm^3)	14.5 ± 11.5	10.6 ± 4.2	0.024	1.08	1.01–1.18	0.047
Albumin (g/dL)	3.92 ± 0.50	3.69 ± 0.46	0.018	2.78	1.14–7.42	0.031
Treatment duration, over 48 weeks	$n = 27$ (75%)	$n = 51$ (52.6%)	0.023	2.53	1.07–6.49	0.042

P-values were obtained by logistic regression model.
CI, confidence interval; G1H, genotype 1 with high viral load; IL28B, interleukin 28B; SVR, sustained virological response.