Table 3 Risk factors for HCC incidence in patients with sustained virological response

Author (reference)	SVR patients	Observation period (years)	Risk factor	Hazard ratio or development rate of HCC per 100 person-years
Yoshida *et al.* 1999[3]	789	4.3	Liver fibrosis, F0–1/F2/F3/F4	0.11/0.10/1.29/0.49‡
Makiyama *et al.* 2004[34]	1197	5.9	Male	5.9
			Age, ≥50 years	7.4
			Liver fibrosis, ≥F3	2.3
Ikeda *et al.* 2005[29]	1056	4.7†	Age, ≥60 years	3.1
			AST, >100	3.1
			Platelets, $<15 \times 10^4/\mu L$	2.8
Tokita *et al.* 2005[30]	126	5.5	Age, ≥65 years; alcohol, ≥27 g/day; liver fibrosis, ≥F3	
Tanaka *et al.* 2007[35]	266	9.9	Age, ≥55 years; liver fibrosis, ≥F3; liver steatosis, ≥2	
Akuta *et al.* 2011[36]	1273	1.1†	HCV genotype 1b with Gln70 or His70	10.5
			Liver fibrosis, ≥F3	9.0
			Age, ≥55 years	3.1
Hung *et al.* 2011[18]	1027	4.3†	DM	
Arase *et al.* 2013[20]	1900	8.1	Liver fibrosis, CH/LC	0.16/1.82‡
			CH, age, 10 years	2.6
			Male	3.4
			Alcohol	2.7
			Type 2 DM	4.8
			LC, alcohol	3.8
Asahina *et al.* 2013[31]	913	6.1	Post-IFN ALT, ≥40, post-IFN AFP, ≥10	
Oze *et al.* 2014[17]	1425	3.3	Age, ≥65 years.	5.8
			Post-IFN AFP, ≥5	8.1

†Median.
‡Development rate of HCC per 100 person-years.
AFP, alpha-fetoprotein; CH, chronic hepatitis; DM, diabetes mellitus; Gln, Glutamine; HCC, hepatocellular carcinoma; His, Histidine; LC, liver cirrhosis; SVR, sustained virological response.

Sato *et al.*[41] conducted a retrospective, multicenter study of 130 patients who developed cancer after achieving SVR and reported that the proportions of patients who developed cancer were 50% at less than 5 years, 29% at 5–10 years, 9% at 10–15 years and 3% at 15 years or more. Nagaoki *et al.*[40] conducted a retrospective, single-institution study of 41 patients who developed HCC after achieving SVR and reported that the proportions of patients who developed HCC were 49% at less than 5 years, 22% at 5–10 years, 22% at 10–15 years and 7% at 15 years or more. In both studies, HCC development within 5 years after the end of antiviral therapy accounted for approximately half of the patients.

In multivariate analyses of risk factors for HCC development within 5 years after the end of treatment, significant factors reported were low albumin levels at baseline (<3.9 g/dL; HR, 2.6) and high AFP levels at baseline (≥10 ng/mL; HR, 2.8).[41] Another study showed that older age at the end of antiviral therapy (≥55 years; HR, 26.6) and excessive alcohol drinking (80 g/day for ≥5 years; HR, 8.9) were significant factors.[40] Furthermore, Toyoda *et al.* reported that more than 50% of the patients who developed cancer had HCC of more than 20 mm in diameter, and 30% or more of the patients had multiple HCC because more patients in the SVR group dropped out of the periodical follow up after the end of treatment than those of the non-SVR group,[37] which has been identified as an issue. Given the above, long-term HCC surveillance should be conducted even after SVR to antiviral therapy.

CONCLUSION

ALTHOUGH PATIENTS WITH hepatitis C who achieve SVR to antiviral treatment show a suppressive effect for HCC, some do develop cancer after achieving SVR. Antiviral treatment for hepatitis C has changed from IFN monotherapy to PEG IFN plus RBV

Table 4 HCC pattern in patients with sustained virological response

Author (reference)	SVR patients	HCC patients	Age, median (range)	Male/female	HCV genotype 1/2	Liver fibrosis	IFN treatment	Time to HCC diagnosis (years)	HCC size ≤20/>20 mm	HCC number solitary/multiple
Toyoda *et al.* 2000[37]	363	8	61 (49–65)	6/2	2/6	F1/2/3: 3/2/3	IFN	2.7 (1.6–7.1)	2/6	5/3
Tokita *et al.* 2005[30]	126	5	71 (53–74)	5/0	1/4	F2/3: 1/4	IFN	4.3 (2.1–8.3)	4/1	3/2
Ikeda *et al.* 2006[38]	2515	38	64 (38–77)	34/4	6/12	F0/1/2/3/4: 0/9/10/10/6	IFN	4.7 (1.4–9.0)	21/16†	31/7
Tanaka *et al.* 2007[35]	266	7	68 (59–78)	6/1	4/2	F1/2/3/4: 1/2/3/1	IFN	5.4 (2.9–9.6)		
Hirakawa *et al.* 2008[39]	1193	23	62 (51–76)	21/2	9/14	F1/2/3/4: 2/12/0/9	IFN, PEG IFN; PEG IFN/RBV	3.1 (0.1–12.9)	12/9	18/5
Ogawa *et al.* 2013[14]	557	13	65 (50–77)	6/7	7/6	CH, 6/LC, 7	PEG IFN/RBV	1.5 (0.5–3.8)		
Nagaoki *et al.* 2011[40]	–	41	67 (54–87)	35/6		F1/2/3/4: 3/17/7/6	IFN	<5/5–10/10–15/≥15: 20/9/9/3	17/24	21/20
Sato *et al.* 2013[41]	–	130	<60/≥60=38/92	107/23		CH, 83/LC, 46	IFN	<1/1–5/5–10/10–15/≥15: 26/50/38/12/4		

†≤30 mm/>30 mm.
CH, chronic hepatitis; HCC, hepatocellular carcinoma; IFN, interferon; LC, liver cirrhosis; PEG IFN, pegylated interferon; RBV, ribavirin; SVR, sustained virological response.

combination therapy or PEG IFN, RBV plus protease inhibitor therapy, which has contributed to increased SVR rates. With the advance of antiviral therapy, more SVR patients with chronic HCV infection have been at increased risk of developing HCC, such as elderly patients and patients with advanced liver fibrosis. In particular, the AFP level at 24 weeks after SVR is important; careful monitoring for the development of liver cancer is needed for patients with AFP levels of 5 ng/mL or more. Counseling on daily lifestyle habits, such as improvement of glucose metabolism disorders, lipid metabolism disorders and restrictions on alcohol intake, is also important to suppress HCC incidence.

Antiviral therapy with only HCV-specific direct-acting antivirals (DAA) is expected to be utilized in the future. However, it is not known whether DAA-based treatment can suppress HCC to the level of IFN-based treatment in patients who have achieved SVR to the respective treatment. Further research is required to clarify this.

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REFERENCES

1 Ikeda K, Saitoh S, Arase Y *et al.* Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. *Hepatology* 1999; 29: 1124–30.
2 Kasahara A, Hayashi N, Mochizuki K *et al.* Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. *Hepatology* 1998; 27: 1394–402.
3 Yoshida H, Shiratori Y, Moriyama M *et al.* Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. *Ann Intern Med* 1999; 131: 174–81.
4 Imai Y, Kawata S, Tamura S *et al.* Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Osaka Hepatocellular Carcinoma Prevention Study Group. *Ann Intern Med* 1998; 129: 94–9.
5 Okanoue T, Itoh Y, Minami M *et al.* Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in an advanced stage: a retrospective study in 1148 patients. Viral Hepatitis Therapy Study Group. *J Hepatol* 1999; 30: 653–9.

6 Serfaty L, Aumaitre H, Chazouilleres O *et al.* Determinants of outcome of compensated hepatitis C virus-related cirrhosis. *Hepatology* 1998; 27: 1435–40.
7 Shiratori Y, Ito Y, Yokosuka O *et al.* Antiviral therapy for cirrhotic hepatitis C: association with reduced hepatocellular carcinoma development and improved survival. *Ann Intern Med* 2005; 142: 105–14.
8 Gramenzi A, Andreone P, Fiorino S *et al.* Impact of interferon therapy on the natural history of hepatitis C virus related cirrhosis. *Gut* 2001; 48: 843–8.
9 Bruno S, Stroffolini T, Colombo M *et al.* Sustained virological response to interferon-alpha is associated with improved outcome in HCV-related cirrhosis: a retrospective study. *Hepatology* 2007; 45: 579–87.
10 Nishiguchi S, Shiomi S, Nakatani S *et al.* Prevention of hepatocellular carcinoma in patients with chronic active hepatitis C and cirrhosis. *Lancet* 2001; 357: 196–7.
11 Bruno S, Silini E, Crosignani A *et al.* Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: a prospective study. *Hepatology* 1997; 25: 754–8.
12 Hamada H, Yatsuhashi H, Yano K *et al.* Impact of aging on the development of hepatocellular carcinoma in patients with posttransfusion chronic hepatitis C. *Cancer* 2002; 95: 331–9.
13 Kurokawa M, Hiramatsu N, Oze T *et al.* Effect of interferon alpha-2b plus ribavirin therapy on incidence of hepatocellular carcinoma in patients with chronic hepatitis. *Hepatol Res* 2009; 39: 432–8.
14 Ogawa E, Furusyo N, Kajiwara E *et al.* Efficacy of pegylated interferon alpha-2b and ribavirin treatment on the risk of hepatocellular carcinoma in patients with chronic hepatitis C: a prospective, multicenter study. *J Hepatol* 2013; 58: 495–501.
15 Watanabe S, Enomoto N, Koike K *et al.* Cancer preventive effect of pegylated interferon alpha-2b plus ribavirin in a real-life clinical setting in Japan: PERFECT interim analysis. *Hepatol Res* 2011; 41: 955–64.
16 Cardoso AC, Moucari R, Figueiredo-Mendes C *et al.* Impact of peginterferon and ribavirin therapy on hepatocellular carcinoma: incidence and survival in hepatitis C patients with advanced fibrosis. *J Hepatol* 2010; 52: 652–7.
17 Oze T, Hiramatsu N, Yakushijin T *et al.* Post-treatment levels of alpha-fetoprotein predict incidence of hepatocellular carcinoma after interferon therapy. *Clin Gastroenterol Hepatol* 2014; 12: 1186–95.
18 Hung CH, Lee CM, Wang JH *et al.* Impact of diabetes mellitus on incidence of hepatocellular carcinoma in chronic hepatitis C patients treated with interferon-based antiviral therapy. *Int J Cancer* 2011; 128: 2344–52.
19 Tateyama M, Yatsuhashi H, Taura N *et al.* Alpha-fetoprotein above normal levels as a risk factor for the development of hepatocellular carcinoma in patients infected with hepatitis C virus. *J Gastroenterol* 2011; 46: 92–100.
20 Arase Y, Kobayashi M, Suzuki F *et al.* Effect of type 2 diabetes on risk for malignancies includes hepatocellular carcinoma in chronic hepatitis C. *Hepatology* 2013; 57: 964–73.
21 Kawamura Y, Arase Y, Ikeda K *et al.* Diabetes enhances hepatocarcinogenesis in noncirrhotic, interferon-treated hepatitis C patients. *Am J Med* 2010; 123: 951–6 e1.
22 Asahina Y, Tsuchiya K, Tamaki N *et al.* Effect of aging on risk for hepatocellular carcinoma in chronic hepatitis C virus infection. *Hepatology* 2010; 52: 518–27.
23 Ikeda K, Arase Y, Saitoh S *et al.* Interferon beta prevents recurrence of hepatocellular carcinoma after complete resection or ablation of the primary tumor-A prospective randomized study of hepatitis C virus-related liver cancer. *Hepatology* 2000; 32: 228–32.
24 Miyake Y, Takaki A, Iwasaki Y, Yamamoto K. Meta-analysis: interferon-alpha prevents the recurrence after curative treatment of hepatitis C virus-related hepatocellular carcinoma. *J Viral Hepat* 2010; 17: 287–92.
25 Shen YC, Hsu C, Chen LT, Cheng CC, Hu FC, Cheng AL. Adjuvant interferon therapy after curative therapy for hepatocellular carcinoma (HCC): a meta-regression approach. *J Hepatol* 2010; 52: 889–94.
26 Tanimoto Y, Tashiro H, Aikata H *et al.* Impact of pegylated interferon therapy on outcomes of patients with hepatitis C virus-related hepatocellular carcinoma after curative hepatic resection. *Ann Surg Oncol* 2012; 19: 418–25.
27 Hagihara H, Nouso K, Kobayashi Y *et al.* Effect of pegylated interferon therapy on intrahepatic recurrence after curative treatment of hepatitis C virus-related hepatocellular carcinoma. *Int J Clin Oncol* 2011; 16: 210–20.
28 Hsu YC, Ho HJ, Wu MS, Lin JT, Wu CY. Postoperative peg-interferon plus ribavirin is associated with reduced recurrence of hepatitis C virus-related hepatocellular carcinoma. *Hepatology* 2013; 58: 150–7.
29 Ikeda M, Fujiyama S, Tanaka M *et al.* Risk factors for development of hepatocellular carcinoma in patients with chronic hepatitis C after sustained response to interferon. *J Gastroenterol* 2005; 40: 148–56.
30 Tokita H, Fukui H, Tanaka A *et al.* Risk factors for the development of hepatocellular carcinoma among patients with chronic hepatitis C who achieved a sustained virological response to interferon therapy. *J Gastroenterol Hepatol* 2005; 20: 752–8.
31 Asahina Y, Tsuchiya K, Nishimura T *et al.* alpha-fetoprotein levels after interferon therapy and risk of hepatocarcinogenesis in chronic hepatitis C. *Hepatology* 2013; 58: 1253–62.
32 Aleman S, Rahbin N, Weiland O *et al.* A risk for hepatocellular carcinoma persists long-term after sustained virologic response in patients with hepatitis C-associated liver cirrhosis. *Clin Infect Dis* 2013; 57: 230–6.
33 Morgan TR, Ghany MG, Kim HY *et al.* HALT-C Trial Group. Outcome of sustained virological responders with histologically advanced chronic hepatitis C. *Hepatology* 2010; 52: 833–44.

34 Makiyama A, Itoh Y, Kasahara A *et al.* Characteristics of patients with chronic hepatitis C who develop hepatocellular carcinoma after a sustained response to interferon therapy. *Cancer* 2004; 101: 1616–22.
35 Tanaka A, Uegaki S, Kurihara H *et al.* Hepatic steatosis as a possible risk factor for the development of hepatocellular carcinoma after eradication of hepatitis C virus with antiviral therapy in patients with chronic hepatitis C. *World J Gastroenterol* 2007; 13: 5180–7.
36 Akuta N, Suzuki F, Hirakawa M *et al.* Amino acid substitutions in hepatitis C virus core region predict hepatocarcinogenesis following eradication of HCV RNA by antiviral therapy. *J Med Virol* 2011; 83: 1016–22.
37 Toyoda H, Kumada T, Tokuda A *et al.* Long-term follow-up of sustained responders to interferon therapy, in patients with chronic hepatitis C. *J Viral Hepat* 2000; 7: 414–9.
38 Ikeda M, Fujiyama S, Tanaka M *et al.* Clinical features of hepatocellular carcinoma that occur after sustained virological response to interferon for chronic hepatitis C. *J Gastroenterol Hepatol* 2006; 21: 122–8.
39 Hirakawa M, Ikeda K, Arase Y *et al.* Hepatocarcinogenesis following HCV RNA eradication by interferon in chronic hepatitis patients. *Intern Med* 2008; 47: 1637–43.
40 Nagaoki Y, Aikata H, Miyaki D *et al.* Clinical features and prognosis in patients with hepatocellular carcinoma that developed after hepatitis C virus eradication with interferon therapy. *J Gastroenterol* 2011; 46: 799–808.
41 Sato A, Sata M, Ikeda K *et al.* Clinical characteristics of patients who developed hepatocellular carcinoma after hepatitis C virus eradication with interferon therapy: current status in Japan. *Intern Med* 2013; 52: 2701–6.

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Original Article

Renal impairment during the treatment of telaprevir with peginterferon and ribavirin in patients with chronic hepatitis C

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Aim: Renal damage has been reported as an important complication during combination treatment of peginterferon (PEG IFN), ribavirin (RBV) and telaprevir (TVR) for chronic hepatitis C. However, very little is known about this complication. We investigated the role TVR plays in renal damage during this triple therapy.

Methods: Twenty-five chronic hepatitis C patients with genotype 1 and high viral load received TVR in combination with PEG IFN and RBV for 12 weeks followed by treatment with PEG IFN and RBV. Renal function of these patients was prospectively evaluated for 16 weeks.

Results: Creatinine clearance decreased significantly during PEG IFN/RBV/TVR treatment. Consequently, serum creatinine and cystatin C significantly rose during PEG IFN/RBV/TVR treatment. Serum creatinine returned to pretreatment levels after the termination of TVR. The increase of serum creatinine and cystatin C from baseline significantly correlated with serum TVR level at day 7, which was determined by starting dose of TVR per bodyweight . When the patients were classified according to the starting dose of TVR per bodyweight, renal impairment was observed only in the high-dose (TVR ≥33 mg/kg per day) group, not in the low-dose (TVR <33 mg/kg per day) group.

Conclusion: These results suggest that TVR dose per bodyweight is important for the occurrence of renal impairment in PEG IFN/RBV/TVR treatment.

Key words: chronic hepatitis C, peginterferon, renal impairment, ribavirin, telaprevir

INTRODUCTION

HEPATITIS C VIRUS (HCV) infection affects approximately 170 million people worldwide and 1.5 million people in Japan. Approximately 30% of individuals infected with HCV progress to decompensated cirrhosis or hepatocellular carcinoma, resulting in a fatal outcome.[1,2] It is a significant public health challenge to diagnose and appropriately treat individuals with the HCV infection to improve the prognosis.[3,4]

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Telaprevir (TVR), a potent non-structural protein 3/4A protease inhibitor, has recently been approved for the treatment of persons infected with genotype 1 HCV in the USA, EU and Japan. The worldwide studies demonstrate that patients who received TVR in combination with peginterferon (PEG IFN)-α-2b and ribavirin (RBV) achieved significantly higher rates of sustained virological response than those who received PEG IFN and RBV.[5–8]

Several adverse events are known to become an obstacle to continuity of PEG IFN/RBV/TVR treatment.[9–11] Anemia and skin disorders are frequent adverse events, which were observed in 90.8% and 82.3%, respectively, in studies in Japan.[9] Recently, alteration of renal function, such as an increase of serum

creatinine and uric acid, during PEG IFN/RBV/TVR treatment has been increasingly reported. Serious adverse events such as acute renal failure have also been reported. Renal impairment often forces reduction of drug dose, leading to diminished treatment effect.

Recently, Suzuki *et al.* reported that the treatment regimen of 1500 mg/day TVR reduced the increase of serum creatinine level during the treatment compared with a dose of 2250 mg/day TVR.[12] They suggested that several treatment-related adverse events could be avoided by dose adjustment of TVR. Here, we investigated the relation between alteration of renal function and serum TVR level. We show that adjustment of TVR starting dose according to bodyweight would avoid renal damage.

METHODS

Patients and study design

TWENTY-FIVE PATIENTS WITH chronic hepatitis C were included in this prospective study. The inclusion criteria of the study were as follows: (i) infection by genotype 1b; (ii) serum HCV RNA levels of 5.0 $\log_{10}$ IU/mL or higher determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan); (iii) more than 20 years old at the time of entry; and (iv) completion of 4 weeks of therapy. The exclusion criteria in this study were as follows: (i) co-infection with hepatitis B virus or HIV; (ii) co-existing chronic liver diseases such as autoimmune hepatitis and primary biliary cirrhosis; (iii) presence of cirrhosis; (iv) impaired renal function (creatinine clearance <50 mL/min); (v) serum hemoglobin level less than 12 g/dL; and (vi) platelet count lower than $10 \times 10^3/\mu L$.

All 25 patients enrolled in this study participated in either of the two clinical trials conducted by the Osaka Liver Forum from March 2012 to November 2012. One was a randomized, double-arm, open-label study comparing the efficacy of TVR at starting doses of 2250 mg/day and 1500 mg/day. The other study was a single-arm, open-label study evaluating the efficacy of TVR at a starting dose of 2250 mg/day. In both studies, patients received PEG IFN/RBV/TVR therapy for 12 weeks followed by PEG IFN/RBV therapy for 12 weeks. The protocols were approved by the institutional review board and written informed consent was obtained from each patient. Patients who withdrew from the clinical trials within 4 weeks were not included in this study. As a result, TVR was administrated at a dose of 2250 mg/day (750 mg every 8 h after food) in 14 patients and 1500 mg/day (500 mg every 8 h after food) in 11 patients. Patients received PEG IFN-α-2b (Pegintron; MSD, Tokyo, Japan) at a starting dose of 1.5 μg/kg s.c. every week. RBV (Rebetol; MSD) was given at a starting dose of 600–1000 mg/day based on bodyweight (<60 kg, 600 mg; 60–80 kg, 800 mg; >80 kg, 100 mg), according to a standard treatment protocol for Japanese patients.

Dose modification followed the manufacturer's drug information on the intensity of the hematological adverse effects. The PEG IFN-α-2b doses were reduced to 50% of the assigned dose when the neutrophil count fell below 750/μL or the platelet count fell below 8×10^4/μL, and the agent was discontinued when the neutrophil count fell below 500/mm^3 or the platelet count fell below $5 \times 10^4/\mu L$. The initial dose of RBV was reduced by 200 mg/day in the case of a hemoglobin level of less than 13 g/dL at baseline. The RBV dose was reduced by 200 mg/day in patients receiving 600 or 800 mg/day (by 400 mg/day in those receiving 1000 mg) when the hemoglobin level was less than 12 g/dL and was reduced by an additional 200 mg/day when the hemoglobin level was less than 10 g/dL. The RBV dose was also reduced by 200 mg/day if the hemoglobin level dropped by 1 g/dL or more within 1 week, TVR was withdrawn when the hemoglobin level was less than 8.5 g/dL. PEG IFN/RBV were withdrawn or interrupted when the hemoglobin level was less than 8.5 g/dL. Dose modification and interruption of TVR were allowed if serious adverse events appeared. The use of erythropoietin was not allowed.

Renal function was evaluated for 4 weeks in 25 patients. Their adherence to TVR for the first week was 100%, and their degree of adherence to TVR for the full 4 weeks ranged 71–100%. Twenty of them received TVR for more than 10 weeks and their renal function was followed for 16 weeks.

Baseline characteristics of the patients are summarized in Table 1.

Measurements of TVR

Serum concentrations of TVR were determined by using a high-performance liquid chromatographic apparatus fitted with a mass spectrometer.[10]

Single nucleotide polymorphism genotyping

Interleukin (IL)-28B (rs8099917) and inosine triphosphate pyrophosphatase (rs1127354) were determined as reported previously.[15–17]

Table 1 Baseline characteristics of the patients treated with the pegylated interferon/ribavirin/telaprevir triple therapy

	$n = 25$
Sex (male/female)	13/12
Age (years)	66 (31–71)†
Bodyweight (kg)	57.0 (40.5–82.5)†
Hypertension (presence/absence)‡	6/19
Diabetes mellitus (presence/absence)‡	4/21
IL-28B; rs8099917 (TT/TG/GG)	20/5/0
ITPA; rs1127354 (CC/CA/AA)	23/1/0§
ISDR substituted animo acid sites (0–1/≥2)	18/4§
Core amino acid 70 (wild/mutant)	19/6
Core amino acid 91 (wild/mutant)	16/9
History of interferon therapy (naïve/relapse/non-responder)	5/16/4
Hepatitis C virus RNA ($\log_{10}$IU/mL)	6.8 (5.1–7.6)†
Alanine aminotransferase (IU/L)	36 (10–150)†
Creatinine (mg/dL)	0.77 (0.47–1.17)†
Cystatin C	1.02 (0.78–1.50)†
Creatinine clearance (mL/min per 1.73 m^2)	86 (58–128)†
Hemoglobin (g/dL)	14.4 (12.1–17.0)†
White blood cell (/μL)	4530 (2310–7330)†
Platelet ($\times 10^4$/μL) (median [range])	15.5 (10.1–31.1)†

†Median (range).
‡The diagnoses of hypertension and diabetes mellitus were made by the reported criteria.[13,14]
§Inosine triphosphate pyrophosphatase (ITPA) and interferon sensitivity-determining region (ISDR) substituted animo acid sites were not determined in one and three patients, respectively.

NS5A IFN-sensitivity determining region and core amino acid

Amino acid substitutions in the NS5A and HCV core were determined as described previously.[18,19]

Measurements of creatinine clearance

Creatinine clearance was calculated using the formula as follows:[20]

Creatinine clearance (mL/min) =
(urinary creatinine (mg/dL) × urine volume [mL])/
(serum creatinine [mg/dL] × min) ×
(1.73/body surface area).

Statistical analysis

SPSS version 17.0 software (SPSS, Chicago, IL, USA) was used for statistical analysis. Comparisons between the two groups were performed using Student's *t*-test or χ^2-test as appropriate. Repeated measures ANOVA was used for comparisons between pre- and post-treatment variables. Correlations were determined by using Pearson's linear regression analysis. All *P*-values were two-sided and a *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Alteration of renal function during treatment

CREATININE CLEARANCE SIGNIFICANTLY decreased at day 7 compared with the pretreatment level (69.9 ± 17.6 vs 84.8 ± 21.2 mL/min per 1.73 m^2, $P < 0.001$), and persisted at reduced level during PEG IFN/RBV/TVR treatment (Fig. 1a). Consequently, serum creatinine and cystatin C levels significantly rose at day 7 compared with the pretreatment level (creatinine, 0.90 ± 0.21 vs 0.78 ± 0.19 mg/dL, $P < 0.001$; and cystatin C, 1.21 ± 0.25 vs 1.03 ± 0.15 mg/dL, $P < 0.001$), and persisted at elevated level during PEG IFN/RBV/TVR treatment (Fig. 1b,c). Serum creatinine returned to pretreatment level after the termination of TVR administration (Fig. 2).

Relation between renal function and TVR

At day 7, increases of serum creatinine and cystatin C from baseline showed significant correlation with serum TVR concentration ($r = 0.543$, $P = 0.005$, and $r = 0.585$, $P = 0.003$, respectively) (Fig. 3). Serum TVR level at day 7 strongly correlated with the starting dose of TVR per bodyweight ($r = 0.775$, $P < 0.001$) (Fig. 4). At day 7, increases of serum creatinine and cystatin C showed significant correlation with the starting dose of TVR per bodyweight ($r = 0.741$, $P < 0.001$, and $r = 0.715$, $P < 0.001$, respectively) (Fig. 5).

Effect of TVR dose per bodyweight on renal function

All the patients were classified into two groups according to the median starting dose of TVR per bodyweight which was 33 mg/kg. Clinical profiles of the high-dose group ($n = 15$, TVR ≥33 mg/kg per day) and low-dose group ($n = 10$, TVR <33 mg/kg per day) did not differ significantly. Serum creatinine in the high-dose group significantly rose during PEG IFN/RBV/TVR treatment, whereas those in the low-dose group did not show significant change (Fig. 6). The rates of rapid virological response in both groups were similar (100% in the

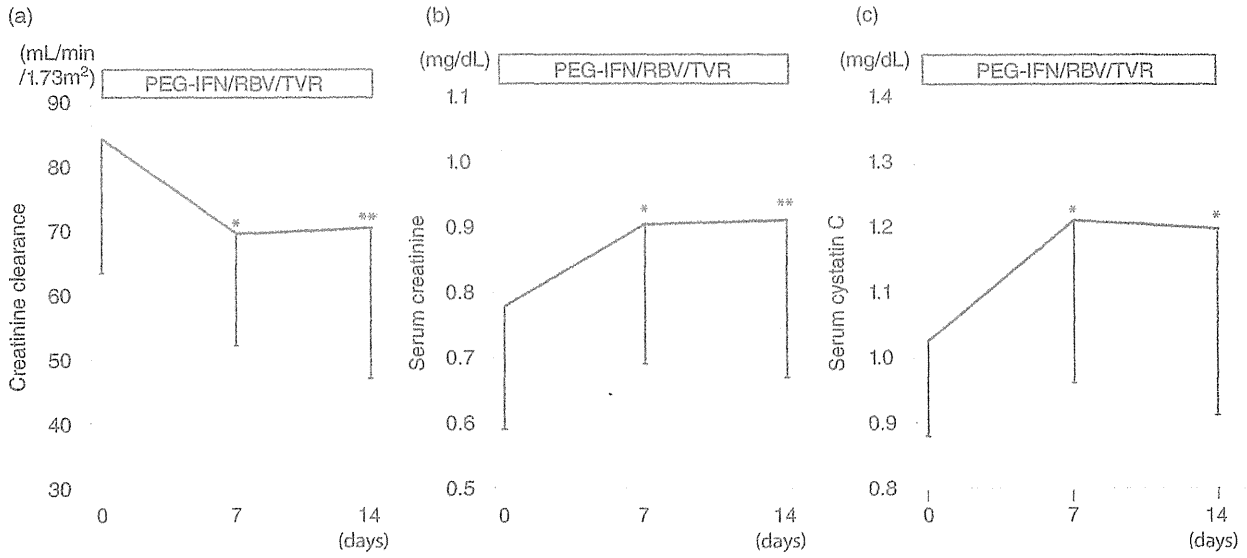

Figure 1 Change of creatinine clearance (a), serum creatinine (b) and serum cystatin C (c) from baseline during the pegylated interferon (PEG IFN)/ribavirin (RBV)/telaprevir (TVR) triple therapy. Creatinine clearance was measured on admission. Each circle and bar represents mean values ± standard deviations, respectively. $^{*}P \leq 0.001$, $^{**}P \leq 0.02$ (vs day 0, paired Student's *t*-test).

low-dose group and 83.3% in high dose-group). Dose modification, interruption or withdrawal of TVR due to deterioration of renal function were observed in three patients of the high-dose TVR group and in none of those of the low-dose TVR group.

DISCUSSION

RENAL IMPAIRMENT IS often observed during PEG IFN/RBV/TVR treatment. Termination of TVR usually leads to restoration of renal function, although persistent renal damage is sometimes reported. Similar to previous reports, in our study, serum creatinine and cystatin C significantly increased during PEG IFN/RBV/TVR treatment, and returned to pretreatment level after termination of TVR. Increases of serum creatinine and cystatin C showed significant correlation with serum TVR level. These observations suggest that TVR would play an important role in treatment-related renal damage.

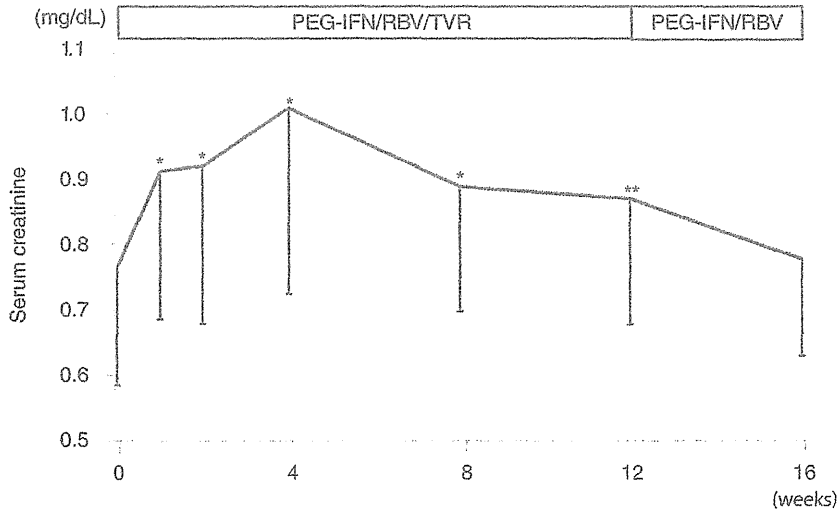

Figure 2 Change of serum creatinine from baseline during the pegylated interferon (PEG IFN)/ribavirin (RBV)/telaprevir (TVR) triple therapy followed by treatment with PEG IFN and RBV. Each circle and bar represent mean values ± standard deviations, respectively. $P < 0.001$, $^{**}P \leq 0.02$ (vs day 0, paired Student's *t*-test).

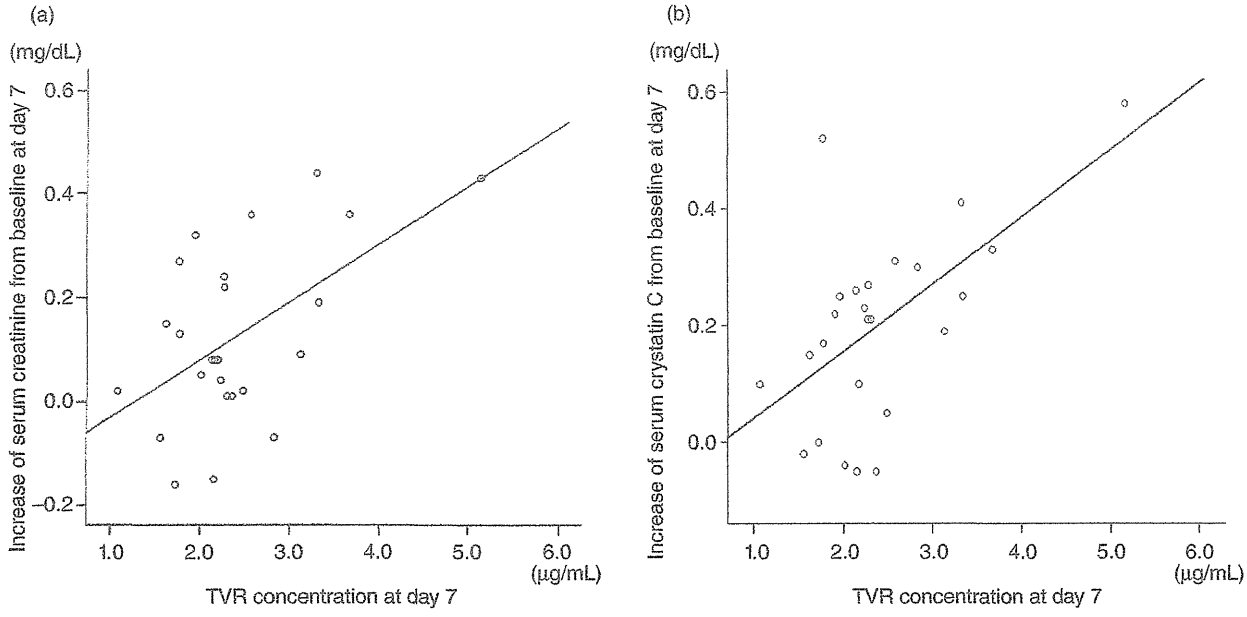

Figure 3 Relationship between the increase of serum creatinine (a) and cystatin C (b) from baseline and serum telaprevir (TVR) concentration at day 7. The increase of serum creatinine and cystatin C from baseline at the day 7 correlated with serum TVR concentration at day 7 ($r = 0.543$, $P = 0.005$, and $r = 0.585$, $P = 0.003$, respectively).

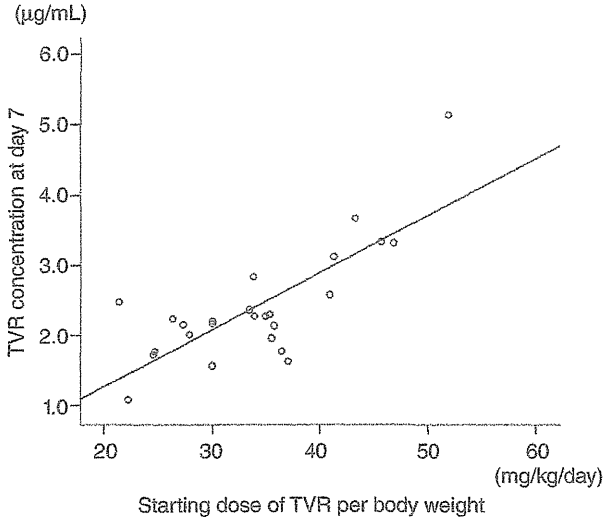

Figure 4 Relationship between serum telaprevir (TVR) concentration at day 7 and TVR starting dose per bodyweight. Serum TVR level strongly correlated with the starting dose of TVR per bodyweight ($r = 0.775$, $P < 0.001$).

Telaprevir is metabolized mainly by cytochrome P450 isoenzyme CYP3A in the liver.[21] However, besides CYP3A, interactions with renal and hepatic drug transporters should not be neglected, and can also be expected to play an important role. Recently, Kunze *et al.* reported that TVR exhibited significant inhibition of the human renal drug transporters, organic cation transporter 2 (OCT2) and multidrug and toxin extrusion transporter 1 (MATE1).[22] Tubular secretion of many organic cations is mediated by uptake across the basolateral membrane by OCT2 and by efflux across the apical membrane by MATE. Although the accurate mechanism of renal damage due to TVR still remains unclear, these observations suggested the direct effect of TVR on renal function.

In our study, serum TVR level strongly correlated with the starting dose of TVR per bodyweight. These findings suggest that adjustment of TVR dose according to bodyweight would avoid renal impairment during treatment. When the patients were classified according to the median starting dose of TVR per bodyweight, renal impairment was observed only in the high-dose group (TVR ≥33 mg/kg per day), not in the low-dose group (TVR <33 mg/kg per day). Suzuki *et al.* reported that dose reduction of TVR given per day from 2250 mg to

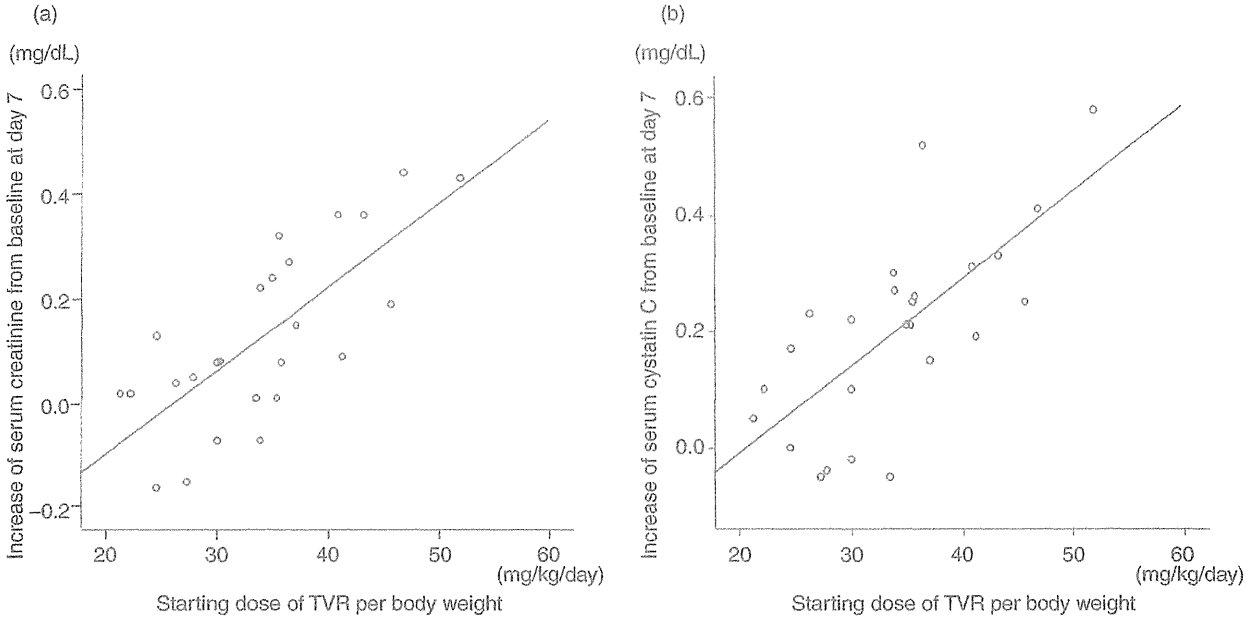

Figure 5 Relationship between the increase of serum creatinine (a) and cystatin C (b) from baseline at the day 7 and telaprevir (TVR) starting dose per bodyweight. The increase of serum creatinine and cystatin C from baseline at the day 7 correlated with the starting dose of TVR per bodyweight ($r = 0.741$, $P < 0.001$, and $r = 0.715$, $P < 0.001$, respectively).

1500 mg may decrease the treatment-related adverse events, specifically anemia and creatinine increase, without the attenuation of treatment effect. Taken together, these findings suggest that adjustment of TVR dose according to bodyweight would improve the continuity and effect of PEG IFN/RBV/TVR treatment.

Our study has several limitations. First, the patients included in this study were relatively elderly compared with previous studies. Seventeen out of 25 patients were over 60 years old, and four patients were over 70 years old. Generally, renal function is thought to decrease with aging. Moreover, drug-induced renal dysfunction is

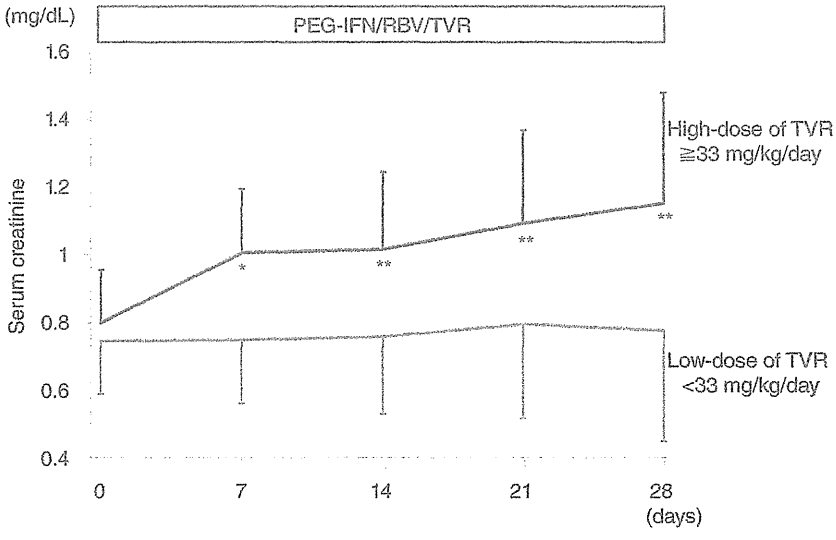

Figure 6 Changes of serum creatinine from baseline during the pegylated interferon (PEG IFN)/ribavirin (RBV)/telaprevir (TVR) triple therapy categorized by the median starting dose of TVR per bodyweight. The high-dose and low-dose groups included the patients with TVR starting dose of ≥33 mg/kg per day ($n = 15$) and those with TVR starting dose of <33 mg/kg per day ($n = 10$), respectively. Each circle and bar represents mean values ± standard deviations, respectively. $^{*}P < 0.001$, $^{**}P < 0.005$ (vs day 0, paired Student's *t*-test).

more prone to occur in elderly persons. The effect of TVR on renal function in our study may be influenced by this sample bias. Second, the sample size of our study was relatively small. Other factors such as age, pretreatment serum creatinine level, hypertension and diabetes mellitus may be associated with renal impairment during PEG IFN/RBV/TVR therapy. The sample size of this study was not sufficient to investigate these associations. A future larger study is necessary to draw definite conclusions.

In conclusion, we showed that TVR dose per bodyweight was significantly related to the occurrence of renal damage during PEG IFN/RBV/TVR treatment. Adjustment of TVR dose according to the bodyweight of patient may improve the effect of the treatment.

REFERENCES

1 Hoofnagle JH. Course and outocome of hepatitis C. *Hepatology* 2002; 36: S21–9.
2 Seeff LB. Natural history of chronic hepatitis C. *Hepatology* 2002; 36: S35–46.
3 Kasahara A, Tanaka H, Okanoue T *et al.* Interferon treatment improves survival in chronic hepatitis C patients showing biochemical as well as virological responses by preventing liver-related death. *J Viral Hepat* 2004; 11: 148–56.
4 Imai Y, Kawata S, Tamura S *et al.* Relationship of interferon therapy and hepatocellular carcinoma in patients with chronic heaptitis C. *Ann Intern Med* 1998; 129: 94–9.
5 Jacobson JM, NcHutchinson JG, Dusheiko G *et al.* Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; 364: 2405–16.
6 Zeuzem S, Andreone P, Pol S *et al.* Telaprevir for retreatment of HCV infection. *N Engl J Med* 2011; 364: 2417–28.
7 Sherman KE, Flamm SL, Afdhal NH *et al.* Response-guided telaprevir combination treatment for hepatitis C virus infection. *N Engl J Med* 2011; 365: 1014–24.
8 Kumada H, Toyota J, Okanoue T, Chayama K, Tsubouchi H, Hayashi N. Telaprevir with peginterferon and ribavirin for treatment-naïve patients chronically infected with HCV of genotype 1 in Japan. *J Hepatol* 2012; 56: 78–84.
9 Hayashi N, Okanoue T, Tsubouchi H, Toyota J, Chayama K, Kumada H. Efficacy and safety of telaprevir, a new protease inhibitor, for difficult-to-treat patients with genotype 1 chronic hepatitis C. *J Viral Hepat* 2012; 19: e134–e42.
10 Yamada I, Suzuki F, Kamiya N *et al.* Safety, pharmacokinetics and resistant variants of telaprevir alone for 12 weeks in hepatitis C virus genotype 1b infection. *J Viral Hepat* 2012; 19: e112–19.
11 Toyota J, Ozeki I, Karino Y *et al.* Virological response and safety of 24-week telaprevir alone in Japanese patients infected with hepatitis C virus subtype 1b. *J Viral Hepat* 2013; 20: 167–73.
12 Suzuki F, Suzuki Y, Sezaki H *et al.* Exploratory study on telaprevir given every 8h at 500mg or 750mg with peginterferon-alpha-2b and ribavirin in hepatitis C patients. *Hepatol Res* 2013; 43: 691–701.
13 Chobanian AV, Bakris GL, Black HR *et al.* Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA* 2003; 289: 2560–72.
14 American Diabetes Association. Standards of medical care in diabetes-2013. *Diabetes Care* 2013; 36 (Suppl 1): S4.
15 Tanaka Y, Nishida N, Sugiyama M *et al.* Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; 41: 1105–9.
16 Suzuki F, Suzuki Y, Akuta N *et al.* Influence of ITPA polymorphisms on decreases of hemoglobin during treatment with pegylated interferon, ribavirin, and telaprevir. *Hepatology* 2011; 53: 415–21.
17 Ohnishi Y, Tanaka T, Ozaki K, Yamada R, Suzuki H, Nakamura Y. A high-throughput SNP typing system for genomewide association studies. *J Hum Genet* 2001; 46: 471–7.
18 Enomoto N, Sakuma I, Asahina Y *et al.* Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med* 1996; 334: 77–81.
19 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: 403–10.
20 Kakuda H, Kanasaki K, Koya D, Takekoshi N. The administration of pitavastatin augments creatinine clearance associated with reduction in oxidative stress parameters: acute and early events. *Clin Exp Nephrol* 2013; 17: 240–47.
21 Kiser JJ, Burton JR, Anderson PL, Everson GT. Review and management of drug interactions with boceprevir and telaprevir. *Hepatology* 2012; 55: 1620–8.
22 Kunze A, Huwyler J, Camenisch G, Gutmann H. Interaction of the antiviral drug telaprevir with renal and hepatic drug transporters. *Biochem Pharmacol* 2012; 84: 1096–102.

EXPERT REVIEWS

Simeprevir for the treatment of chronic hepatitis C genotype 1 infection

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Simeprevir is a second-wave hepatitis C virus NS3/4A protease inhibitor that was designed to optimize its antiviral activity, safety, drug-drug interactions, and pharmacokinetic profile. When used to treat patients with hepatitis C virus genotype 1, simeprevir is coadministered with peginterferon and ribavirin for 12 weeks, followed by double therapy with Peg-IFN and ribavirin for an additional 12 or 36 weeks. Phase III studies achieved a sustained virologic response in 80–90% of treatment-naïve patients (International Phase III studies QUEST-1/2: 80/81%; Japanese Phase III trial CONCERTO-1: 89%). Unlike with the first protease inhibitors, telaprevir or boceprevir, used in triple therapy, when using simeprevir the frequency of clinically problematic adverse events such as anemia, rash, and digestive symptoms is almost comparable to that of double therapy. The advent of simeprevir has enabled interferon therapy, which started as monotherapy in early 1990s, to reach its maximum efficacy and arrive at what can be considered its final form at least in genotype 1b.

KEYWORDS: direct acting antiviral • hepatitis C virus • peginterferon • protease inhibitor • Simeprevir

An estimated 130–150 million people worldwide are infected with the hepatitis C virus (HCV) [1]. The majority of these individuals are infected as adults through blood exposure, and although 20–30% develop only a transient infection, the other 70–80% develop a persistent infection. Spontaneous clearance of the virus after the development of a persistent infection is rare (~0.2% per year), and infections lasting 20–30 years can lead to decompensated liver cirrhosis or liver cancer. In the USA and Japan, these diseases are the leading indications for liver transplantation because of end-stage liver disease. Therefore, it is important to completely eradicate the virus to defer the development of liver disease during the persistent infection stage.

Interferon monotherapy was introduced as an antiviral therapy for hepatitis C in the early 1990s, and it has made achieving a sustained virologic response (SVR; defined as the inability to detect HCV RNA at 24 weeks after completing treatment) possible in roughly 1 of 3 or 4 cases. However, it was not effective for patients with HCV genotype 1, particularly those with a high viral load, and the SVR rate was found to be approximately 5–10%, which led to these cases being called 'difficult-to-treat hepatitis C' (FIGURE 1). In the 2000s, two-drug combination therapy with peginterferon (Peg-IFN) and ribavirin (RBV) became the standard antiviral therapy for chronic HCV infection. With this treatment, the SVR rate for genotype 1 patients after 48 or 72 weeks of treatment became 40–50% and the rate for genotype 2 patients after 24 weeks of treatment became approximately 80% [2]. The release of the protease inhibitor telaprevir (TVR), and also boceprevir (BOC), as a treatment for genotype 1 patients in 2011 ushered in the era of triple therapy, drastically shrinking the treatment duration to 24 weeks at least for some naive patients and relapsers and improving the SVR rate to roughly 80% [3]. Although TVR and BOC had problematic aspects including complex drug interactions and strong side effects such as anemia and skin manifestations, simeprevir (SMV), a second-wave protease inhibitor with very limited side effects, was approved at the end of 2013 [4]. This paper reviews the use of triple-drug therapy with SMV for the treatment of patients with HCV genotype 1.

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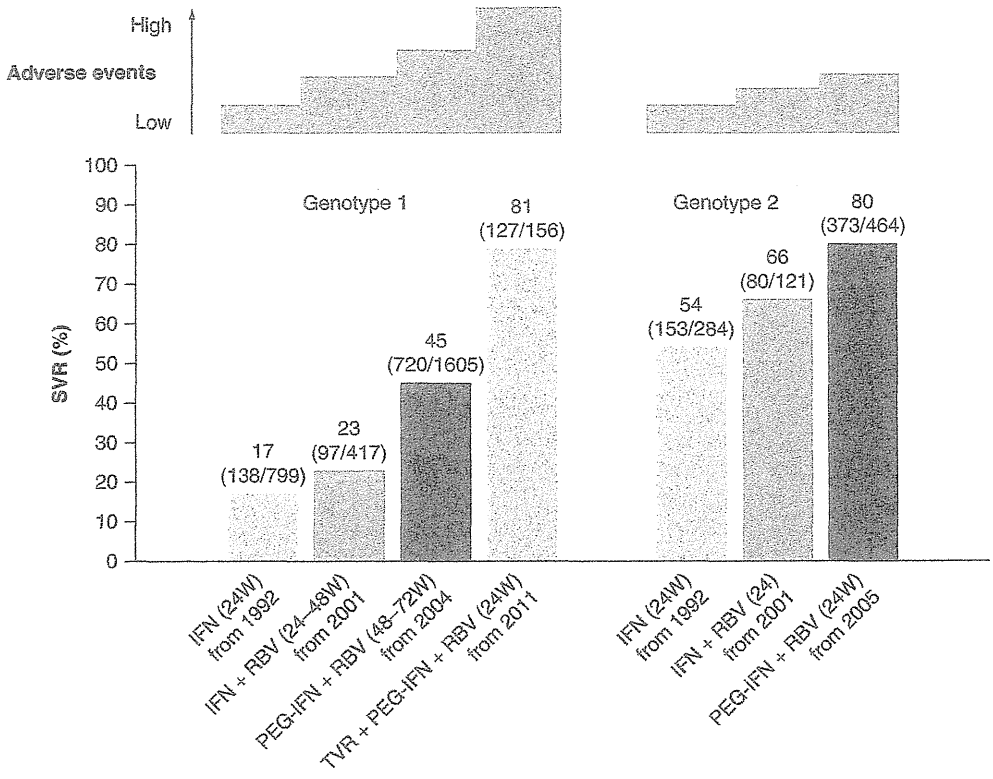

Figure 1. Changes to interferon treatment before the advent of simeprevir. Sustained virologic response rates are from therapies using postmarket IFNs conducted at Osaka University and associated facilities. Advancements in IFN therapy have led to improvements in the sustained virologic response rates for patients with hepatitis C genotype 1 and genotype 2, but the frequency of side effects has also increased. In particular, decreased compliance with long-term treatment and the increased side effects associated with additional dosage of telaprevir are problematic for patients with genotype 1.
IFN: Interferon; Peg-IFN: Peginterferon; RBV: Ribavirin; SVR: Sustained virologic response; TVR: Telaprevir.

History of NS3/4A protease inhibitor development

IFN and RBV have long been used as the primary antiviral drugs to treat hepatitis C [2] as both drugs are nonselective HCV inhibitors. IFN is a cytokine prototype discovered in the 1950s, and RBV is an early nucleoside analog antiviral drug synthesized in 1970. Although HCV was discovered in 1989, its discovery in itself did not lead directly to the development of new drugs for treating hepatitis C infection. The main reason for this was that although the genetic structure of the virus had been characterized, no efficient assay system for HCV replication was developed quickly – it took 10 years after the discovery of the virus for a replicon that could reproduce HCV proliferation *in vitro* to be created in 1999 [5]. Another important step that laid the groundwork for research on new drugs for treating HCV infection was that the structures of HCV proteases, which are essential to HCV replication, were elucidated in 1996 [6]. HCV NS3 protease was shown to undergo inhibition by the N-terminal substrate products. This made it possible to design functional molecules to target the active sites of the proteases. In the 2000s, the conformation of the substrate peptides predicted from the structures of proteases was altered to a nonpeptide form, and after the antivirus activity of small molecules that could be taken orally was screened using replicons, a new era of clinical development of drugs with direct antiviral activity against HCV (direct-acting antivirals) began.

One of these HCV-selective antiviral drug prototypes was BILN 2061 (Figure 2). The BILN 2061 molecule is a noncovalent protease inhibitor with a macrocyclic structure resembling a lid placed from above on the active site of the NS3/4A HCV protease [7]. BILN 2061 was the first drug to inhibit HCV replication in humans, but subsequent studies on monkeys revealed cardiotoxicity at high doses [8], and thus its development was suspended. Another molecule developed slightly after BILN 2061 was VX-950, which is a covalent protease inhibitor with a linear structure that binds to the bottom part of the groove of the active site of the NS3/4A HCV protease. VX-950 immediately gained attention because of its strong HCV inhibitory effect both *in vitro* and *in vivo*, and following clinical trials, it was released for clinical use as TVR in 2011. The release of TVR improved the SVR rate for hepatitis C genotype 1 after 24 weeks of combined treatment with Peg-IFN + RBV to 73%

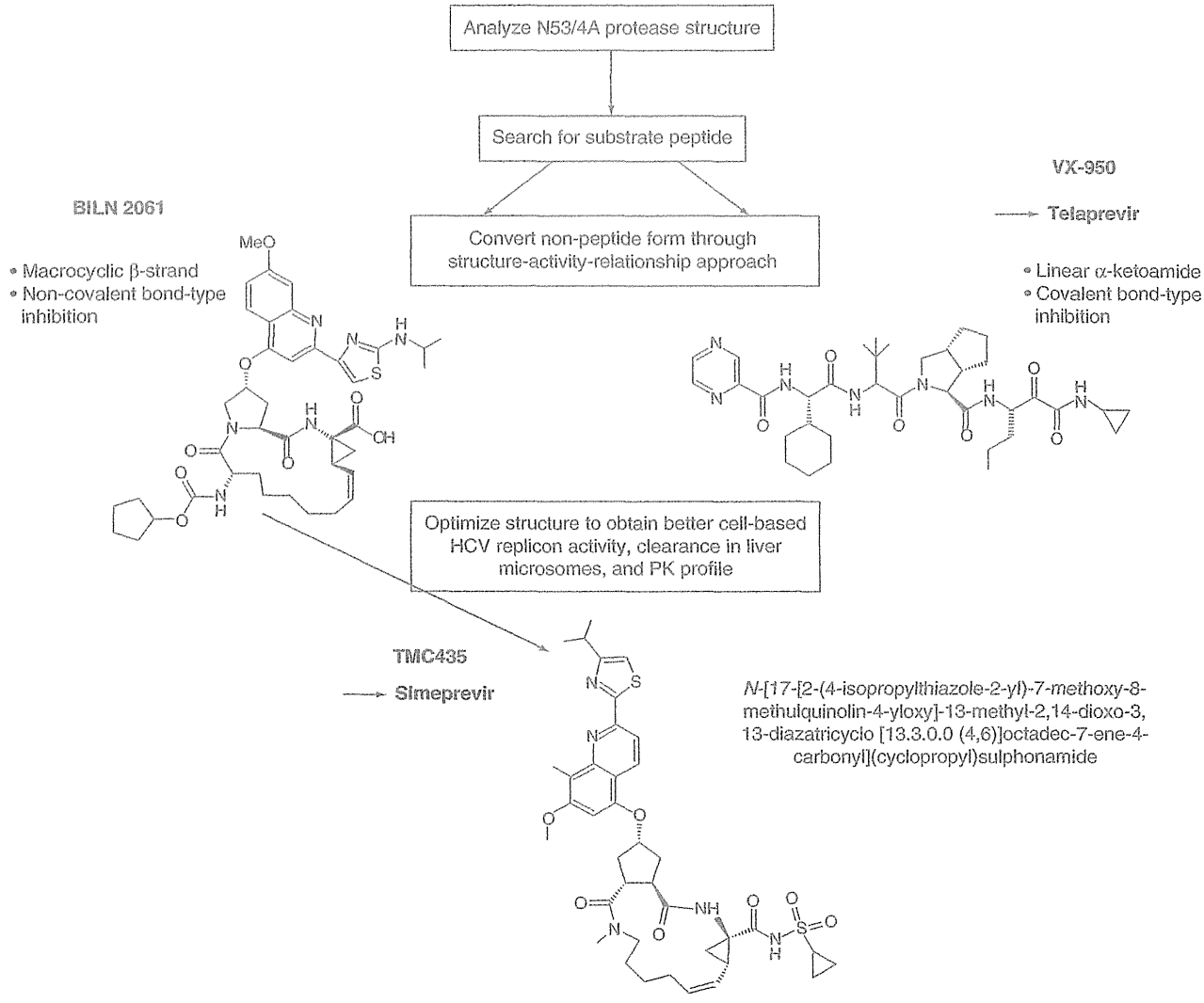

Figure 2. Structure of protease inhibitors and development of simeprevir.
HCV: Hepatitis C virus; PK: Pharmacokinetic.

(in a Japanese Phase III development study). Although TVR is certainly a first-in-class drug, particular caution must be taken with its use because of its complex interactions with many other drugs and its unique side effect profile that includes anemia, skin reactions and kidney damage [3,9].

Although development of the macrocyclic molecule BILN 2016 was suspended, several other drugs are being developed based on its structure with the conformation changed to avoid toxicity. One of these is SMV (code name, TMC435), and other similar drugs currently in clinical development include faldaprevir and vaniprevir. To differentiate them from the first drugs TVR and BOC (the latter not approved in Japan), they are second-wave protease inhibitors. SMV is based on a lead compound developed from BILN 2061 and was selected after performing tests including an enzyme inhibitory activity test using recombinant proteins, an inhibitory effect on replication test using a replicon, a clearance test using human liver microsomes, a permeability test using Caco-2 cells and a pharmacokinetic test in rats [10]. Although SMV was approved for clinical use after TVR, its molecular structure has been carefully optimized as described above, making it highly superior with respect to its safety, drug interactions and pharmacokinetic profile.

Clinical development of SMV

Preclinical studies

Protease inhibitors are classified as linear or macrocyclic based on their molecular structure. TVR and BOC, which have already been approved in Europe and the USA, possess linear keto-amide structure, whereas SMV possesses macrocyclic structure. SMV has a molecular weight of 750 Da and can be taken orally. This drug is a strong inhibitor of the NS3/4A protease

that originates in HCV genotypes 1a and 1b (the enzyme inhibition constant [Ki] of SMV against NS3/4A proteases is 0.5 nmol/l in genotype 1a [H77 strain] and 1.4 nmol/l in genotype 1b [Con1 strain]) and strongly suppresses replication in genotype 1b replicon cells (EC_{50} = 8.1–25.2 nM) and genotype 1a replicon cells (EC_{50} = 28.4 nM) [11]. In combination with other drugs in a replicon system, it has synergistic antiviral effects with IFNs and NS5B polymerase inhibitors, as well as additive effects with RBV. Studies on the drug distribution among organs in rats showed high accumulation in the liver (liver-to-plasma ratio, 32:1–65:1). The plasma concentration 8 h after administration reached the same EC_{99} value measured in the replicon, and the concentration remained at EC_{50} even after 24 h, indicating that a once-daily dose of the drug could yield sufficiently long-lasting effects. It is known that the mutations to the NS3 domain that confer resistance to SMV differ from those that confer resistance to TVR. Specifically, the antiviral effect of SMV is not affected by substitutions of amino acids 36, 54 and 170, which confer resistance to TVR, whereas substitutions of amino acids 43, 80, 155, 156 and 168 are known to confer resistance to SMV [12]. Mutations at position 155 and 156 also confer resistance to TVR, but the others are not related to TVR resistance.

Phase I/IIa studies

A randomized double-blind placebo-controlled trial in 49 healthy adults and a nonplacebo-controlled open-label study in six patients with hepatitis C (TMC435350-C101 study) were Phase I studies of a single administration of TMC435 conducted outside Japan [13]. Healthy adults were administered a single dose from 50 to 600 mg in an ascending dose study and continuous doses of 100 (once daily), 200 (once daily), 200 (twice daily) and 400 mg (once daily) over 5 days. The increase in blood concentration after the start of administration was greater than the increase in dose. Some subjects experienced issues such as headache and photosensitivity while taking the drug, but no serious adverse events were observed. Patients with hepatitis C (4 genotype 1a, 2 genotype 1b) were administered a 200 mg dose once daily for 5 days. Three days after the start of treatment, blood levels of HCV RNA fell by a median magnitude of 3.46 $\log_{10}$ IU/ml (1.6–3.8) and ultimately decreased by at least 3 $\log_{10}$ IU/ml in all six subjects. Although no viral breakthrough was observed in the 8 days after starting administration, new protease domain variants were detected in all six subjects after starting treatment. The 24 h area under the curve after 5 days of treatment was approximately threefold higher than that of healthy subjects, suggesting that the pathology of hepatitis C may influence elimination of the drug.

A study where TMC435, Peg-IFNα-2a and RBV were administered to patients with hepatitis C, genotype 1 was conducted outside Japan as a Phase II proof-of-concept study (OPERA-1 study [TMC435-C201 study]). Considerable mean reductions in HCV RNA levels were observed after 4 weeks of triple therapy; specifically, 3.64 $\log_{10}$ IU/ml in the placebo group, 4.74 $\log_{10}$ IU/ml in the 25 mg TMC435 group, 5.52 $\log_{10}$ IU/ml in the 75 mg group and 5.44 $\log_{10}$ IU/ml in the 200 mg group [14]. To assess the efficacy of treatment in patients with other genotypes, single doses of TMC435 (200 mg once daily) were administered for 7 days to 37 treatment-naive patients with HCV genotypes 2–6 (TMC435-C202 study) [15,16]. Although this considerably reduced the viral load in subjects with genotypes 4–6, it only reduced HCV RNA by roughly half in subjects with genotype 2 and did not reduce the viral load in any subject with genotype 3.

Phase IIb studies

The international PILLAR study (TMC435-C205 study) and the Japanese DRAGON study (TMC435-C215) began as Phase IIb studies in 2009 at around the same time (Tables 1 & 2) [17,18]. In the DRAGON study, 92 IFN therapy-naive patients with chronic hepatitis C genotype 1 infection and a high viral load were randomly assigned to a group that received triple therapy with SMV + Peg-IFNα-2a + RBV for 12 weeks followed by Peg-IFNα-2a + RBV (600–1000 mg depending on body weight) for 12 weeks, a group that received triple therapy with SMV + Peg-IFNα-2a + RBV for 24 weeks, or a control group that received Peg-IFNα-2a + RBV for 48 weeks [18]. The 12-week and 24-week groups were both subdivided into 50 mg and 100 mg SMV dose groups, which are lower doses than used in the PILLAR study, which is discussed in a later section. The DRAGON study protocol called for response-guided therapy (RGT). Total Peg-IFN + RBV duration would be 24 weeks if HCV RNA was <1.4 $\log_{10}$ IU/ml at week 4 and undetectable at week 12, 16 and 20, and if this criteria were not met total Peg-IFN + RBV duration was 48 weeks. As a result, only one patient was treated for 48 weeks. The SVR rates in the SMV groups (listed by 12/24 week subgroups) were 78/77% in the 50 mg/day groups and 77/92% in the 100 mg/day groups, indicating that treatment with SMV was significantly more efficacious than treatment with the Peg-IFNα-2a + RBV (46%). Although adverse events such as decreased hemoglobin levels and drug eruptions occurred, most events were grade 1 or 2, and the frequency of events did not differ between the TMC435 and control groups. Transient hyperbilirubinemia after 1–2 weeks of treatment was a side effect observed in the SMV groups, but it was not found to be associated with any other clinical symptoms or laboratory test parameters such as aspartate aminotransferase or alanine aminotransferase levels. These results suggest that in Japanese treatment-naive patients infected with HCV genotype 1 with high viral load, triple therapy with SMV is superior to double therapy with respect to its SVR rate with a comparable safety and tolerability.

The PILLAR study, an international study of IFN treatment-naive patients with HCV genotype 1, had a similar treatment design to the DRAGON study but used higher doses of SMV (75 and 150 mg) and RBV (1000–1200 mg depending on body weight) [17]. The SVR rates of the SMV groups in

Table 1. Summary of international Phase IIb/III studies.

Phase	IIb	IIb	III	III	III
Patient population	Naïve	Experienced (relapsers/partial responders/null responders)	Naïve	Naïve	Experienced (relapsers)
Study design	5 arms	7 arms	2 arms	2 arms	2 arms
Simeprevir dose (mg)	75/150	100/150	150	150	150
Patient number	386	462	394	391	393
Genotype 1b (%)	55	57	44	58	58
Cirrhosis (%)	Excluded	Included (18)	Included (12)	Included (8)	Included (15)
Peg-IFN	α2a	α2a	α2a	α2a/α2b	α2a
Treatment duration (weeks)	24 (RGT)	48	24 (RGT)	24 (RGT)	24 (RGT)
Met RGT criteria (%)	79–86	NA	85	91	93
SVR† (SMV arms) (%)	75–86	38–59 (null responders) 48–86 (partial responders) 77–89 (relapsers)	80	81	79
(Control PR48 arm) (%)	65	19 (null responders) 9 (partial responders) 37 (relapsers)	50	50	37
Ref.	[17]	[19]	[20]	[21]	[22]

†SVR shown is determined at 24 weeks for PILLAR and ASPIRE and at 12 weeks for QUEST-1/2 and PROMISE after the end of treatment.
Peg-IFN: Peginterferon; RGT: Response-guided treatment; SMV: Simeprevir; SVR: Sustained virologic response.

the PILLAR study (listed by 12/24 week subgroups) were 82/75% in the 75 mg/day groups and 81/86% in the 150 mg/day groups, indicating that treatment with SMV was more effective than treatment with the Peg-IFNα-2a + RBV (65%). According to RGT criteria, 79–86% of SMV-treated patients completed treatment by week 24; 85–96% of these subsequently achieved SVR. No clear relationship between the dosing period of SMV and the SVR rate was observed. The SVR rate was lower for genotype 1a than for genotype 1b in the 75 mg dose group, but did not differ between genotypes in the 150 mg dose group.

In SMV/Peg-IFN/RBV triple therapy, the effect of prior treatment is currently the most important factor influencing the SVR. Outside Japan, a second Phase IIb study was conducted in patients who had previously received Peg-IFN/RBV therapy (ASPIRE study [TMC435-C206 study]) [19]. The dosing period for each regimen was set to 48 weeks. The control group received Peg-IFNα-2a + RBV for 48 weeks, whereas the experimental groups received Peg-IFNα-2a + RBV for 48 weeks along with 100 mg/day or 150 mg/day of SMV for the first 12 weeks, the first 24 weeks or the entire 48 weeks of the study. The SVR rates of the SMV groups (listed by 12/24/48 week subgroups) were 70/66/61% in the 100 mg/day groups and 67/72/80% in the 150 mg/day groups, respectively, significantly higher than the 23% of the control group; the SVR rate did not differ with respect to the SMV dosing period. However, the effect of prior treatment strongly influenced the SVR rate, which (for 100 mg/150 mg doses) was 85/85% for relapsers, 57/75% for partial responders (≥2 log reduction in HCV RNA at week 12 of previous treatment) and 46%/51% for null responders (<2 log reduction in HCV RNA at week 12 of previous treatment). The SVR rate did not differ between genotypes 1a and 1b in prior relapsers, but was lower for genotype 1a in prior partial responders and prior null responders. Transient hyperbilirubinemia and rash were observed more frequently in the SMV group than in the control group. However, the incidence of clinically problematic major adverse events did not differ between the treatment groups. In the ASPIRE study, unlike the PILLAR study, 18% of subjects had liver cirrhosis, but no particularly problematic adverse events were observed in subjects with advanced fibrosis.

Phase III studies

Following the success of the Phase II studies, Phase III studies of triple therapy with SMV + Peg-IFN + RBV were initiated. Outside Japan, the QUEST-1 and QUEST-2 studies were conducted in treatment-naive patients, [20,21], and the PROMISE study was conducted in prior relapsers [22]. In Japan, the CONCERTO-1 study [23] was conducted in treatment-naive patients, the CONCERTO-2 and CONCERTO-3 studies [24] were conducted in treatment-experienced patients and the CONCERTO-4 study [25] was conducted separately and used Peg-IFNα-2b. Studies outside Japan included subjects with liver cirrhosis, whereas Japanese studies did not. In addition,

Table 2. Summary of Japanese Phase IIb/III studies.

Phase	IIb	III	III	III	III
Patient population	Naïve	Naïve	Experienced (nonresponders†)	Experienced (relapsers)	All
Study design	5 arms	2 arms	2 arms	1 arm	3 arms
SMV dose (mg)	50/100	100	100	100	100
Patient number	92	183	106	49	79
Genotype 1b (%)	100	98	97	98	99
Cirrhosis	Excluded	Excluded	Excluded	Excluded	Excluded
Peg-IFN	α2a	α2a	α2a	α2a	α2b
Treatment duration (weeks)	24 (RGT)	24 (RGT)	24 (RGT)	24 (RGT)	24 (RGT) for naïve/relapsers 48 for nonresponders
Met RGT criteria (%)	83–90	92	74–81	96	92 for naïve 97 for relapsers
SVR‡ (SMV arms) (%)	77–92	89	36–51	90	92 for naïve 39 for nonresponders 97 for relapsers
(Control PR48 arm) (%)	46	57	NA	NA	NA
Ref.	[18]	[23]	[24]	[24]	[25]

†Nonresponder means partial responder plus null responder.
‡SVR shown is determined at 24 weeks after the end of treatment.
Peg-IFN: Peginterferon; RGT: Response-guided treatment; SVR: Sustained virologic response; SMV: Simeprevir.

about half of the subjects in studies outside Japan were genotype 1a, whereas most subjects in Japanese studies were genotype 1b.

The QUEST-1 (n = 394) and QUEST-2 (n = 391) studies compared the treatment efficacy of SMV (150 mg/day, 12 weeks) + Peg-IFN + RBV in patients with hepatitis C genotype 1 infection (including those with compensated cirrhosis) with the control treatment of Peg-IFN + RBV. The SVR rate for triple therapy with SMV + Peg-IFN + RBV was 80–81% overall and 82–90% for genotype 1b. By *IL28B* genotype (rs12979860), the SVR rate was 94–96% for the CC allele, 76–80% for the CT allele and 58–65% for the TT allele. By the degree of fibrosis (METAVIR score), the SVR rate was 83–85% for F0-2, 67–78% for F3 and 58–65% for F4.

The CONCERTO-1 study conducted in Japan compared the treatment efficacy of SMV (100 mg/day, 12 weeks) + Peg-IFNα-2a + RBV in 183 treatment-naive patients with hepatitis C genotype 1 infection with the control treatment of Peg-IFNα-2a + RBV. While the SVR rate of control arm was 57% (34/60), that for triple therapy with SMV/Peg-IFNα-2a/RBV was 89% (109/123) overall, 87% (20/23) for subjects aged ≤45 years, 90% (70/78) for those 44–64 years and 86% (19/22) for those ≥65 years (but ≤70), indicating no difference with respect to age. By *IL28B* genotype (rs8099917), the SVR rate was 94% (77/82) for the major, favorable, allele TT and 78% (32/41) for the minor alleles TG/GG. Although the rate was significantly higher for the TT allele, the efficacy of treatment for the TG/GG alleles was also relatively high. The incidence of serious adverse events was lower in the study group than in the control group (3.3 vs 10%). Although a transient increase in bilirubin levels was observed in subjects soon after treatment began in the study group, but not in the placebo group, this increase was not accompanied by an increase in transaminase and subsided in all cases as treatment continued. In the CONCERTO-4 study, a clinical trial of SMV-based triple therapy using Peg-IFNα-2b, the SVR rate for treatment-naive subjects was 92% (44/49), almost the same as that achieved with SMV-based triple therapy using Peg-IFNα-2a (CONCERTO-1 study).

The CONCERTO-2 study was conducted in 106 prior nonresponders infected with the hepatitis C genotype 1. Subjects were administered Peg-IFNα-2a + RBV for 24 weeks along with SMV (100 mg/day) for the first 12 weeks or the full 24 weeks. The SVR rate was 51% (27/53) and 36% (19/53), respectively. The SVR rate was 50% (7/14) for the TT allele and 42% (39/92) for the TG/GG alleles, indicating no clear relationship between the IL28B genotype (rs8099917) and therapeutic efficacy. The CONCERTO-3 study was conducted in 49 prior relapsers infected with the hepatitis C genotype 1. Subjects were administered Peg-IFNα-2a + RBV for 24 weeks along with SMV (100 mg/day) for the first 12 weeks as with CONCERTO-1. The SVR rate was 90%

(32/35) overall and was high for all IL28B genotypes (rs8099917) at 91% (32/35) for the TT allele and 86% (12/14) for TG/GG alleles. In the CONCERTO-4 study, a clinical trial of SMV-based triple therapy using Peg-IFNα-2b, the SVR rate was 97% (28/29) for prior relapsers and 38% (10/26) for nonresponders, almost the same as the rates achieved with SMV-based triple therapy using Peg-IFNα-2a (CONCERTO-2, 3 studies).

The four CONCERTO studies examined genetic mutations of the NS3 protease domain in 87 cases of treatment failure defined by viral breakthrough, virologic stopping due to insufficient antiviral efficacy, HCV RNA-positive status at completion of treatment or relapse following completion of treatment. Mutations conferring SMV resistance were detected in approximately 90% of treatment failure cases, and almost all were due to substitutions of amino acid 168, with approximately 90% involving D168V (single D168V substitutions and multiple substitutions including D168V). It should be noted here that, in total, 98% of subjects in these CONCERTO studies had genotype 1b, while the non-Japanese PILLAR and ASPIRE studies similarly reported the involvement of D168 in almost all SMV-resistant mutations in genotype 1b. A study of NS3 polymorphism before treatment showed that the main polymorphs were S122G (16–27%), S122T (3–13%) and Q80L (8–10%); it should be noted that, in Japanese studies, almost all patients are genotype 1b. However, susceptibility to SMV did not differ between these polymorphs in an *in vitro* system, and no differences were observed in the efficacy for the S112G polymorph observed in the CONCERTO studies. Moreover, almost no D168 polymorphism was present before treatment. The therapeutic efficacy of triple therapy with SMV is known to be slightly lower in genotype 1a patients than in genotype 1b patients due to the Q80K mutation seen in a proportion of genotype 1a patients; of note proportion of Q80K differs substantially within genotype 1a patients ranging from 5 to 10% depending on region [20,22]. Indeed, in the QUEST-1 study, the patients with genotype 1a and Q80K mutation had a similar efficacy in the triple therapy arm compared with control arm. It is also known that in genotype 1a patients, in contrast with genotype 1b patients, R155K is the most common resistance mutation that appears with treatment failure.

Conclusion

Triple therapy with SMV + Peg-IFN + RBV yields a high SVR rate (≥80%) after a standard 24-week treatment period in patients with hepatitis C genotype 1 infection. High percentage of subjects who met RGT criteria and the high SVR rates in these subjects support the fixed treatment duration recommended in the USPI based on Phase III trial data. The side effect profile of this treatment, in contrast with that of TVR, is comparable to the side effect profile of double therapy with Peg-IFN + RBV, although it should also be noted that SMV still has several side effects such as hyperbilirubinemia and photosensitivity. In addition, the therapeutic efficacy depends on factors such as responsiveness to IFN (*IL28B* genotype or responsiveness to prior treatment) and the stage of liver fibrosis, but efficacy remains relatively high for patients with minor *IL28B* genotypes, prior nonresponders and patients with advanced liver disease. The incidence of side effects is also comparable to that of double therapy with Peg-IFN + RBV, making it an excellent treatment and an easy choice for a large number of patients.

Expert commentary

IFN therapy for hepatitis C was started in the 1990s, but the therapeutic efficacy for genotype 1 patients with high viral load was soon discovered to be low, leading to such cases being called 'difficult-to-treat hepatitis C.' In the 2000s, Peg-IFN + RBV became the standard treatment, but genotype 1 cases required a longer treatment period (48–72 weeks) than genotype 2 cases, and the therapeutic efficacy was only 40–50%. Today, the addition of SMV to therapy has shortened the treatment period for patients with chronic hepatitis C genotype 1 to 24 weeks, and superior, or equal at least, treatment results can now be expected from Peg-IFN + RBV therapy in patients with genotype 2. It should also be noted that the addition of SMV has not yet been reported to increase the incidence of clinically important adverse events. The advent of triple therapy with SMV has led to IFN therapy, which has improved the method of treating difficult-to-treat cases of hepatitis C since its development in the 1990s, to what can be considered nearly its final form. Both SMV + Peg-IFN + RBV and sofosbuvir (SOF) + Peg-IFN + RBV therapies have been approved in the USA for genotype 1 cases, [26], but the SOF triple therapy regimen is not being developed in Japan and thus SMV + Peg-IFN + RBV is the first-line treatment for genotype 1 cases.

Hepatitis C treatments that are planned for release include not only other types of triple therapy with IFN, but also treatments such as daclatasvir (DCV) + asunaprevir (ASV) and SOF + ledipasvir (LDV) that omit IFN. [27,28]. Antiviral therapy for hepatitis C must be started as soon as possible to control the progression of liver disease and the development of liver cancer, particularly in aged patient populations like that of Japan. At the same time, secondary options must also be considered in advance because treatment failure occurs in 10–20% of cases. In the era of direct-acting antiviralss, it is important to avoid creating resistant viruses to the greatest possible extent. It is generally believed that treatments that include IFN present a high barrier to the emergence of resistant viruses. In patients who developed virologic failure to SMV + Peg-IFN + RBV, mutations can develop, more specifically, D168V for genotype 1b and R155K for genotype 1a. Although these two resistance mutations are shared by the second-wave protease inhibitors, their susceptibility to SOF + LDV will likely be maintained. The fact that such secondary options are available is yet another reason why SMV + Peg-IFN + RBV combination therapy can be considered an excellent treatment method.

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Key issues

- Simeprevir (SMV) is a second-wave hepatitis C virus NS3/4A protease inhibitor that was designed to optimize its antiviral activity, drug–drug interactions and pharmacokinetics.
- SMV is coadministered with peginterferon (Peg-IFN) and ribavirin (RBV) for 12 weeks, followed by double therapy with Peg-IFN and RBV for an additional 12 or 36 weeks for genotype 1 patients.
- SMV + Peg-IFN + RBV therapy yields a high sustained virologic response rate (≥80%) for treatment-naive patients.
- The efficacy of SMV + Peg-IFN + RBV therapy depends on factors such as responsiveness to IFN (IL28B genotypes for naive patients or responsiveness to prior treatment for experienced patients) and the stage of liver fibrosis.
- Therapeutic efficacy of triple therapy with SMV is slightly lower in genotype 1a patients than in genotype 1b patients due to the Q80K mutation seen in a proportion of genotype 1a patients.
- In patients who developed virological failure to SMV + Peg-IFN + RBV therapy, mutation can develop specifically D168V for genotype 1b and R155K for genotype 1a, which may have little influence to give the effect of the later therapy such as a combination of NS5A inhibitor and NS5B inhibitor.

References

1. WHO. Available from: www.who.int/mediacentre/factsheets/fs164/en/index.html
2. Hayashi N, Takehara T. Antiviral therapy for chronic hepatitis C: past, present, and future. J Gastroenterol 2006;41(1):17-27
3. Muir AJ. Telaprevir for the treatment of chronic hepatitis C infection. Expert Rev Anti Infect Ther 2011;9(12):1105-14
4. You DM, Pockros PJ. Simeprevir for the treatment of chronic hepatitis C. Expert Opin Pharmacother 2013;14(18):2581-9
5. Lohmann V, Körner F, Koch J, et al. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. Science 1999;285(5424):110-13
6. Kim JL, Morgenstern KA, Lin C, et al. Crystal structure of the hepatitis C virus NS3 protease domain complexed with a synthetic NS4A cofactor peptide. Cell 1996; 87(2):343-55
7. Lamarre D, Anderson PC, Bailey M, et al. An NS3 protease inhibitor with antiviral effects in humans infected with hepatitis C virus. Nature 2003;426(6963):186-9
8. Reiser M, Hinrichsen H, Benhamou Y, et al. Antiviral efficacy of NS3-serine protease inhibitor BILN-2061 in patients with chronic genotype 2 and 3 hepatitis C. Hepatology 2005;41(4):832-5
9. Mauss S, Hueppe D, Alshuth U. Renal impairment is frequent in chronic hepatitis C patients under triple therapy with telaprevir or boceprevir. Hepatology 2014; 59(1):46-8
10. Raboisson P, de Kock H, Rosenquist A, et al. Structure-activity relationship study on a novel series of cyclopentane-containing macrocyclic inhibitors of the hepatitis C virus NS3/4A protease leading to the discovery of TMC435350. Bioorg Med Chem Lett 2008;18(17):4853-8
11. Lin TI, Lenz O, Fanning G, et al. In vitro activity and preclinical profile of TMC435350, a potent hepatitis C virus protease inhibitor. Antimicrob Agents Chemother 2009;53(4):1377-85
12. Lenz O, Verbinnen T, Lin TI, et al. In vitro resistance profile of the hepatitis C virus NS3/4A protease inhibitor TMC435. Antimicrob Agents Chemother 2010;54(5): 1878-87
13. Reesink HW, Fanning GC, Farha KA, et al. Rapid HCV-RNA decline with once daily TMC435: a phase I study in healthy volunteers and hepatitis C patients. Gastroenterology 2010;138(3):913-21
14. Manns M, Reesink H, Berg T, et al. Rapid viral response of once-daily TMC435 plus pegylated interferon/ribavirin in hepatitis C genotype-1 patients: a randomized trial. Antivir Ther 2011;16(7):1021-33
15. Moreno C, Berg T, Tanwandee T, et al. Antiviral activity of TMC435 monotherapy in patients infected with HCV genotypes 2-6: TMC435-C202, a phase IIa, open-label study. J Hepatol 2012;56(6):1247-53
16. Lenz O, Vijgen L, Berke JM, et al. Virologic response and characterisation of HCV genotype 2-6 in patients receiving TMC435 monotherapy (study TMC435-C202). J Hepatol 2013;58(3):445-51
17. Fried MW, Buti M, Dore GJ, et al. Once-daily simeprevir (TMC435) with pegylated interferon and ribavirin in treatment-naïve genotype 1 hepatitis C: the randomized PILLAR study. Hepatology 2013;58(6):1918-29
18. Hayashi N, Sero C, Kato M, et al. Once-daily simeprevir (TMC435) with peginterferon/ribavirin for treatment-naïve hepatitis C genotype 1-infected patients in Japan: the DRAGON study. J Gastroenterol 2014;49(1):138-47
19. Zeuzem S, Berg T, Gane E, et al. Simeprevir Increases Rate of Sustained Virologic Response Among Treatment-Experienced Patients With HCV Genotype-1 Infection: a Phase IIb Trial. Gastroenterology 2014;146(2):430-41
20. Jacobson GL, Dore GR, Foster MW, et al. Simeprevir (TMC435) with peginterferon/ribavirin for chronic HCV genotype-1 infection in treatment-naïve patinets: results from QUEST-1, a phase III trial. EASL 2013, Late-braker #1425
21. Manns M, Marcellin P, Fred Poordad FP, et al. Simeprevir (TMC435) with peginterferon/ribavirin for treatment of chronic HCV genotype-1 infection in treatment-naïve patients: results from

QUEST-1, a phase II trial. EASL 2013, Late-breaker #1413

22. Forns X, Lawitz E, Zeuzem S, et al. Simeprevir with peginterferon and ribavirin leads to high rates of SVR in patients with HCV genotype 1 who relapsed after previous therapy: a phase III trial. Gastroenterology 2014. [Epub ahead of print]
23. Hayashi N, Izumi N, Kumada H, et al. Simeprevir with peginterferon/ribavirin for treatment-naïve hepatitis c genotype 1 patients in Japan: CONCERTO-1, a Phase III trial. J Heparol 2014. [Epub ahead of print]
24. Izumi N, Hayashi N, Kumada H, et al. Once-daily simeprevir with peginterferon and ribavirin for treatment-experienced HCV genotype 1-infected patients in Japan: The CONCERTO-2 and -3 studies. J Gastroenterol 2014;49(5):941-53
25. Hiramatsu N, Takehara T, Hayashi N, et al. Pooled analysis of CONCERTO Studies: Simeprevir (TMC435) Combination Therapy for Genotype-1 HCV Infection (Poster). APASL; 2014
26. Lawitz E, Lalezari JP, Hassanein T, et al. Sofosbuvir in combination with peginterferon alfa-2a and ribavirin for non-cirrhotic, treatment-naive patients with genotypes 1, 2, and 3 hepatitis C infection: a randomised, double-blind, phase 2 trial. Lancet Infect Dis 2013;13(5):401-8
27. Chayama K, Takahashi S, Toyota J, et al. Dual therapy with the nonstructural protein 5A inhibitor, daclatasvir, and the nonstructural protein 3 protease inhibitor, asunaprevir, in hepatitis C virus genotype 1b-infected null responders. Hepatology 2012;55(3):742-8
28. Lawitz E, Poordad FF, Pang PS, et al. Sofosbuvir and ledipasvir fixed-dose combination with and without ribavirin in treatment-naive and previously treated patients with genotype 1 hepatitis C virus infection (LONESTAR): an open-label, randomised, phase 2 trial. Lancet 2014; 383(9916):515-23